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An Update on Glomerulopathies

Edited by Sharma S. Prabhakar

AN UPDATE ON GLOMERULOPATHIES – CLINICAL AND TREATMENT ASPECTS

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Notice

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Meet the editor

Dr. Sharma S. Prabhakar is a distinguished nephrologist currently at Texas Tech University Health Sciences Center, where he is a tenured professor in the Departments of Medicine and Cell Physiology and Molecular Biophysics, and the Chief of Nephrology Division and Vice Chairman, Department of Medicine. He is an established researcher examining pathophysiologic mechanisms of diabetic nephropathy and of insulin resistance in vitro and animal models. In the area of clinical research he initiated and is the principal investigator of a number of clinical studies. Dr. Prabhakar has over 100 publications including original articles, reviews, book chapters and published abstracts in prestigious journals, such as the American Journal of Physiology, Journal of American Society of Nephrology, and Kidney International. He has recently published a reference book entitled "Advances in the Pathogenesis of Diabetic Nephropathy". In recognition of his excellence in practice of medicine, Dr. Prabhakar was awarded an endowed chair by the University Medical Center. He is active in several professional societies and organizations and is the current President of American Federation for Medical Research.

Contents

Foreword

The study of the fundamental and clinical aspects of glomerular disease has expanded exponentially over the last several decades. Fresh insights into old clinico-pathological entities emerge frequently and continuously. New disease entities also arise as the sophistication of investigation expands. Novel disease-specific treatments are applied often with impressive results while older, more empiric therapeutic strategies are in a constant state of evaluation and re-evaluation. Thus a book dealing with recent advances in the field of glomerulopathies from both clinical and therapeutic aspects is a welcome addition to our knowledge base. *An Update on Glomerulopathies - Clinical and Treatment Aspects* builds on the background provided by An Update on Glomerulopathies - Etiology and Pathogenesis.

In 24 concise, focused and well-formulated chapters the broad scope of clinical and therapeutic aspects of primary and secondary glomerular disease is reviewed and updated. The Editor, Dr. Sharma Prabhakar has chosen both the topics and the authors wisely. The contributions will have great appeal and value to the practicing clinician who frequently must face diagnostic and management challenges in this arena of medicine. While not exhaustive in coverage, the topics included embrace an impressive breadth of clinical experience in these disorders. The contributions also examine contemporary issues in sufficient detail to serve as an excellent guide for the clinician as well as trainees in nephrology. *An Update on Glomerulopathies - Clinical and Treatment Aspects* illustrates how far we have come in our understanding of clinical issues in glomerular disease, but also shows how much more needs to be understood.

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Preface

As the complexities of pathobiology of the glomerular diseases continue to unfold, the resultant scientific knowledge has lent itself to development of novel therapeutic targets and innovative preventive and therapeutic strategies. This book is a sequel to a similar one devoted to Etiology and Pathogenesis, and a broad discussion of advances in the clinical and treatment aspects of various glomerular disorders is the focus of this book. While most individual chapters serve as updates of clinical and treatment aspects of the respective glomerulopathies, they are by no means comprehensive discussion of these clincal conditions. The reader is referred to currents textbooks for the basic background clinical information of glomerulopathies and to enrich such knowledge from this reference book.

The book contains twenty four chapters divided conveniently into five sections. The first section deals with primary glomerulopathies and has six chapters. The first chapter is a rather comprehensive discussion on the clinical aspects of membranous nephropathy with a detailed review of the currently available treatment options. This is followed by a very exhaustive narrative of focal and segmental glomerulosclerosis with lucid illustrations. Membranoproliferative glomerulonephritis is a complex disorder which is also seen is several systemic conditions and is very elegantly discussed by Matthew Pickering et al. There are two chapters on IgA nephropathy, the most common glomerulopathy worldwide. Francois Berthoux et al. authored an excellent overall review of the clinical features and management of this condition, while Dimitrios Kirmizis et al. present a comprehensive review of the genetic determinants of IgA nephropathy. Finally, Maria Pia Rastaldi provides a crisp and clear discussion on the important subject of rapidly progressive glomerulonephritis with current management strategies.

The second section is devoted to glomerulopathies complicating infectious conditions and includes six chapters. Gurmeet Singh authored a concise yet informative review on the postinfectious glomerulonephritis. In addition, there is a separate chapter on

the renal and non-renal sequelae of S pyogenes infections by Luiza Guilherme et al., while W. Michael McShan et al. provided a focused overview on the genomics of post streptococcal glomerulonephritis. The atypical clinical features of poststreptococcal glomerulonephritis are discussed in a chapter written by Toru Watanabe. Finally, there are two chapters that provide recent insights into glomerular complications of Hepatitis C and HIV infections respectively.

Systemic autoimmune disorders and vasculitides constitute major causes of glomerular disease and often renal failure. The third section deals with such conditions and includes six chapters. Marco Zaffanello provided an excellent overview of the clinical aspects and management of Henoch-Schönlein Purpura in children, while Chi Chiu Mok reviewed the glomerular involvement in systemic lupus erythematosis in an elaborate manner. There are two other chapters covering other autoimmune conditions leading to glomerular disease, namely anti-glomerular basement membrane disease with a detailed description of the clinical features and management (Kouichi Hirayama et al.) and autoimmune glomerulonephritis with special focus on mixed bone marrow chimerism in the treatment (Emiko Takeuchi). Two other chapters discuss pulmonary renal syndrome, with Martin Kimmel et al. discussing the differential diagnosis of the condition in a chapter, while Mitra Naseri describes in the chapter, the rapidly progressive glomerulonephritis specifically leading to pulmonary renal syndrome.

The fourth section includes four chapters discussing the glomerular involvement in some major metabolic and systemic conditions. The first of these is diabetic glomerulopathy, which is the leading cause of renal failure resulting in end stage renal disease in the western hemisphere. This chapter is a very comprehensive review of pathogenesis and clinical features, besides being an exhaustive review of the currently available and emerging therapeutic options. Hequn Zou et al. wrote an excellent review on the glomerular involvement in a related disorder, the metabolic syndrome, a condition that is regarded by many as a distinct disease and is on the rapid rise to epidemic proportions. Daniel Fischman et al. authored a chapter on glomerular disease in cystic fibrosis, a systemic metabolic condition, while in another chapter, Han-Seung Yoon et al. reviewed milder forms of Alport syndrome, a hereditary glomerulopathy.

The final section has two chapters which relate to some general aspects of glomerular diseases. Gertruida van Biljon reviewed in a special chapter, glomerular disease leading nephrosis in children in South Africa. Finally, Toshihiko Ishimitsu described

in a concise and clear manner the significance of and approaches to blood pressure control in the context of glomerular disease.

In summary, *An Update on Glomerulopathies - Clinical and Treatment Aspects* is a systemic overview of recent advances in clinical aspects and therapeutic options in major syndromes of glomerular pathology. While by no means this book replaces currently available textbooks in nephrology, it is the expectation of the Editor that it will form an excellent reference tool for practicing and academic nephrology community. The Editor expresses deep and sincere gratitude to all the authors for their valuable contributions which facilitated the prompt compilation of this invaluable resource.

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Part 1

Primary Glomerulopathies

1

Membranous Nephropathy

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1. Introduction

Membranous nephropathy (MN), a very common cause of nephrotic syndrome, is a glomerulopathy defined histopathologically by the presence of immune complexes on the extracapillary side of the glomerular basement membrane (GBM). Idiopathic membranous nephropathy (IMN) is an antibody-mediated glomerular disease with no defined etiology, histologically characterized by uniform thickening of glomerular basement membrane (GBM), caused by subepithelial immune complex deposits. Most cases of MN are idiopathic, for instance approximately 75% of the cases of MN in developed countries are idiopathic, or primary membranous nephropathy (IMN). MN can be secondary to a wide spectrum of infections, tumors, autoimmune diseases or exposure to drugs or toxic agents. Examples include systemic lupus erythematosus, hepatitis B antigenemia or other chronic infections, and historically graft vs. host disease, sickle cell anemia, a number of drugs and toxins such as therapeutic gold salts, penicillamine, tumors, and agents containing mercury
Idiopathic MN is a glomerulus-specific autoimmune disease and second only to focal glomerulosclerosis, is a leading primary cause of the nephrotic syndrome in adults. The name, '*membranous* nephropathy' reflects the pathological observation in light microscopy of thickening in the GBM between and around immune deposits that occur beneath the podocyte foot processes. The histological hallmarks of the disease were first described by Jones and Mellors and Ortega' over 60 years ago. These include "spikes," stained by methenamine silver, of normal GBM that extend between the immune deposits, a fine granular distribution of immunoglobulin (lg) G and the complement component C3 in a capillary-loop pattern revealed by immunofluorescence, and the presence of electron-dense subepithelial immune deposits indicated by electron microscopy (EM). Idiopathic MN most commonly occurs in patients between the ages of 30 and 60 years, with men twice as likely to be affected as women. However, MN does occur in children as well as in the very elderly. Up to 70% of patients present with the nephrotic syndrome and the others garner clinical attention due to abnormalities in urine sediment such as proteinuria. Microscopic hematuria is observed in up to 50% of cases although red cell casts are rare. Hypertension and impaired renal function are uncommon at the outset of the disease and are more likely to occur with disease progression

2. Pathogenesis

Idiopathic membranous nephropathy (IMN) is an antibody-mediated glomerular disease that is histologically characterized by uniform thickening of glomerular basement membrane (GBM), caused by subepithelial immune complex deposits. The immune

deposits consist of IgG, mainly IgG4 and IgG1 of antigens that have long escaped identification, and the membrane attack complex of complement C5b-9 (MAC). The formation of subepithelial immune deposits and complement activation are responsible for functional impairment of the glomerular capillary wall, causing proteinuria .Most data on the pathogenesis of MN comes from an animal model, the Heymann model of experimental MN in rats, which suggests that the podocyte is the target of injury. Studies show that there is in-situ binding of a circulating antibody to antigen in the subepithelial space. In the Heymann nephritis model, megalin was identified as the antigenic target. However, megalin, which is a member of the low-density lipoprotein receptor family and is expressed with clathrin at the base of podocyte foot processes (the site of immune complex formation) in rats, is an unlikely antigen for human MN.

In Heymann nephritis model of MN, rats are immunized against an antigenic fraction derived from rat proximal tubular brush border and develop subepithelial deposits virtually identical to those observed in human disease. The target antigen is a large transmembrane endocytic receptor known as megalin. In the rat (but not in human beings) megalin is additionally present on the foot processes of podocytes, allowing circulating antimegalin antibodies to cross the GBM, bind megalin at the podocyte cell surface, and ultimately form subepithelial immune deposits in situ. Complement, activated by the immune deposits, leads to insertion of the terminal complement components C5b-9 (the membrane attack complex) into the podocyte cell membrane, causing cell injury, effacement of the foot processes, and proteinuria.

In 2002, Debiec and Ronco identified neutral endopeptidase (NEP) as the responsible antigen in a rare subset of patients with alloimmune antenatal membranous nephropathy. This discovery supplied proof of concept that a human podocyte antigen could serve as a target for nephritogenic antibodies, as shown some 20 years earlier for the rat podocyte megalin in Heymann nephritis, the well established experimental model of membranous nephropathy.

Debiec and Ronco studied the development of neonatal MN in infants born of mothers genetically lacking neutral endopeptidase (NEP), a membrane-associated podocyte antigen that digests peptides. Because the fetus did not lack NEP, fetomaternal alloimmunization occurred and anti-NEP antibodies (often in very high titers) developed in the mothers. These antibodies (often of the IgG4 or IgG1 subclasses, similar to human idiopathic MN) crossed the placental barrier and interacted with the NEP, heavily expressed on the normal fetal podocyte. In situ immune complexes (containing both IgG1 and IgG4) developed in the newborn infant (or soon after birth) and typical MN ensued, along with proteinuria and nephrotic syndrome. The finding of the C5b-C9 membrane attack complex in the deposits, suggesting that this spontaneous human alloimmune disease also might be complement-dependent, was similar to what had been proposed for Heymann nephritis.

In 2009 Beck and et al identified circulating autoantibodies reactive with the transmembrane glycoprotein M-type phospholipase A2 receptor (PLA2R) in the majority of cases of adult IMN. This protein is expressed by the human podocyte, again suggesting a mechanism of disease that fits the paradigm established in Heymann nephritis. These anti-PLA2R autoantibodies were highly specific for IMN, and were not present in normal individuals, in patients with other causes of the nephrotic syndrome, or in cases of secondary MN. Levels of circulating anti- PLA2R antibodies parallel the course of clinical disease, declining or disappearing before a partial or complete remission of proteinuria, and reappearing with recurrence of nephrotic syndrome

T cells play a significant role in the pathogenesis. The presence of IgG4, which is a product of the type 2 response T helper cells (Th2) and an upregulation of cytokines, such as interleukins (IL) -4 and -10, suggest Th2 involvement. This CD4, T-cell dependent humoral response leads to subsequent Ig deposition and complement activation.

More recently Stanescu et al (2011) performed independent genome-wide association studies of single-nucleotide polymorphisms (SNPs) in patients with idiopathic membranous nephropathy from three populations of white ancestry (75 French, 146 Dutch, and 335 British patients). The patients were compared with racially matched control subjects; population stratification and quality controls were carried out according to standard criteria. In a joint analysis of data from the 556 patients studied (398 men), they identified significant alleles at two genomic loci associated with idiopathic membranous nephropathy. Chromosome 2q24 contains the gene encoding M-type phospholipase A_2 receptor (PLA_2R1) (SNP rs4664308, $P=8.6\times10^{-29}$), previously shown to be the target of an autoimmune response. Chromosome 6p21 contains the gene encoding HLA complex class II HLA-DQ alpha chain 1 (HLA-DQA1) (SNP rs2187668, $P=8.0\times10^{-93}$). The association with HLA-DQA1 was significant in all three populations ($P=1.8\times10^{-9}$, $P=5.6\times10^{-27}$, and $P=5.2\times10^{-36}$ in the French, Dutch, and British groups, respectively). The odds ratio for idiopathic membranous nephropathy with homozygosity for both risk alleles was 78.5 (95% confidence interval, 34.6 to 178.2). They concluded that an HLA-DQA1 allele on chromosome 6p21 is most closely associated with idiopathic membranous nephropathy in persons of white ancestry. This allele may facilitate an autoimmune response against targets such as variants of *PLA2R1*, findings which suggest a basis for understanding this disease and illuminate how adaptive immunity is regulated by HLA.

2.1 Natural history and prognosis of idiopathic MN

MN is a chronic disease, with spontaneous remission and relapses. In the United States and Europe, MN remains the second or third leading cause of ESRD among the primary glomerulonephritis types. Spontaneous remissions occur in up to 30% of cases and usually occur within the first 2 yrs after presentation. The percentage of patients going into spontaneous remission is much lower in patients with higher grades of proteinuria at presentation (*e.g.*, proteinuria >8 g/24 h). The remaining two thirds are divided into those with persistent proteinuria who maintain renal function long term, or who progress to renal failure. In white patients with NS, 10-yr kidney survival of 70% has been reported. Although the percentage of the IMGN population that progresses to end-stage renal failure remains relatively small, the absolute numbers are large. It affects people predominantly in their 30s and 40s, and has an enormous long-term impact on their quality of life and productivity. Because they have single-organ disease rather than multisystem organ failure (as is seen in diabetes), they survive longer on dialysis and after renal transplantation. However, even though these patients survive longer, they continue to function at a lower level in comparison with the age and gender matched normal population, and rarely returns to the same level of productivity or quality of life as their peers. Even in patients who do not progress to ESRD, complications often occur, including life-threatening thromboembolic phenomena and accelerated vascular disease. These may be due to an underlying specific defect in coagulation and/or tissue repair and/or the long-term sequelae of their prolonged nephrotic condition.

Today, once the diagnosis is made, the management of edema, BP, and hyperlipidemia is effective in almost all IMGN patients. The impact of the control of these factors alone on the

natural history is expected to be positive but is currently unknown. This is partly due to the unusual phenomena of up to 30% of IMGN patients experiencing spontaneous remission. This wide variation in outcome is one of the factors that has led meta-analysis and systematic reviews of this disease to reach varying conclusions about the impact of immunosuppressive treatment on patient and renal survival and on proteinuria remission rates.

Female gender and low grade proteinuria is associated with good prognosis and associated with spontaneous remission. End-stage renal disease occurs at a 2-3:1 male:female ratio. Also, Asians with IMN appear to have a more favorable long-term prognosis than their non-Asian counterparts.

The Toronto Glomerulonephritis Registry created a model for identifying patients at risk for progression of renal insufficiency, taking into account the initial creatinine clearance (CrCl), the slope of the CrCl, and the lowest amount of proteinuria during a 6-month period. According to this model, patients who present with a normal CrCl (proteinuria <4 g/24 h), and stable renal function over 6 months are considered to be at low risk for progression. On the other hand, patients with persistent proteinuria (>8 g/24 h) have a 66−80% probability of progression to ESRD within 10 years, independent of the degree of renal dysfunction. Other factors associated with poor prognosis include older age, tubular interstitial changes on kidney biopsy and a high degree of glomerulosclerosis.

2.2 Clinical manifestations

MN affects patients of all ages and races, but is generally more common in men than women. It most commonly occurs in middle age, with peak incidence between the ages of 40-60. In contrast to primary MN, secondary forms of MN are most commonly encountered in young children and in individuals who are older than 60. At presentation, 60-70% of patients have nephrotic syndrome with the remaining 30-40% of patients presenting with subnephrotic range proteinuria (<3.5 g/24 h). 60 % of patients who present with subnephrotic range proteinuria will progress to full nephrotic syndrome in 1-2 years (Daniel Cattran 2006). Microscopic hematuria is common in MN (30 to 40%), but macroscopic hematuria and red cell casts are rare and should suggest other diagnoses .The majority of patients with MN are normotensive at presentation, however hypertension is present in 10-20 % of patients. Less than 20% present with renal insufficiency.

Serologic evaluation of all patients with MN should include anti-nuclear antibody HbsAg and hepatitis C virus antibody studies. Workup for malignancy is also warranted, to the extent that testing should be guided by the patient's age and whether there is a history of tobacco use.

2.3 Primary (idiopathic) vs. secondary forms of membraneous nephropathy

In developed countries, MN is primarily idiopathic, implying that known secondary causes have been effectively ruled out. Secondary forms of MN have been linked to multiple different agents and conditions. MN occurring post-hematopoietic stem cell transplantation (HSCT) may be a humoral manifestation of chronic graft-versus-host disease; it is the most common cause of post-HSCT nephrotic syndrome, and like idiopathic, post-HSCT MN disproportionately affects males. MN may recur in up to 42% of renal allografts with slowly progressive proteinuria; it is also possible for *de novo* MN to occur, perhaps as *an* alloimmune reactivation to minor histocompatibility antigens on the allograft podocytes.

Finally, MN may briefly occur early in infancy as a result of feto-maternal alloimmunization. Idiopathic MN must be distinguished from the various secondary causes, since treating or eliminating those underlying conditions are often sufficient to cause nephrotic syndrome remission.

The most common secondary form of MN in the United States (US) is membranous lupus nephritis (LN), designated class V LN by the International Society of Nephrology-Renal Pathology Society, and is seen in -10%-20% of LN cases (picture of LMN). The disease may occur in isolation and pre-date other symptoms or serological abnormalities suggestive of lupus. Thus, even in the absence of positive serological markers such as antinuclear antibodies (ANAs), membranous LN should remain a possibility in any young woman with a biopsy diagnosis of MN. Features that distinguish idiopathic MN from membranous LN and other secondary forms of MN include the glomerular location of the immune deposits, the predominance of a particular IgG subclass, and other pathological features. Clues to the diagnosis of membranous LN include the presence of subendothelial and mesangial deposits, in addition to the predominant subepithelial deposits, and a "full house" pattern of staining for IgG, IgA, IgM, C3, and Clq on immunofluorescence. In idiopathic MN the predominant IgG subclass found in the glomerular deposits is IgG4, whereas in many secondary forms, IgGl, IgG2, and IgG3 predorninate. Finally, an ultrastructural finding of tubuloreticular structures in the glomerular endothelium suggests lupus, although these structures can also be found in other non-idiopathic forms of MN.

Currently, renal biopsy is the sole means for diagnosis of MN and distinguishing it from other causes of nephrotic syndrome. The results of routine serological studies, including complement levels, are all normal in idiopathic MN. Possibly, antibodies to the human phospholipase A2 receptor (PLA2R) found in many patients with idiopathic MN may allow a serological diagnosis of MN, but this test is only available in research settings. Secondary causes of MN may be suggested by the presence of ANA, hepatitis B virus (HBV)-antigenemia, or concurrent infection with schistosomiasis or secondary syphilis. Hypocomplementemia may occur in lupus or HBV-associated MN, but normal complement levels do not rule out these diagnoses. Associations of MN with malignancy have been found in older individuals seemingly more frequent than chance. Therefore, in older individuals with newly diagnosed MN, tests to exclude malignancy is reasonable.

A number of secondary processes can also cause MN that are clinically and histologically similar to IMN. Worldwide, chronic infections such as hepatitis B, malaria, syphilis, and schistosomiasis are the most important causes of secondary MN. Systemic lupus erythematosus can give rise to a membranous form of glomerular disease, classified as class V lupus nephritis. Other autoimmune diseases such as rheumatoid arthritis, autoimmune thyroid diseases, and Sjogren's syndrome can all be associated with MN. Historically, certain medications used for the treatment of rheumatoid arthritis such as gold salts, penicillamine, and some NSAIDs were causally linked to MN. Solid tumors are associated with secondary MN more often than chance alone would predict, and on rare occasions remissions and relapses of the glomerular disease have been noted to occur with removal or relapse of the malignancy.

Finally, MN can occur de novo after renal transplantation or allogeneic hematopoietic stem cell transplantation, perhaps reflecting alloimmunization to a minor histocompatibility antigen expressed in the glomerulus. Secondary forms of MN often exhibit histopathological clues that distinguish them from IMN, although this is not always the case. As opposed to the exclusively subepithelial and intra-membranous deposits seen in IMN, secondary forms,

especially membranous lupus nephritis, often have mesangial and subendothelial deposits. Tubuloreticular inclusions may also been seen within the glomerular endothelium on electron microscopy in lupus-associated MN. The IgG subclasses found within the glomerular deposits also differ. In contrast to the predominant IgG4 found in IMN, IgG2 and IgG3 are typically most abundant in secondary (lupus- and malignancy-associated) forms of MN. Finally, the nature of the electron dense material itself may herald a secondary cause. A form of MN characterized by spherular structures within the subepithelial deposits has been described that appears to be distinct from its idiopathic cousin.

Infectious
• Hepatitis B
• Hepatitis C
• Streptococcal Infections
• Malaria
• Schistosomiasis
• Syphilis
• Leprosy
• Tuberculosis
• Cytomegalovirus
Drugs
• Captopril
• Clopidogrel
• Mercury
• Penicillamine
• NSAIDs
• Gold
Autoimmune Diseases
• Systemic Lupus erythematosis
• Rheumatoid Arthritis
• Thyroiditis
• Sjogren's Disease
• Psoriasis
• Sarcoidosis
• Mixed Connective Tissue Disease
Neoplasms
• Carcinomas of the bladder, breast, pancreas, prostate, stomach cancer, lung cancer.
• Hematological malignancies: Lymphoma, Chronic Lymphoctic Leukemia
Others
• Sickle Cell
• Diabetes Mellitus
• Post-Transplant
• Hematopoietic Stem-cell transplant

Table 1. Secondary Causes of MN

2.4 Post-transplantation membranous glomerulopathy

Idiopathic MN recurs in 10–30% of patients after kidney transplantation. De novo MN, which is the most common de novo glomerulopathy in renal allografts, affects 2–9% of renal allografts. De novo MN occurs 2-3 years post-transplantation, while recurrent MN occurs after 1-2 years. The exact pathogenesis of de novo MN is unknown. Recurrence of membranous nephropathy is preceded by nephrotic range proteinuria. Recurrent membranous nephropathy usually presents sooner after transplantation (within 2 years) than *de novo* membranous nephropathy (after 2 years). Some data suggests that the actual risk of recurrence reaches 29% 3 years after transplantation. Half of the cases of recurrent membranous nephropathy progressed to end-stage renal disease within a decade. There is one case report in literature where the de novo MN was linked to antibody mediated rejection: the patient had donor specific antibody-DQ7. Remission of proteinuria was associated with a fall in the anti-DQ7 titer. This raised the possibility that *de novo* membranous nephropathy could be a particular manifestation of chronic antibody - mediated rejection (Menon, Shina et al 2010)

2.5 Histopathologic considerations of membranous nephropathy

The subtle nature of the light microscopic findings in some cases of Membranous Glomerulonephritis (MGN) and presence of basement membrane thickening in other glomerular diseases lead to the uncertainty in the diagnosis of MGN in its earlier days of evolution as a pathologic entity (Heptinstall R). Only after the development of electron microscopy and immunologic techniques MGN was distinguished with certainty from other causes of the nephrotic syndrome including minimal change disease and its variants and certain forms of chronic glomerulonephritis (Heptinstall).

3. Light microscopy

3.1 Glomeruli

The characteristic changes of MGN are seen in glomerular capillary walls. The other compartments may have secondary changes but are usually minor until the advanced stages of disease. The light microscopic appearance may be subtle, especially in early cases; however, in these cases immunopathology and electron microscopy can easily establish the diagnosis. Capillary loops may appear round and rigid in more advanced stages on hematoxylin and eosin stain (Fig.1).

The earliest sign by light microscopy is “moth-eaten” appearance of the GBM on silver stains (fig.2).

3.2 Immunopathology

The immunofluorescence characteristic of MGN is granular capillary wall staining for immunoglobulins and complement. IgG is present in almost all cases, and C3 staining is seen in approximately three quarter of cases (Fig. 3). The most important and invariable deposit is IgG, and even when other immunoglobulin or complement reactants are seen, they have a weaker staining and only segmental presence (Jenette JC 1983).

Mesangial deposits are usually not seen. The presence of prominent mesangial deposits or full house positivity with other immunoglobulins or complements should suggest MGN secondary to systemic lupus erythematosus.

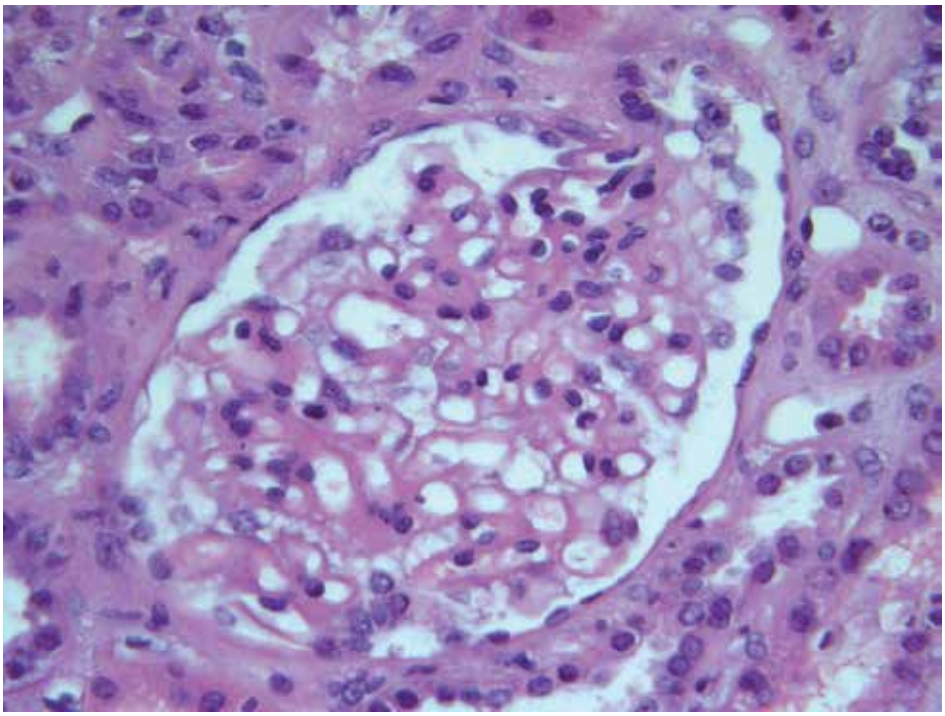


Fig. 1. Membranous glomerulonephropathy. Capillary loops may appear round and rigid in advanced cases. (H&E, 40X)

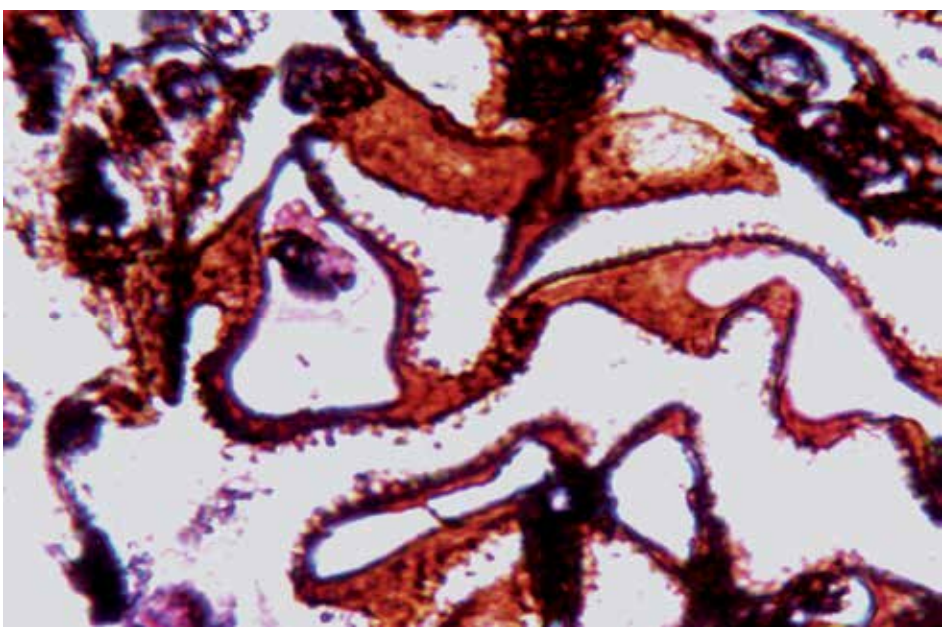


Fig. 2. Membranous glomerulonephropathy. Linear projections or "spikes" protrude from the outer surface of the GBM on silver stains (Periodic acid methenamine silver, 100X)

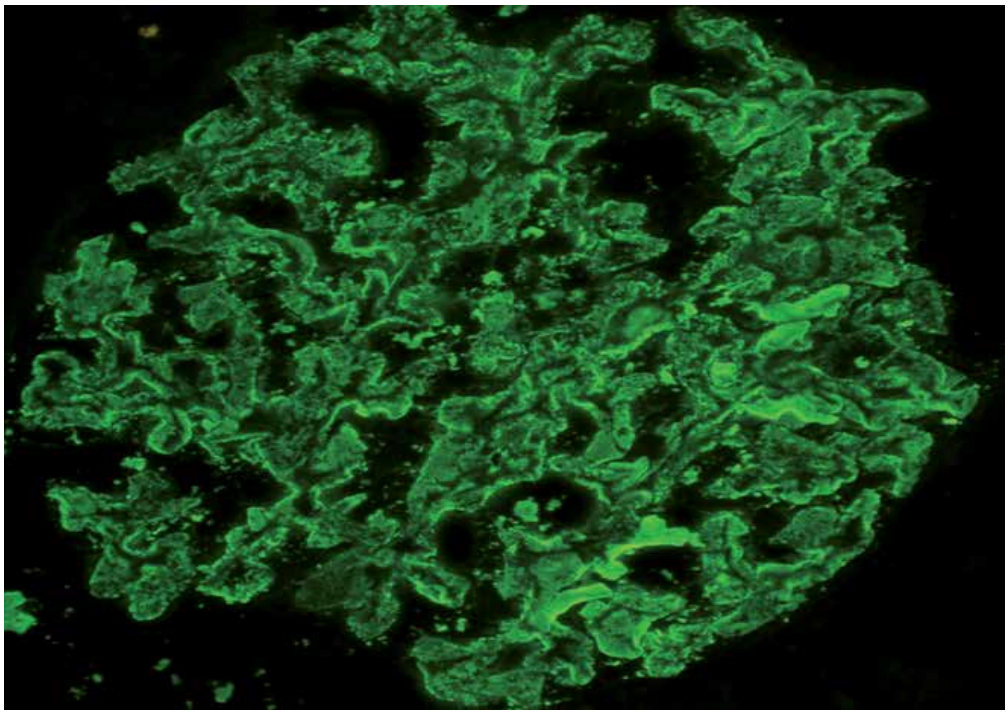


Fig.3. Membranous glomerulonephropathy. IgG is positive (2+) by immunofluorescence.

3.3 Electron microscopic findings

Electron microscopy findings helped define MGN by demonstrating the subepithelial and intramembranous (depending on the stage) location of electron-dense deposits (Gartner HV 1974). Electron-dense deposits are seen on the epithelial side of glomerular capillary loops (subepithelial). The location of electron-dense deposits at different levels in the GBM in the course of the disease led to the hypothesis that there is a sequence of changes in the GBM following initial subepithelial deposition (Ehrenreich T 1968).

3.4 Tubules

Tubular changes in MGN include progressive atrophy as the glomerular lesion progresses.

3.5 Interstitium

In uncomplicated cases, interstitial fibrosis may be seen without prominent inflammation or tubular atrophy. Development of interstitial fibrosis may reflect a progression of the glomerular lesion (Magill AB 1995).

3.6 Differential diagnosis

The light microscopic differential includes all glomerular diseases that have thickening of the glomerular basement membrane. In context of diseases associated with the nephrotic syndrome, the differential diagnosis includes minimal change disease, focal segmental glomerulosclerosis, membranoproliferative glomerulonephritis, diabetes mellitus and amyloidosis. In the past, before the development of electron microscopy and immunologic

techniques, many of these distinctions were made on clinical grounds or not at all. However, currently, characteristic histologic, immunopathologic, and ultrastructural findings can reliably distinguish it from other causes of nephrotic syndrome.

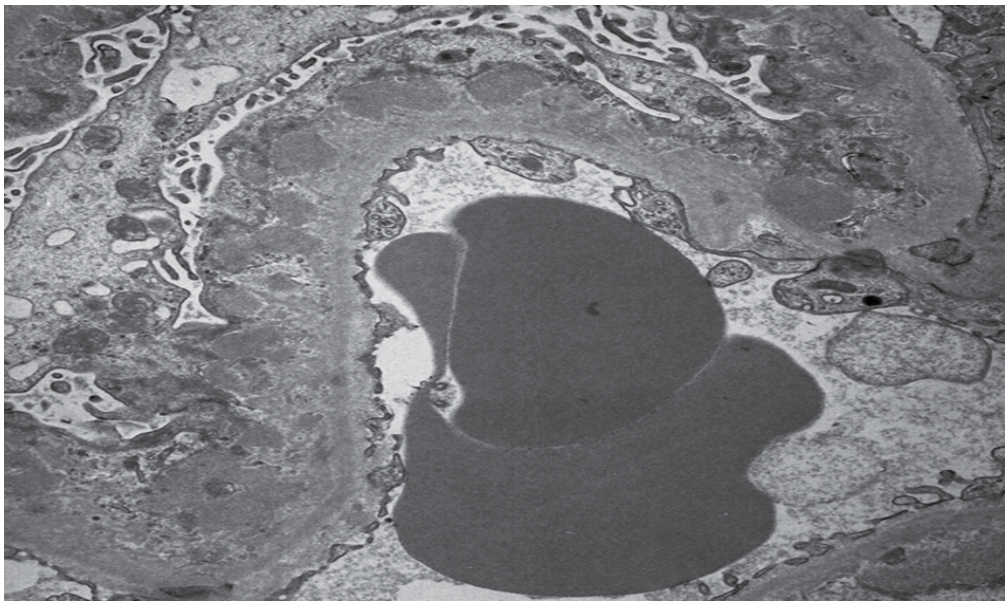


Fig. 4. Electron-dense deposits are present in subepithelial location.

3.7 Prognostic indicators

Ehrenreich and Churg (5) described the stages of membranous transformation as the morphologic representations of progression of the disease. Favorable outcomes are generally related to early stages (I and II) of membranous transformation. However, several later studies (Wahrmann 1989) did not find a relation between glomerular stage and outcome.

4. Natural history and prognosis of idiopathic MN

Although spontaneous remission of nephrotic syndrome occurs in about a third of patients, end-stage renal failure is observed in about 40% of patients after 10 years. Predicting the clinical course of a patient with MN at disease presentation is impossible given the variable and fluctuating disease course. A widely appreciated yet oversimplified view is that one-third of all patients will spontaneously remit without treatment, another third will remain proteinuric with preserved renal function and the final third will progress to end-stage renal disease (ESRD). Young females and those with subnephrotic levels of proteinuria are most likely to experience spontaneous remission, justifying several months of observation prior to any initiation of treatment in the absence of problematic clinical features. Baseline demographic differences in natural-history studies lead to a blurred prognostic picture. Several risk factors for MN progression have been proposed: older age at onset, male sex, nephrotic-range proteinuria (especially >8 g), and increased serum creatinine at presentation. As with most renal diseases, progression correlates with the amount of

tubulointerstitial disease on renal biopsy, and a tubulointerstitial disease score has been included as a prognostic variable in several studies. Although the rate of renal decline may not differ in comparison with MN patients having preserved renal function, patients with a higher serum creatinine or increased interstitial disease at presentation will reach ESRD in a shorter time; therefore, it is advisable to consider early treatment in these patients. Asians with IMN appear to have a more favorable long-term prognosis than their non-Asian counterparts.

Achieving complete remission predicts an excellent long-term renal prognosis and those patients have nearly universal renal survival at 10 years, whereas the number falls to 90% with partial remission, and 45% with no remission. Cattran and his colleagues (Cattran 2005) proposed a prognostic model dividing patients with into low-, moderate-, and high-risk groups based on their degree of proteinuria and clinical course over 6 months of observation. Those with normal renal function and lower amounts of proteinuria <4 g daily) over 6 months constitute a group at low risk for developing progressive renal insufficiency from the disease. Intermediate levels of proteinuria (4-8 g daily) with stable renal function over 6 months define a group at moderate risk. The highest-risk patients are those with >8 g of daily proteinuria for 6 months, and/or reduced renal function at outset or deterioration of renal function over 6 months. The risk of further renal deterioration in this group is at least 75%.

A number of adult studies have allowed practitioners to characterize prognostic factors in adult patients with MN. Laluck et al. showed that low-grade, subnephrotic proteinuria and female gender were associated with spontaneous remission. Ideally, only patients unlikely to spontaneously remit and those at risk for significant renal deterioration should be treated. Male gender, age >50 years, persistent high-grade proteinuria, impaired renal function at onset, presence of segmental glomerular sclerosis, and tubulointerstitial damage on the kidney biopsy have been considered to be poor prognostic factors in adult idiopathic MN .

The Toronto Glomerulonephritis Registry created a model for identifying patients at risk for progression of renal insufficiency, taking into account the initial creatinine clearance (CrCl), the slope of the CrCl, and the lowest amount of proteinuria during a 6-month period. According to this model, patients who present with a normal CrCl, proteinuria <4 g/24 h, and stable renal function over 6 months are considered to be at low risk for progression. On the other hand, patients with persistent proteinuria (>8 g/24 h) have a 66−80% probability of progression to ESRD within10 years, independent of the degree of renal dysfunction

5. Treatment of idiopathic membranous nephropathy

5.1 General outlines

Treatment goals in IMN are to prevent loss of renal function and to prevent the complications of the nephrotic syndrome (eg, hyperlipidemia, volume overload hypertension, and thrombophilia). Opinions vary on how best to obtain the desired results, and the literature concerning the treatment of IMN is still unclear. The relatively low incidence of MN hampers recruitment into clinical trials, and the variable natural history of the disease adds further treatment complications. In addition, substantial risks for treatment are associated with established immunosuppressive agents and newer, potentially less toxic agents (eg, mycophenolate or rituximab) have been introduced for the treatment of MN without the benefit of long-term clinical trials. A meta-analysis on 1025 patients with MN from 18 randomized clinical trials concluded that immunosuppressive treatment had no

benefits in patient or renal survival; however some data suggest that the treatment is warranted. Because of the high rate of spontaneous remission in MN, newly diagnosed patients with nephritic syndrome and normal renal function should initially receive conservative therapy with an ACE inhibitor or ARB, diuretics, salt restriction, and statins. If a patient remains proteinuric with normal renal function, such conservative treatment can be continued, but those patients who remain frankly nephrotic after 6 months or who initially present with (or develop) renal dysfunction should be treated with an immunosuppressive agent.

The treatment of MN depends on patient presentation and disease progression after diagnosis is made by biopsy. The two leading immunomodulatory therapies used are alkylating agents (cyclophosphamide or chlorambucil) and calcineurin inhibitors (cyclosporine or tacrolimus), both typically given orally or intravenous with corticosteroids . Given the limited efficacy, high rate of relapse, and toxicities of alkylating agents, calcineurin inhibitors, and corticosteroids, other therapies for MN are needed. Recently, rituximab has surfaced as a potential treatment option for MN. This monoclonal antibody directed against the B cell antigen CD20 may be beneficial in MN on the basis of experimental evidence that B cell activation is a key step in the pathogenesis of MN. Furthermore, rituximab is generally well tolerated with a limited short-term toxicity profile. A significant amount of literature is emerging on the benefits of rituximab in MN as primary treatment and as treatment for IMN refractory to other immunosuppressant regimens.

The treatment of membranous nephropathy in patients with normal renal function remains controversial. However in patients with deteriorating renal function, a combination therapy with steroids and cytotoxic agents is considered beneficial . It remains unclear if therapy is effective in more advanced renal failure since there is no published data on such cases. There are some cases reported in literature with near end-stage renal failure in whom treatment resulted in clinical improvement. Thus therapy is effective in patients with primary membranous nephropathy and advanced azotemia especially in those who had never been treated.((Prabhakar S et al 1996)

5.2 Alkylating agents

Corticosteroids as monotherapy for treatment of IMN is not effective, instead, typical immunosuppressive regimens for idiopathic MN combine corticosteroids with alkylating agents for 6-12 months. Treatment with cyclophosphamide or chlorambucil in conjunction with corticosteroids is supported by randomized controlled trials (RCTs) cumulative data suggest that 30%-40% of those treated will achieve complete remission, with 30%-50% attaining partial remission and only 10% developing progressive renal disease," Relapse occurs in approximately 25%- 30% within 5 years of discontinuing the alkylating agent, but often responds to a repeat course of immunosuppressive therapy arm. A 6-month regimen consisting of alternating months of corticosteroids and alkylating agents has both short-term and long-term beneficial effects on proteinuria and renal survival. Ponticelli and colleagues found that this regimen increased remission rates at the final follow-up visit from 36% in untreated patients to 76%, and improved 10-year renal survival from 60% to 92%. The long-term outcomes of a randomized, controlled trial from India (Jha et al) found the same result. Some studies indicated that a delay in therapy did not lead to differences in efficacy. Studies showed immunosuppressive therapy markedly lessened the decline in renal function.

Despite the favorable results of alkylating agents in IMN, many physicians are reluctant to use these drugs, because of increased risk of infection and myelosuppression, particularly

those with reduced GFR. Cancer risk is increased when alkylating agents are used for a long time. There are some reports of increased risk of wegener granulomatosis with cyclophosphamide when the dose is more than 36 gmr (equivalent to 100 mg daily for one year) were associated with a 9.5-fold increased risk of bladder cancer. Use of cyclophosphamide for long time have also been associated with an increased risk of lympho-proliferative disorders. Relapses occur in 25–30% of patients within 5 years of discontinuation of therapy with alkylating agents. While this rate of relapse is lower than that observed after discontinuation of cyclosporine, it is still disconcerting since relapses generally necessitate increased immunosuppression. Despite reduction in proteinuria, these agents failed to show beneficial effects on overall mortality or risk of ESRD.

5.3 The calcineurin inhibitors (CNIs): Cyclosporine and tacrolimus

Cyclosporine is an alternative, clinically validated immunosuppressive agent used in the treatment of IMN. In one randomized clinical trial (RCT), 51 patients with steroid-resistant IMN, treatment with cyclosporine plus steroids for 6 months with tapering over 4 weeks resulted in a 75% complete or partial remission rate, versus only 22% in the placebo (steroids alone) group. Typically, many patients in cyclosporine based treatment regimens achieved partial remissions, and many relapsed after discontinuing treatment. Another similarly-sized trial compared 12 months of cyclosporine and corticosteroids to cyclosporine alone." Although both groups achieved -80% remission rates at 12 months, the relapse rate was lower in the group receiving adjunctive corticosteroids from the beginning. Longer courses of cyclosporine (1-2 years) with a slow taper may be necessary to avoid a high rate of relapse. Other investigators demonstrated that treatment with tacrolimus in heavily nephrotic patients resulted in higher remission rates compared with conservative treatment alone; however, nearly half of these patients had a nephritic relapse within several months of tapering tacrolimus. Cyclosporine reduces proteinuria and the rate of decline in renal function in patients with IMN. These effects have been demonstrated in patients with preserved renal function, in those with declining or impaired renal function and also in patients resistant to other immunosuppressants. In some studies almost 50% of patients who had achieved remission relapsed within 1 year of cyclosporine withdrawal, especially in the first 6 months. In high-risk patients with declining renal function a 12 month treatment of cyclosporine led to a 50% reduction in proteinuria in half of the patients, and slowed the rate of renal deterioration compared with placebo. Notably, no prospective, randomized, head-to-head comparisons of cyclosporine and alkylating agents have been conducted in IMN.

On the basis of the available data, extended therapy seems to enhance the likelihood of remission. In one analysis, the majority of complete remissions occurred after at least 6 months of therapy, and the number increased as treatment continued for more than 12 months. Thereafter, the combination of low-dose cyclosporine (1.4–1.5 mg/kg per day; trough levels >100 ng/ml) and prednisolone (0.1 mg/kg per day) might be more beneficial than cyclosporin monotherapy for maintaining remission and preventing relapse.

Several investigators have evaluated whether tacrolimus could provide similar efficacy to cyclosporine in IMN. Tacrolimus is considered to be more potent than cyclosporine, has a more favorable cardiovascular risk profile and leads to better long-term renal function after renal transplantation. Studies showed the overall remission rate achieved with tacrolimus is similar to that reported with cyclosporine but the rate of complete remission is higher with tacrolimus. This difference might be, in part, related to the long duration of therapy used in

these studies (18 months, compared with 26 weeks in the study of cyclosporine by Cattran et al). The nephrotoxic effects of calcineurin inhibitors are of concern, particularly if long-term treatment is required as a result of relapses. Managing the use of these agents in patients with reduced GFR can be difficult. Due to this issue, Ponticelli and Villa recommend alternative agents in patients with impaired renal function (creatinine clearance <60 ml/min), severe hypertension or severe interstitial fibrosis and tubular atrophy. Finally, the extent to which calcineurin inhibitors affect the underlying immune process rather than merely modifying disease expression is unclear. In view of the broad range of toxic effects and the high rates of relapse associated with the use of steroids, alkylating agents and calcineurin inhibitors, alternative treatments have been investigated

6. Antimetabolites

6.1 Mycophenolate mofetil

Mycophenolate is another agent used for MN treatment with varying results. Initial studies suggested that mycophenolate could reduce proteinuria in patients with MN resistant to other conventional therapies. However, a recent RCT detected no effect of mycophenolate monotherapy in patients with normal renal function and nephrotic levels of proteinuria, when compared to conservative antiproteinuric therapy. Corticosteroid treatment with mycophenolate therapy achieved a 1-year cumulative remission rate of 66% in a group of MN patients with moderate renal dysfunction, but was inferior to alkalating agents and steroids in a historically treated control group and demonstrated a relapse rate of nearly 40%. However, a small RCT revealed similar effects from 6 months of mycophenolate and steroids compared with chlorambucil and steroids at 15 months of follow-up. Given these small studies and lack of consistently demonstrated superior efficacy, mycophenolate is not a first-line agent for the treatment of MN, but may be considered with adjunctive corticosteroids, if standard therapies are not effective or cannot be tolerated.

Clinical efficacy studies of mycophenolate mofetil (MMF) in IMN have produced mixed results in a multicenter study (Chan *et al.)* randomized 20 newly diagnosed patients with persistent proteinuria ≥3 g per day to undergo 6 months of treatment with either MMF plus prednisolone or with a regimen of chlorambucil alternating monthly with corticosteroids. The groups achieved similar remission rates (65%) and experienced few relapses, which suggests that MMF in conjunction with steroids has similar efficacy to a modified Ponticelli regimen. An open-label trial in the Netherlands evaluated the efficacy of MMF in patients considered to be at high risk of disease progression. The outcomes of 32 patients treated for 1 year with MMF 2 g per day and steroids were compared with those of historic matched controls treated with oral cyclophosphamide plus corticosteroids for 1 year. Patients in both groups had reduced GFR at baseline (median approximately 40 ml/min) and median proteinuria was >8 g/g creatinine. The two groups achieved similar remission rates (approximately 70%), but the relapse was higher in the MMF group such that by the end of follow-up, patients in the MMF arm were less likely to be in remission than those in the cyclophosphamide . Both treatments resulted in stabilization or improvement of renal function in the majority of patients, and infections and hospitalization occurred at a similar frequency in the two groups. Although the investigators concluded that MMF did not seem to be as effective as, or any better tolerated than cyclophosphamide, this study does suggest that a prolonged course of MMF might be of benefit even in patients with unfavorable baseline characteristics.

In contrast to the above-mentioned studies, responses to MMF in other multicenter randomized controlled trials the have been poor. Firm recommendations regarding the use of this agent as initial therapy are difficult to make. MMF might be a reasonable option when the toxic effects of alkylating agents and high-dose steroids are of particular concern or when severe azotemia prohibits use of calcineurin inhibitors. Studies in large numbers of patients with prolonged follow-up are needed to determine the long-term effectiveness of MMF for maintenance of remission and preservation of renal function. Additional information is also needed to fully evaluate the adverse effect profile of MMF. MMF is also associated with pregnancy loss and congenital malformations and it can also increase the risk of lymphoma and infection. Cases of JC-virus-associated progressive multifocal leukoencephalopathy in patients with systemic lupus erythematosus receiving MMF have elicited concern. All these considerations must be weighed in the decision to use MMF in IMN,

6.2 Azathioprine

Before the use of MMF became widespread, azathioprine was tested as a treatment for IMN in several small studies, with mixed results. A combination of azathioprine and corticosteroids was reported to be beneficial in high-risk patients with declining renal function. Some patients experienced reduction in proteinuria and stabilization or improvement of renal function. However, these studies were case series with no control groups and the combined number of patients analyzed was small. In contrast to these favorable findings, a recent retrospective review indicated that azathioprine had no long-term benefit in IMN. Due to the conflicting evidence regarding the efficacy of azathioprine in IMN and the popularity of MMF, azathioprine is unlikely to be tested in future randomized trials in this setting.

7. Alternative agents

Due to the often severe adverse or nephrotoxic effects associated with cyclophosphamide and cyclosporine, several newer and potentially less toxic agents are under evaluation for the treatment of MN. Several small studies indicate the potential efficacy of rituximab, mycophenolate, or synthetic adrenocorticotrophic hormone (ACTH) in MN; unfortunately, none are large RCTs nor do they provide long-term follow-up data

7.1 Rituximab

Rituximab is a monoclonal anti-CD20 antibody that depletes B cells, Its rationale for use is provided by the suggested pathophysiological basis for MN of autoantibodies targeting a suspected glomerular antigen. Rituximab has been used in treatment of non-Hodgkin Lymphoma and others diseases. Although Rituximab appears to induce remission with an initial efficacy comparable to alkylating agents and corticosteroids, long-term data on dialysis-free survival have not been reported. In an open label trial of rituximab with a group of 15 high-risk idiopathic MN patients, there were 2 complete and 6 partial remissions at final follow-up. Others reported the effects of treatment with 4 weekly doses of rituximab on 50 consecutive patients with persistent nephrotic levels of proteinuria despite 6 months of conservative therapy," Ten patients achieved a full remission after treatment; however, they were more likely female and with lower baseline serum creatinine values, which is a population of high spontaneous remission. Recently, a small RCT study

conducted in Spain demonstrated that rituximab was of benefit in 13 Spanish patients with idiopathic MN and CNI dependence, allowing successful weaning of the nephrotoxic CN. A review of the published literature about rituximab describing the use of rituximab in MN highlights that, while promising, the existing literature consists of too few patients, heterogeneous populations, and insufficient follow-up to recommend the use of rituximab outside the research setting.

There may be potentially fatal mucocutaneous reactions, such as stevens–Johnson syndrome and toxic epidermal necrolysis, can occur following rituximab exposure. Severe infections are infrequent, occurring in only 1–2% of patients. Of great concern, rare cases of progressive multifocal leukoencephalopathy have been reported with rituximab use, particularly as part of a multidrug immunosuppressive regimen. Physicians and patients need to be aware of the presenting features of this devastating demyelinating disease of the central nervous system, which include altered mental status, visual symptoms, motor deficits and ataxia. The preliminary results of rituximab treatment are encouraging, but concerns remain before this agent can be recommended for routine use in IMN. So far, no randomized, controlled trials have been conducted to clarify the role of rituximab in the treatment of IMN. Adequately powered, randomized, controlled trials with prolonged follow-up are needed to determine the long-term course of the disease following B-cell reconstitution; rates of relapse; subsequent redosing regimens; and effects on renal survival. Further studies must clarify whether rituximab should be used as monotherapy or in combination with other immunosuppressive drugs to achieve maximum anti proteinuric effect and durable remission. The preliminary small, uncontrolled study suggests that the addition of rituximab to tacrolimus can induce sustained remission of the nephrotic syndrome, allowing early tacrolimus withdrawal and thereby overcoming the issue of tacrolimus dependence.

7.2 Adrenocorticotrophic hormone (ACTH)

Several small, uncontrolled trials have reported beneficial effects of synthetic adrenocorticotropic hormone ACTH in patients with IMN. One small, randomized, controlled trial by Ponticelli *et al.* compared treatment with ACTH for 1 year to a 6-month regimen of methylprednisolone alternating monthly with a cytotoxic agent in 32 (mostly medium-risk) patients with IMN. The probability of complete or partial remission did not differ substantially between the groups (87% versus 93%), and the number of remissions, mean time to response and number of relapses were also comparable between the groups. The results suggest that prolonged ACTH treatment could be equivalent to the combined use of cytotoxic drugs and steroids. The side effects of ACTH include glucose intolerance, fluid retention, hypertension, diarrhea, bronze discoloration of the skin, dizziness and fatigue, all of which resolve after discontinuation of treatment. Extensive studies with long follow-up are needed to confirm the preliminary data on the use of ACTH in IMN. Further investigation is also required to find the mechanisms by which ACTH seems to decrease proteinuria and alter apolipoprotein metabolism. These effects are probably not entirely attributable to an increase in endogenous cortisol synthesis, since steroid monotherapy has not been shown to be effective in IMN. ACTH therapy can be effective in patients who are unresponsive to steroids. On the other hand, the endogenous cortisol liberated by the actions of exogenous ACTH might act differently and perhaps more effectively than orally administered steroid

7.3 Sirolimus

The role of sirolimus in IMN has been evaluated in two small pilot studies, with unfavorable results. No remissions occurred during therapy, but one patient achieved a partial remission after cessation of therapy. Severe adverse events, including pneumonitis, infection, persistent proteinuria and azotemia, necessitated discontinuation of the drug in the majority of cases. These trials were prematurely terminated owing to the unfavorable risk–benefit ratio. An open-label trial of sirolimus in 11 patients with a variety of chronic glomerulopathies and declining renal function, including three with membranous nephropathy, was associated with acute kidney injury in more than half of the patients; this event generally occurred within weeks of starting sirolimus. Thus, sirolimus does not seem to have a role in the treatment of IMN

7.4 Eculizumab

Eculizumab is a fully humanized monoclonal antibody directed against the complement protein C5, approved for the treatment of Paroxysmal Nocturnal Hematuria. Ecluzimab inhibit C5a and C5 b thus preventing complement activation. Treatment with eculizumab improves the quality of life and reduces the need of transfusions and the risk of thrombosis in patients with PNH. However, eculizumab can increase the risk of meningococcal infections perhaps due to the reduction in the levels of C5 activity. Patients should therefore be vaccinated or revaccinated with a meningococcal vaccine at least 2 weeks before receiving the first dose of eculizumab. Other side effects include headache, nasopharingitis, back pain and cough; nausea may occur in the period following injection. The mechanism of action of eculizumab renders this monoclonal antibody potentially attractive for treating patients with IMN, as the terminal components of the complement C5b--C9 play a prominent role in mediating the inflammation and the damage of podocytes and glomerular basement membrane. However, a RCT conducted in IMN failed to show any advantage over placebo of eculizumab 8 mg/kg every other week or every 4 weeks. Further trials are needed to establish whether a different dosage or more prolonged treatment may obtain therapeutic results in IMN

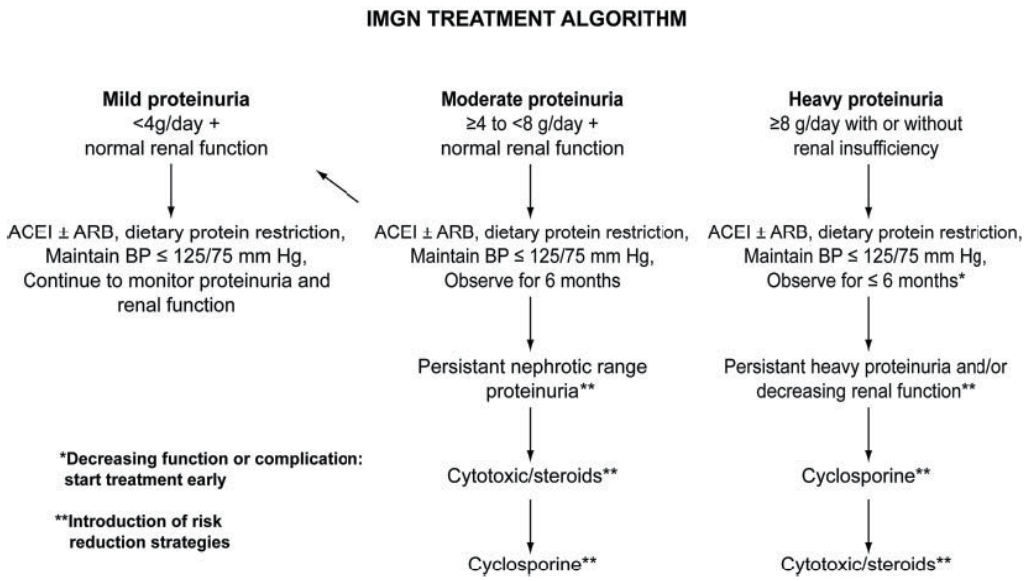
8. Intravenous high-dose immunoglobulins [IVIG]

IVIG have been used in high dose in treatment of IMN. IVIG interferes with complement-mediated immune damage by binding to C3b and C4b,by this mechanism preventing glomerular injury. This mechanism may be involved in IMN, as suggested by a study in passive Heymann nephritis, in which treatment with systemic immunoglobulin obtained a decrease in proteinuria, associated with a decreased glomerular deposition of C3c and C5b--9, without changes in the amount, size or distribution of the subepithelial immune complexes . A few anecdotal uncontrolled studies suggested a possible benefit of IVIG therapy in IMN.

8.1 Anticoagulation

Use of anticoagulation in nephrotic syndrome is controversial issue. Nephrotic syndrome (NS) is associated with high risk of for thromboembolic complications, including deep venous thrombosis, renal vein thrombosis, and pulmonary embolism; this risk seems to be greater for IMN especially in patient with low albumin and previous history of thrombo-embolic disease. Analyses showed that in patients with IMN the benefits of oral

anticoagulation outweigh the risks. However, before prescribing anticoagulants the physician should take into account the severity of the NS (as assessed by serum albumin concentration), pre-existing thrombotic states, and the overall likelihood of serious bleeding events consequent to oral anticoagulation The optimal duration of prophylactic anticoagulation is unknown but should probably last for as long as NS persists (Ponticelli C et al 2010).



[Cattran et al 2010].

9. Future directions

If anti-PLA2R or other MN-specific autoantibodies can be demonstrated to be tightly associated with immunological disease activity in idiopathic MN, a serologic immunoassay would have several potential applications. it could use the anti- PLA2R as an initial assay for the diagnosis of idiopathic MN without kidney biopsy. Serial assays for the presence and titer of anti-PLA2R prior to therapeutic intervention in clinical trials could help reduce uncertainty as to whether rapid responders represent a true therapeutic effect or a spontaneous remission. Anti-PLA2R could also be followed during treatment to assess the efficacy of immunosuppressive therapy and to determine the length of treatment. It could also be useful in partial remission, when residual proteinuria could be caused either by ongoing but attenuated immune activity or by structural glomerular changes without immune activity.

10. Summary

Membranous Nephropathy is a common cause of nephrotic syndrome in adults of all races and ethnicities. Its molecular pathogenesis is increasingly well understood, and identification of PLA2R as a target antigen may allow better diagnosis, better following of

the disease course, and improved decision-making regarding necessity and duration of treatment. Treatment should be provided to those at high risk of progression to ESRD, including patients with persistent severe proteinuria or a documented loss of renal function. At present, alkylating agents and cyclosporine are the only clinically validated treatments with sufficient follow-up data; however, as the roles of tacrolimus, rituximab, mycophenolate, and ACTH grow, these agents may become the new treatments of choice for idiopathic MN.

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2

Focal Segmental Glomerulosclerosis

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1. Introduction

Focal segmental glomerulosclerosis (FSGS) as the name implies is a histopathological pattern of lesions, where the "focal" refers to, involving minority of glomeruli and the "segmental" refers to, involving a portion of the glomerular capillary tuft caused by injury to podocytes (Fig 1A). Clinically it manifests proteinuria which can progress to nephrotic syndrome and eventually to end stage renal failure.

1.1 Historical aspects

Karl T. Fahr, a German pathologist, described "Progressive lipoid nephrosis" in 1925 which is currently recognized as FSGS. Arnold Rich (1957) was the first to report segmental sclerosis in juxtamedullary glomeruli in autopsy cases of children with nephrosis and uremia. In this report, he hypothesized that the progression to end stage renal disease (ESRD) in a subset of children with idiopathic nephrotic syndrome was because of the development of glomerular sclerosis. Churg et al published histopathological classification of nephrotic syndrome in children for the International Study of Kidney Disease in Children (ISKDC) in 1970, and the disease entity of FSGS was emphasized as a clinicopathological entity separate from minimal change disease (MCD) by its marked resistance to steroids and progression to ESRD. Habib (1973) described clinical and histopathological features of this entity as a separate disease entity using the term 'focal glomerular scleroses'.

Brown et al (1978) reported the malignant form of FSGS, which is characterized by FSGS with rapid decline in renal function. Howie et al. (1984) described the glomerular tip lesion in FSGS, as glomeruli with segmental lesions at the outer 25% with adhesion or prominence of podocytes at the tubular neck. Patients with the glomerular tip lesion often develop nephrotic syndrome and have excellent response to steroids, favorable outcome, and their clinical course is similar to that of patients with MCD. Schwartz et al. (1985) reported another form of FSGS-related cellular lesion characterized by glomerular extracapillary epithelial hypercellularity and endocapillary hypercellularity with foam cells and infiltrating leukocytes, and this was considered to represent an early stage in the development of FSGS. Soon after a collapsing form of FSGS as a new clinic-pathological entity was described by Weiss et al. (1986), which is characterized by nephrotic syndrome with progressive irreversible ESRD and by glomerular collapse with epithelial hypercellularity. Subsequently, many cases of renal disease associated with HIV infection, (Rao TK et al 1984) termed as HIV-associated nephropathies, were reported with features similar to the collapsing form of FSGS.

Between 1980–2000, secondary FSGS (Rennke HG et al. 1989 & D'Agati V. 1994) was established as FSGS with recognized etiologic associations, including genetic mutations in podocyte-associated proteins, virus, drug toxicities, and structural–functional adaptations. Finally in 2004, the Columbia classification was proposed by D' Agati, Fogo, Bruijn, and Jenette, as a working classification for FSGS as given in table 2. Lately some celebrity figures like Sean Elliott and Alonzo Mourning (National Basketball Association) have been diagnosed with FSGS which has further enhanced awareness of this disease in public.

1.2 Epidemiology

Focal segmental glomerulosclerosis (FSGS) currently is the leading cause of nephrotic syndrome in adults and children, particularly in the United States, Australia, Brazil, Canada, Kuwait, India and many other countries. (reference from: http://eng.hi138.com/?i295373_The-epidemiology-of-focal-segmental-glomerulosclerosis).
Renal biopsy survey for idiopathic nephrotic syndrome in adults in United States between 1995 to 1997, has revealed that FSGS is the most common cause of nephrotic syndrome, responsible for 35 percent of all cases and more than 50 percent of cases among black population, (67 percent of such cases in black adults were younger than 45 years of age). Idiopathic FSGS is now the most common cause of end stage renal disease (ESRD) caused by primary glomerular disease in the United States in both the black and white populations. The proportion of ESRD attributed to FSGS has increased 11-fold, from 0.2% in 1980 to 2.3% in 2000 (excluding patients with HIV). As per Kitiyakara C. et al (2004) the peak decade for FSGS ESRD incidence is 40 to 49 years among black patients as compared to, 70 to 79 years among white and Asian patients. Males have 1.5- to 2-fold greater risk than females. Recent incidence of end stage renal disease secondary to FSGS is five cases per million population in Caucasian US population and 30-40 cases per million population for African-American population (Hogg R. et al 2007).

2. Etio-pathogenesis

While majority of FSGS cases are still considered idiopathic, the etiologies and mechanisms involved in FSGS development continue to be elucidated. FSGS can be divided into primary or idiopathic and secondary depending upon if the etiology is unknown vs. known respectively. For FSGS to produce nephrotic range or non-nephrotic range proteinuria, alterations of normal glomerular structure and function has to occur. Normal glomerular function requires, that the three major components of glomerular filter, namely *endothelial cells, podocytes, and glomerular basement membrane (GBM)*, be intact and are able to provide a permselective filtration barrier. Specialized tight junctions between podocyte foot processes create a slit diaphragm (SD) which is integral in preventing the loss of protein into Bowman's space (Kimberly et al. 2007 & Asanma et al 2003) . Even though the clinical presentation of FSGS is often heterogeneous, a cardinal feature of the disease is proteinuria, which implies loss of this permselective barrier (Schnaper HW, 2003 & Fogo AB, 2003). Electron microscopic picture clearly reveals distortion of normal architecture (or effacement) of the foot processes of podocytes in FSGS
(Fig 1B).
A key factor in the pathogenesis of FSGS is damage and loss of podocytes. Asanuma K. et al. (2003) described that based on recent insights into the molecular pathology of podocyte injury, at least four major causes have been identified that lead to the uniform reaction of

pododcyte foot processes effacement and proteinuria: (1) interference with the slit diaphragm complex and its lipid rafts (2) direct interference with the actin cytoskeleton (3) interference with the GBM or with podocyte-GBM interaction, and (4) interference with negative surface charge of podocytes. Damage to podocytes triggers apoptosis and the detachment of podocytes from the glomerular basement membrane. The resulting reduction in podocyte number (podocytopenia) leaves the glomerular basement membrane to be exposed, (Fogo AB 2003) which leads to development of maladaptive interactions between the glomerular basement membrane and epithelial cells. This is followed by proliferation of epithelial, endothelial and mesangial cells. The combined reaction of cell proliferation and leakage of proteins into Bowman's space results in deposition of the collagen. Eventually the capillary loop collapses and endothelial cells are lost and the affected part of the glomerular tuft heals by scarring causing a characteristic lesion of FSGS. Thus the lesions initially are limited to a few segments in the glomerulus (segmental) and in a few regions of the kidney (focal) but the disease ultimately progresses to involve the entire kidney leading to end stage renal disease (ESRD).

The precise initial insult that leads to the above cascade of events is still unknown. A 'circulating permeability factor' which leads to glomerular basement membrane injury has been proposed in the pathogenesis of FSGS (Shalhoub RJ, 1974). The following information favors the hypothesis of 'circulating Permeability factor' (1) frequent recurrence of proteinuria after renal transplant [Ingulli E. et al. 1991] (2) the efficacy of extracorporeal techniques such as plasmapheresis in reducing the post renal transplant proteinuria [Artero M. et al 1992 and 1994]. (3) the results of *in vitro* bioassay which detects permeability changes induced by FSGS serum on isolated glomeruli [Savin VJ et al in 1992 and 1996].

(4)Rea et al (2001) demonstrated that the main clinical feature of FSGS i.e. proteinuria, disappeared within one year after transplantation in two recipients of kidneys from a patient with FSGS. Not taking into account ethical and legal implications, good outcome of the FSGS allograft kidneys into non FSGS recipients is another good evidence for humoral genesis/circulating permeability factor, as a cause of the FSGS. (5) Kemper M. et al (2001) demonstrated transmission of glomerular permeability factor from the mother affected by FSGS to her infant during gestation. After birth, proteinuria in the child decreased and then disappeared, suggesting a strong correlation with some circulating factor transmitted from the mother to the child.

At the molecular level, cytokines and vasoactive factors are believed to play a major role in the progression of FSGS. The overexpression of transforming growth factor β (TGFβ) or its downstream proteins, the 'Smads' lead to glomerulosclerosis in animal models. Activation of the renin-angiotensin system upregulates TGFβ, which is considered to cause further progression of the disease (Harris RC et al. 2006). Angiogenic factors, like platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF) seem to play a role in disease progression. This is based upon the rat remnant kidney model (RK model) experimental studies of progressive glomerulosclerosis. In this model, VEGF upregulation soon after the renal injury and later loss of VEGF expression correlates well with progression of the glomerulosclerosis (Kang DH et al. 2001).

Mechanical stress is also believed to play a role in the progression of FSGS. (Hostetter TH. 2003 & Kwoh C. et al 2006) The hyperfiltration due to the defects of the filtration barrier results in increased single nephron glomerular filtration rate (SNGFR), which results in hypertrophy of glomeruli. The hypertrophy exacerbates the mismatch between the glomerular basement membrane and the decreased numbers of podocytes, propagating the

injury further. Another factor in the progression of FSGS is tubulointerstitial injury. Clinically, tubulointerstitial injury is a predictor of the loss of renal function in FSGS (D'Agati VD 2003 & Rodriguez-Iturbe B et al. 2005). The nonspecific entry of proteins into the tubular lumen is one potential source of damage to the tubulointerstitium. Indeed, persistence of nephrotic-range proteinuria is a negative prognostic factor for the progression of FSGS to ESRD (Walls J. 2001). Cytokines (such as TGFβ), when present in the tubules, will recruit monocytes, macrophage, and T-cells. This stimulates other cytokines, including interleukin-1, tumor necrosis factor alpha, and other chemokines. This inflammatory infiltrate leads to mesangial matrix deposition, promoting the collapse of glomeruli. The cellular infiltrate and cytokines also damage tubular epithelial cells, and some tubular epithelial cells may undergo transformation to mesenchymal cells. These mesenchymal cells, as well as recruited and stimulated fibroblasts, result in collagen matrix deposition and tubulointerstitial fibrosis
(Harris RC et al. 2006).

Regardless of the cause of podocyte injury, when the podocytes start dying, leakage of protein across the GBM leading to proteinuria and hypoalbuminemia ensues. At this time cholesterol levels start rising due to increased synthesis of cholesterol by the liver and loss of lipid regulating proteins in the urine, the underlying mechanism of these effects is not completely known yet. The beneficial effects of blocking the renin-angiotensin system may not be limited to their antiproteinuric or antihypertensive effects. As noted earlier, angiotensin stimulates TGFβ, in turn contributing to fibrosis. In addition, angiotensin affects intracellular calcium concentrations and the podocyte cytoskeleton (Harris RC et al. 2006). Inhibition of angiotensin may slow progression by these local mechanisms (Korbert SM, 2003).

With increasing incidence of FSGS, these pathways of podocyte injury and disease progression provide important targets for future intervention. Trials have already been initiated to antagonize cytokines, such as TGFβ (as discussed later in this chapter in treatment section).

Genetic mutations seen in congenital forms of nephrotic syndrome and FSGS enabled researchers to identify specific gene mutations involved in podocyte damage (Tryggvason K. et al. 2006). Mutations of the nephrin gene, a podocyte-specific transmembrane component of the slit diaphragm, are found in congenital Finnish-type nephrotic syndrome, and may lead to loss of normal caliber slit diaphragms. (Kestila M. et al. 1998, Tryggvason K. et al. 2006, Kwoh et al. 2006). In mouse models, mutations of nephrin-like transmembrane genes (NEPH-1) which also localize to the slit diaphragm result in proteinuria and early death. Other proteins which are part of the slit diaphragm complex include: podocin, CD2-associated protein (CD2AP), FAT, P-cadherin,ZO-1, LAP (leucine rich repeat and PDZ domain) protein. (Asanuma K et al 2003, Tryggvason K. et al. 2006). Mutations in podocin (a transmembrane protein that interacts with nephrin, NEPH-1 and CD2AP) have been identified in familial FSGS. Recently, mutations in CD2AP, an immunoglobulin-like protein that is involved in nephrin integration with podocyte cytoskeleton, have also been linked to genetic forms of FSGS (Shih NY. et al. 1999, Kim JM et al 2003, Tyggvason K. et al 2006). In mouse models, the loss of FAT1 and FAT2 (transmembrane proteins with cadherin-like repeats) results in the absence of slit diaphragms, proteinuria, and early death. Alpha-actinin-4, an important structural component of the podocyte cytoskeleton, is mutated in some autosomal dominant forms of FSGS (Kaplan JM et al 2000, Yao J et al. 2004). In addition to the abnormal structural proteins mutations, some other mutations have been

identified in association with FSGS like TRPC6 (Transient receptor potential cation chanel, subfamily C, member 6) which is a cation-selective, ion-channel protein that mediates calcium signals (Winn MP et al. 2005). The role of the other components of the slit diaphragm in the pathophysiology of FSGS is not yet clear.
In summary, these data suggest that mutations in the cytoskeleton and membrane proteins specific to podocytes are responsible for most inherited forms of disease. The frequency of spontaneous mutations in the general population who develop nephrotic syndromes or FSGS still needs to be assessed as it has diagnostic, prognostic and therapeutic implications in case of FSGS. For example, patients who possess genetically defective podocytes should be unresponsive to conventional steroid treatment. Similarly, these patients should not have recurrent FSGS when transplanted with a structurally normal kidney allograft. Furthermore, family members who are potential living donors could be genetically screened for mutations associated with the development of kidney disease and excluded as candidate donors.

3. Secondary FSGS

Secondary FSGS can be seen in a variety of conditions such as renal agenesis, obesity, or sickle cell disease etc where hyperfiltration is a characteristic abnormality. Glomerulomegaly is common in situations with hyperfiltration. Obesity-associated FSGS needs a definitive diagnosis with a renal biopsy to exclude the presence of concurrent early diabetic nephropathy. Secondary FSGS may also result from intravenous drug abuse and reflux disease. It has been reported that toxins, including lithium and pamidronate, and sirolimus are associated with the development of FSGS lesions. Among the viral infections, HIV is the most common cause. Rarer viral causes of secondary FSGS include persistent parvovirus B19, simian virus 40, and cytomegalovirus infection.

4. Clinical presentation

Primary FSGS more likely presents with sudden-onset nephrotic syndrome, whereas secondary FSGS presents more insidiously with subnephrotic range proteinuria and renal insufficiency. But the secondary FSGS from pamidronate toxicity though typically presents with full nephrotic syndrome and acute or subacute renal failure, with collapsing FSGS lesions on biopsy. Physical exam reveals elevated blood pressure and edema. Urine may be foamy and may have hematuria. The relative frequencies of these findings at presentation or biopsy based diagnosis in published series (Rydell JJ et al. 1995 & Chun MJ et al 2004) are as follows. Primary FSGS can present with nephrotic range proteinuria (60 to 75%), microscopic hematuria with variable degrees of proteinuria (30 to 50%), hypertension (45 to 65 %), renal insufficiency (25 to 50 %) and with overlapping of these presentations. Children tend to present with more proteinuria whereas hypertension is more common in adults. A case of FSGS should be considered idiopathic only when other etiologies are thoroughly excluded. Uncommonly Muehrcke's lines (white banding on the nails due to hypoalbuminaemia), xanthelasma and xanthomata (cholesterol deposits in skin) are associated with FSGS as well. Following risk factors need to be considered when doing history and physical examination on such patients: male gender, black race, positive family history, heroin abuse, use of known causative medications, chronic viral infection, a solitary kidney, and obesity.

Primary or idiopathic FSGS: Refer to Table 2

Secondary FSGS:

1. Virus assoiated
   a. HIV associated nephropathy (HIVAN)
   b. Parvovirus B19
   c. SV40
   d. CMV
2. Drug toxicity
   a. Pamidronate
   b. Lithium
   c. Interferon –alpha
   d. Heroin
   e. Sirolimus
3. Secondary FSGS mediated by adaptive structural-functional responses
   a. Reduced renal mass
      Unilateral renal agenesis
      Oligomeganephronia
      Renal dysplasia
      Reflux nephropathy
      Sequela to cortical necrosis
      Surgical renal ablation
      Chronic allograft nephropathy
      Any advanced renal disease with reduction in functioning nephrons
   b. Initially normal renal mass
      Obesity
      Hypertension
      Atheroembolic or other acute vaso-occlusive processes
      Cyanotic congenital heart disease
      Sickle cell anemia
      Anabolic steroids
4. Familial FSGS
   a. Autosomal recessive :
      mutations in genes NPHS1 (coding for nephrin) ,NPHS2 (coding for podocin) and PLCE1 (coding for PLC epsilon1)
      LAMB2 (coding for Laminin beta 2 chain)
   b. Autosomal dominant
      Mutations in genes ACTN1 (coding for alpha actinin4), TRPC6 (coding for TRPC6), INF2 (coding for INF2)
      WT1 (coding for WT1)

Table 1. Etiologic Classification of Focal Segmental Glomerulosclerosis

Differences in clinical manifestations correlate with differences in pathologic phenotypes. Collapsing variant of FSGS often has more severe proteinuria and renal insufficiency but less hypertension than typical variant. Patients with collapsing FSGS frequently have extrarenal manifestations of the disease, a few weeks before the onset of the nephrosis e.g. episodes of upper respiratory infections, diarrhea that are usually ascribed to the viral or other infectious processes. However, the symptoms of fever, anorexia, aches and pains are present only in 20% of patients at the time of onset of nephrosis. Glomerular tip lesion variant (Fig 4) often presents with rapid onset of edema similar to minimal change disease. Glomerular tip lesion patients may develop reversible acute renal failure, at the times of initial presentation when the degree of proteinuria, edema, hypoalbuminemia are at their peak. This behaves like minimal change disease and this rarely happens in any other forms of FSGS. Patients with glomerular tip lesion FSGS tend to be older white males in comparison to younger black males predominance in collapsing variant FSGS.
Distinguishing between primary and secondary FSGS is important, since secondary FSGS should not respond to immunosuppressive therapy. Instead, the inciting condition or toxin should be alleviated or stopped in secondary form FSGS if possible. Unfortunately, distinguishing between primary vs. secondary FSGS can be challenging, since focal sclerosis lesions may be present in a diverse assortment of glomerular, vascular, and tubular injuries, just as global sclerosis represents a common endpoint lesion for the end-stage renal disease.

5. Laboratory findings

Urine analysis may have wide range of findings starting from fatty casts to dysmorphic red blood cells and red blood cell casts. Proteinuria varies from less than 1 gm to 30g/day. Hypoproteinemia is common in patients with FSGS, with total serum protein reduced to varying extents. Hypoalbuminemia may drop as low as below 2 g/dL, especially in patients with the collapsing and glomerular tip variants of FSGS. Cholesterol levels are increased. Serum complement components are typically in normal range in FSGS. CD4 cell count and HIV test is essential in all patients with FSGS, especially those with the collapsing pattern. DNA/PCR for Parvo B19 and CMV test is an essential part of work up to rule out the rare forms of secondary FSGS. Finally renal biopsy is the final step to make the diagnosis of FSGS.

6. Histopathology

The diagnosis of FSGS is not an easy one to make, because the morphologic features of FSGS are nonspecific and can occur in a variety of other conditions or superimposed on other glomerular disease processes. Additionally, because the defining glomerular lesion is focal, it may not be adequately sampled in small needle biopsies. The diagnosis of FSGS is further complicated by the existence of a primary (or idiopathic) form and many secondary forms (Table 1). Before a diagnosis of primary FSGS can be made, secondary forms must be excluded. Idiopathic FSGS must be distinguished from human immunodeficiency virus (HIV) -associated nephropathy, heroin nephropathy and other large group of secondary FSGS caused by structural-functional adaptations mediated by intrarenal vasodilatation, and increased glomerular capillary pressures, (as listed in Table 1). The morphological types are not used to guide treatment but to provide useful prognostic information. Electron microscopy can be used to distinguish primary and secondary FSGS. In primary FSGS, foot process fusion is diffuse and occurs throughout the glomeruli. In secondary FSGS, foot process fusion is mostly limited to the sclerotic areas.

Five main light microscopic patterns of FSGS have been defined, as given below:

Variant	Positive Criteria	Negative Criteria
FSGS NOS	At least one glomerulus with segmental increase in matrix obliterating the capillary lumina. There may be segmental glomerular basement membrane collapse without podocyte hyperplasia.	Exclude perihilar,cellular,tip and collapsing variant.
Perihillar Variant	Perihillar sclerosis and hyalinosis involving >50% of segmentally sclerotic glomeruli.	Exclude cellular,tip and collapsing variant.
Cellular Variant	At least one glomerulus with segmental endocapillary hypercelllularity occluding lumina, with or without foam cells with karyorrhexis.	Exclude tip and collapsing variant.
Tip variant	At least one segmental lesion involving the tip domain (outer 25% of tuft next to origin of the proximal tubule) The tubular pole must be identified in the defining lesion. The lesion must have either an adhesion or confluence of podocyte with parietal or tubular cells at the tubular lumen or neck. The tip lesion may be sclerosing or cellular.	Exclude callasping variant . Exclude any perihilar sclerosis.
Collapsing variant	At least one glomerulus with segmental or global collapse Podocyte hypertrophy/hyperplasia	none

Classified based upon the 'Working Columbia Classification 2004'.

Table 2. Morphological variants of FSGS

Although appearance of the glomerular tuft differs in these forms, all share the common feature of podocyte alterations at the ultrastructural level. At present, it is unclear if these morphologic variants reflect pathogenetic differences or they are the consequence of different severities of podocyte injury or histopathologic evolution. Future studies are needed to address these questions.

Classic Focal Segmental Glomerulosclerosis (Focal Segmental Glomerulosclerosis Not Otherwise Specified) : also called FSGS NOS, or typical FSGS. FSGS NOS requires exclusion of the other more specific subtypes described in the table 2. Light Micrscopic examination reveals accumulation of extracellular matrix which occlude glomerular capillaries, forming discrete segmental solidifications involving affected portion of the glomerular tuft. Also seen is plasmatic insudation of amorphous glassy material beneath the GBM, endocapillary foam cells, and wrinkling of the GBM. Adhesions to Bowman's capsule are common, and overlying visceral epithelial cells often appear swollen over the sclerosing segment. Non sclerotic glomerular lobules appear normal by light microscopy except for mild podocyte swelling.

Immunofluorescence: focal and segmental granular deposition of IgM, and C3 is seen often, but C1 in the distribution of segmental glomerular sclerosis may also be seen. Nonsclerotic glomeruli may have weak mesangial staining for IgM and C3. By electron microscopic examination, segmental sclerotic lesions exhibit increased matrix, wrinkling and retraction of GBM. Accumulation of inframembranous hyaline material but *no immune complex* electron-dense deposits are seen. Overlying the segmental sclerosis, there is usually

effacement of foot processes (Fig 1.B)and podocyte hypertrophy. The adjacent nonsclerotic glomerular capillaries show only foot process effacement.

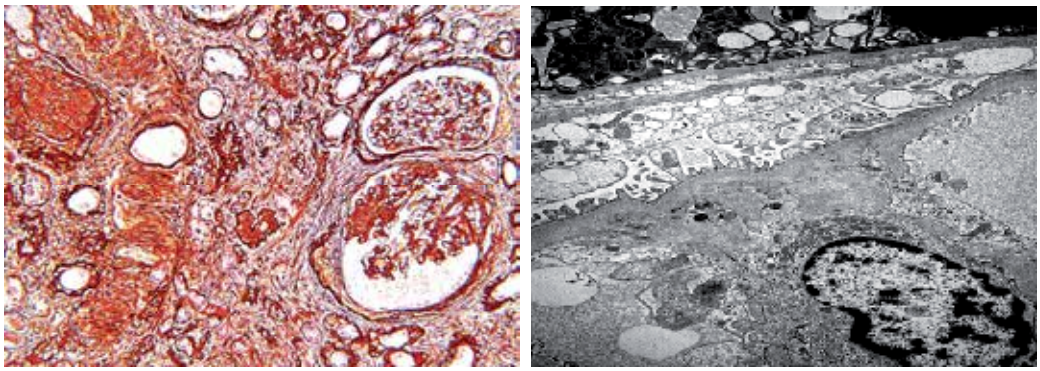

Fig. 1. (A-left) Focal segmental distribution of glomerular lesions. The glomerulus at the top right of the picture is normal. The glomerulus at the bottom shows dense segmental scars with adhesion to Bowman's capsule. (Periodic acid methenamine silver, 20X). (B-Right) Patchy effacement of foot processes is present. No immune complex-type deposits are seen along the GBM. (Electron Microscopy).

Perihilar Variant of Focal Segmental Glomerulosclerosis : This variant is defined as perihilar hyalinosis and sclerosis (Fig 2. A & B) which involves more than 50% of glomeruli with segmental lesions. This category requires that the cellular, tip, and collapsing variants be excluded. Podocyte hyperplasia is uncommon.

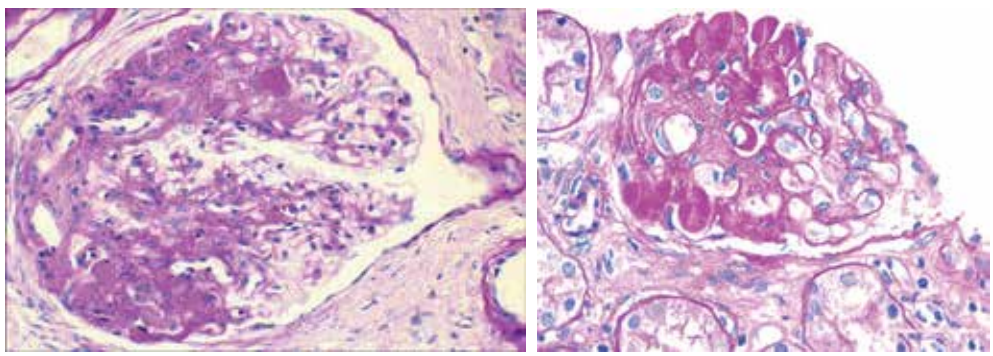

Fig. 2. (A-left): Perihillar variant FSGS (B): Glomerulus with "Hyalinosis": homogeneous, eosinophilic material may be present at the periphery of sclerotic foci, or within the capillary subendothelial space (PAS, 40X). (Fig 2A-obtained from http://www.unckidneycenter.org/kidneyhealthlibrary/fsgs.html#causes)

Immunofluorescence (IF) reveals segmental deposits of IgM and C3 in areas of sclerosis and hyalinosis.

Electron microscopy (EM) demonstrates variable foot process effacement. The perihilar variant may occur in primary or secondary FSGS. It is more common in secondary forms of FSGS mediated by adaptive structural-functional responses, in which it is typically accompanied by glomerular hypertrophy. In this setting, the greater filtration pressures at

the proximal end of the glomerular capillary bed may favor the development of lesions at the vascular pole.

Cellular Variant of Focal Segmental Glomerulosclerosis : This variant is characterized by focal and segmental endocapillary hypercellularity that may mimic a form of focal proliferative glomerulonephritis. Glomerular capillaries are segmentally occluded by endocapillary hypercellularity, including foam cells, infiltrating leukocytes, karyorrhectic debris, and hyaline. There is often hyperplasia of the visceral epithelial cells, which may appear swollen and crowded, sometimes forming pseudocrescents. This variant requires that tip lesions and collapsing lesions be excluded.

IF shows focal and segmental glomerular positivity for IgM and C3 and EM reveals severe foot process effacement.

Collapsing Variant of Focal Segmental Glomerulosclerosis: This variant is defined by at least one glomerulus with segmental or global collapse (Fig 3) and overlying hypertrophy and hyperplasia of visceral epithelial cells. There is occlusion of glomerular capillary lumina by implosive wrinkling and collapse of the GBMs. This lesion is more often global than segmental. Overlying podocytes display striking hypertrophy, hyperplasia and express proliferation markers. Podocytes often contain prominent intracytoplasmic protein resorption droplets and may fill Bowman's space, forming pseudocrescents . Although podocyte hyperplasia is found in both the collapsing and cellular variants of FSGS, collapsing glomerulopathy is distinguished by the absence of endocapillary hypercellularity. In collapsing FSGS, there is prominent tubulointerstitial disease, including tubular atrophy, interstitial fibrosis, interstitial edema, and inflammation. A distinctive feature is the presence of dilated tubules forming microcysts that contain loose proteinaceous casts. This pattern can occur both in primary FSGS and also in secondary FSGS due to HIV,parvovirus B12,pamidronate toxicity and interferon therapy. Endothelial tubuloreticular inclusions are identified in over 90% of patients with HIVinfection collapsing glomerulopathy and interferon therapy, whereas only in 10% cases with idiopathic collapsing glomerulopathy. Besides these conditions endothelial tubuloreticular inclusions are seen commonly in systemic lupus erythematosus nephritis.

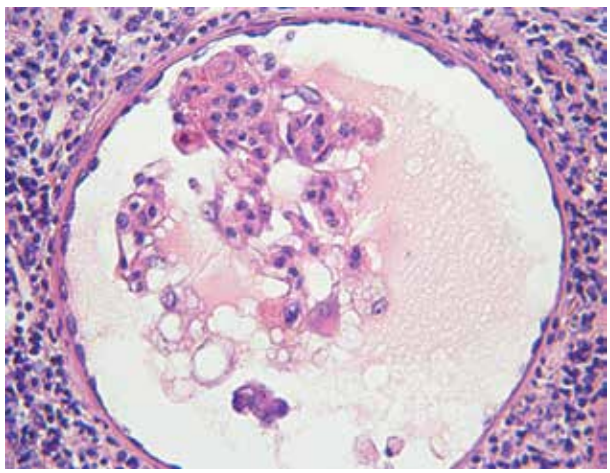

Fig. 3. A glomerulus displays collapsing variant of FSGS , glomerular capillaries show collapse with absent lumen (H&E, 40X).

IF microscopy of collapsing lesions often is positive for IgM and C3. EM reveals severe foot process effacement affecting both collapsed and noncollapsed glomeruli

Tip Variant of Focal Segmental Glomerulosclerosis :

This variant is defined by the presence of at least one segmental lesion involving the tip domain (i.e., the outer 25% of the tuft next to the origin of the proximal tubule). There is either adhesion (Fig 4) between the tuft and Bowman's capsule or confluence of swollen podocytes with parietal or tubular epithelial cells at the tubular lumen or neck. In some cases, the affected segment appears to herniate into the tubular lumen. The segmental lesions may be cellular or sclerosing type. Although initially peripherally located, these lesions may progress more centrally. The presence of perihilar sclerosis or collapsing sclerosis rules out the tip variant.

IF microscopy reveals involved tip area positive for IgM and C3 and EM findings demonstrate foot process effacement.

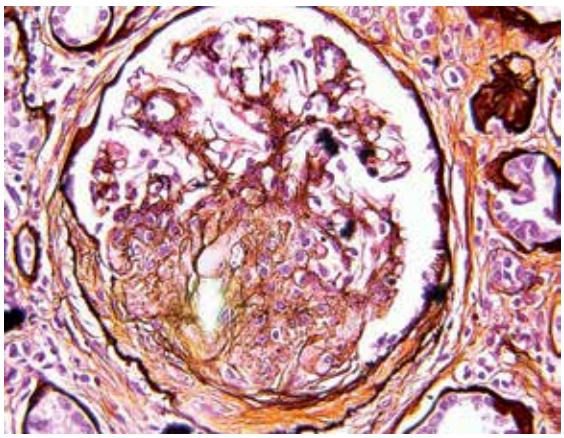

Fig. 4. Sclerotic glomerular segment with a small adhesion; "Glomerular tip lesion" (Periodic acid methenamine silver, 40X)

7. Treatment

Typically the goals of treatment in FSGS include-(1) to reduce, eliminate or at least suppress the proteinuria and (2) to avoid or at least retard the progression to ESRD. Treatment of the FSGS can be arbitrarily divided into (1) **Nonspecific; nutritional management** (2)**Non immunosuppressive therapy**: diuretics and RAS interference. (3) **Immunosuppressive treatment: which includes the use of s**teroids, steroid sparing medications like cyclophosphamide, cyclosporine A, tacrolimus and mycophenolic mofetil. **(4)-Antifibrotic drugs:Pirfenidone, Rosiglitazone (FONT Phase 1 trial completed and now FONT phase II trial is ongoing) (5)-Monoclonal/polyclonal Antibodies: Adalimumab, Rituximab, Fresolmumab (6)-Plasmapheresis**

Nonspecific treatment: Nonspecific treatment goals in FSGS are like any other glomerulopathy associated with nephrotic syndrome, which include maintenance of adequate nutrition, minimization or elimination of proteinuria, and prevention of complications resulting from edema. The mainstay of treatment is reduction in daily salt intake to 2 g of sodium. A high level of protein intake may further aggravate proteinuria, adversely affecting renal function. Current recommendations call for an intake of 1to1.3 g of

high biologic value protein per kilogram of body weight and a reduction of fat intake. Lipid lowering is necessary to reduce cardiovascular risk and to possibly delay the progression of renal disease.

7.1 Non-immunosuppressive drug treatment

Symptomatic relief of edema helps the patient feel better. In most patients, loop diuretics are needed to promote diuresis. Patients with massive edema with impaired oral absorption may require intravenous administration of loop diuretics. In patients with refractory conditions, addition of other diuretics (eg, metolazone) and potassium-sparing agents (eg, spironolactone, triamterene) facilitates diuresis and prevents hypokalemia. Rarely, some patients (especially children) with intractable edema may need intravenous albumin and diuretics in a hospital setting to initiate diuresis. Protracted use of intravenous albumin should be discouraged; the regimen is expensive and ineffective, because most of the infused albumin is lost in the urine. Continuous IV drip of furosemide is preferred over large boluses of IV push to avoid side effects.

Control of hypertension is one of the most important aspects of overall management in FSGS, like any other glomerular disease. Angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) are nonspecific agents that reduce proteinuria because of their antihypertensive and intrarenal hemodynamic effects of reducing glomerular capillary pressure and resistance. ACEIs and ARBs are effective in reducing protein loss even in normotensive patients. These agents do have an effect in slowing down of the disease progression by downregulating the TGFβ by interfering with renin angiotensin system, regardless of any antihypertensive effects.These agents do not abolish proteinuria completely or reverse the primary glomerular disease process per se.

As hypertension develops in most patients with FSGS which further causes deterioration of renal function, the control of blood pressure is an important part of the treatment of FSGS. Many patients, may require combination antihypertensive therapy to maintain blood pressure in the normal range. Lipid lowering therapy like statins is warranted to correct hyperlipidemia to reduce the risk of cardiovascular disease in this subset of patient population.

7.2 Immunosuppressive treatment

Idiopathic FSGS is a difficult disease to treat because of its highly variable clinical course. The specific treatment approach is still empirical, and no consensus has evolved because of a lack of prospective controlled trials. Current evidence is, mostly derived from retrospective analyses, and favors prolonged steroid therapy (6 months or longer) to induce remission in patients with idiopathic FSGS.

Criteria for remission: (1) complete remission is considered when urinary protein excretion of less than 200-300 mg/day, and (2) partial response is considered when urinary protein excretion of 200-3500 mg/day, or a greater than 50% reduction in baseline proteinuria.

Since long-term steroid therapy is not without serious toxicity, the patient counselling regarding the goals of the therapy, possible potential side affects and expected outcomes, is essential before starting such treatment. The current consensus is to initiate therapy with prednisone in a dose of 1 mg/kg (60-80 mg/d) for 2-6 months or longer, depending on patient's response as assessed by presence or absence of edema, 24-hour urine protein excretions, creatinine clearance, serum creatinine, serum albumin, and lipid levels. Literature reveals that 30-60% of patients may undergo complete or partial remission with

this treatment regimen, and relapses are frequent when steroids are discontinued. Blacks and patients with collapsing FSGS are generally refractory to treatment and progress to renal failure. In steroid responsive patients, the goal is to titrate prednisone to the lowest dose needed to stop proteinuria and to prevent relapses. Use of steroids on alternate days can also reduce toxicity. The optimal duration of treatment is uncertain; some authorities recommend use of steroids indefinitely. If no remission after 4 months of corticosteroid treatment, disease is defined as being corticosteroid-resistant (Meyrier A.,2009).

In patients who are refractory to 2-3 months of prednisone therapy, the recommendation is to reduce the steroid dose and to add cyclophosphamide (2.5 mg/kg [150-200 mg/day]), monitor patients for bone marrow suppression, and encourage adequate fluid intake to prevent hemorrhagic cystitis. Prolonged use of cyclophosphamide may lead to gonadal toxicity; therefore, persisting with cyclophosphamide beyond 3 months in patients who do not respond is unwise. Cyclosporine (3.5mg/kg/day) can induce remission and preserve renal function, although relapse occurs in 60% of patients when cyclosporine alone is used (Ponticelli C. et al. 1993 & Cattran DC et al. 1999). Continuing treatment with cyclosporine for 1 year after remission followed by a slow tapering of the dose results in a longer remission (Meyrier A 1994). Low-dose cyclosporine in combination with low-dose prednisone (0.15mg/kg/day) in corticosteroid-resistant patients is more effective than cyclosporine alone (Cattaran DC. Et al 1999 & Meyrier A 2009). Rates of complete (Niaudet P. 1994 & Braun N et al 2008) and partial remission achieved with cyclosporine are greater if low-dose prednisone is given at the same time. Continuous use of cyclosporine for >12 months is associated with a significant increase in tubulointerstitial fibrosis. Its use should, therefore, be limited to patients with creatinine clearance >60 ml/min/1.73 m^2

Isolated reports have suggested that patients who are refractory to steroids and cyclophosphamide, treatment with other immunosuppressive agents, such as tacrolimus and sirolimus, may be beneficial in inducing remission. However, studies using these agents were uncontrolled investigations that were limited to a few patients.

7.3 Tacrolimus

Few studies have reported the use of tacrolimus for Idiopathic FSGS. In the largest study, 25 patients with nephrotic syndrome due to primary FSGS and known resistance to or dependence on cyclosporine (cyclosporine) were given tacrolimus plus prednisolone (prednisone) for 6 months. Seventeen patients had a reduced proteinuria to <3 g/day, and 12 had complete or partial remission. Thirteen patients relapsed after discontinuing tacrolimus; reinstitution of therapy for 1 year resulted in complete remission (5 patients) and partial remission (4 patients). [Segarra A et al 2002].

7.4 Mycophenolate mofetil

Mycophenolate mofetil (MMF) is a reversible inhibitor of inosine monophosphate dehydrogenase (IMPDH) in purine biosynthesis. MMF is selective for the de novo pathway critical to lymphocytic proliferation and activation. Because of favorable results in other glomerular diseases, mycophenolate mofetil has also been evaluated in FSGS. Although the experience is limited, the suggested dose is 750-1000 mg twice daily in patients who are refractory to corticosteroids and in whom calcineurin inhibitors may not be appropriate. However, adequate randomized studies supporting this approach are lacking (Cattran DC et al. 2004). If mycophenolate is being considered, cyclosporine should not be used concurrently. Mycophenolate can be either used alone or in combination with corticosteroids.

7.5 Monoclonal antibody treatment

Rituximab: Rituximab is an anti CD20 chimeric monoclonal antibody. Case reports have suggested rituximab may be effective in treating patients with minimal change nephropathy and FSGS. (Peters HP et al 2008). Four patients with nephrotic syndrome due to minimal change nephropathy or FSGS were treated with rituximab because of failure of or intolerance to the standard immunosuppressive therapy. Other cases of FSGS reported in the literature (6 pediatric patients) that were successfully treated with rituximab were also included. Complete remission was reported within 1 month for a 7-year-old boy with FSGS during treatment with rituximab and concurrent treatment with mycophenolate, low-dose prednisolone (prednisone), and tacrolimus. Controlled trials are needed to further evaluate the efficacy of rituximab in FSGS.

Fresolimumab: Recently a phase one trial (Trachtman H. et al 2011) has been completed which evaluated the safety and pharmacokinetics of single-dose infusions of fresolimumab, a human monoclonal antibody that inactivates all forms of transforming growth factor-beta (TGF-β), in a phase I open-label, dose-ranging study. Patients with biopsy-confirmed, treatment-resistant, primary FSGS with a minimum estimated glomerular filtration rate (eGFR) of 25 ml/min per 1.73m2, and a urine protein to creatinine ratio over 1.8mg/mg were enrolled. All 16 patients completed the study in which each received one of four single-dose levels of fresolimumab (up to 4mg/kg) and was followed for 112 days. Fresolimumab was well tolerated with pustular rash, the only adverse event, developing in two patients. Single-dose fresolimumab was well tolerated in patients with primary resistant FSGS. Additional evaluation in a larger dose-ranging study is necessary.

7.6 Antifibrotic agents

Pirfenidone has been shown to have therapeutic potential in fibrotic diseases, although the mechanism of action is not well understood. It has been shown to reduce transforming growth factor-β 1 production, antagonize TNF-α signaling, and scavenge reactive oxygen species. It also reduces fibrosis and prevents loss of glomerular filtration in animal models of renal disease. An open-label trial evaluated the safety and efficacy of pirfenidone in patients with idiopathic and post adaptive FSGS. Pirfenidone had no effect on BP or proteinuria but it did preserve renal function. Controlled trials are needed to further evaluate the efficacy of pirfenidone in FSGS (Cho ME. et al 2007).

Rosiglitazone: Renal mesangial cells express peroxisome proliferator-activated receptor (PPAR –gamma). PPAR-gamma activation, can exhibit anti-inflammatory effects. Weissgarten et al (2006) demonstrated that PPAR-gamma activation by rosiglitazone resulted in decreased manifestation of inflammatory hallmarks, including inhibition of mesangial cell proliferation, downregulation of apoptosis and blunted responsiveness to angiotensin II in animal models. FONT-I (a phase 1 clinical trial) showed that this agent was safe and well tolerated in 11 patients with biopsy-demonstrated FSGS. The results of further studies are awaited.

Despite all attempts, some patients continue to deteriorate and progress to ESRD. Patients and their families should be counselled in detail regarding the treatment options for ESRD so that they can choose appropriate treatment tailored to their life style among maintenance hemodialysis, continuous ambulatory peritoneal dialysis, or renal transplantation. FSGS may recur in the transplanted kidney, but most centers do not consider this a contraindication for renal transplantation.

Secondary FSGS treatment management is directed toward the etiology or associated disorder. For example, discontinuing pamidronate in pamidronate induced FSGS, and in HIV-associated FSGS, HAART is associated with remission of proteinuria and preservation of renal function. In heroin-associated FSGS, discontinuation of the drug may result in remission of proteinuria and improvement in renal function.

8. Prognosis

Prognosis of idiopathic FSGS is variable. Important prognostic factors are, the amount of proteinuria, the level of plasma creatinine, the morphological subtype, and the response to therapy as listed in table 3. Korbet SM (1999) described that nephrotic patients with FSGS, particularly those with massive proteinuria, have a significantly poorer prognosis than non-nephrotic patients, with 50% progressing to end-stage renal disease (ESRD) over 3-8 years as compared with a 10-year survival of >80%, respectively. In addition, the recurrence rate of this lesion is high in transplanted patients with primary FSGS. When clinical and histological features at presentation have been evaluated by multivariate analysis, the significant positive predictors of progression to ESRD have consistently been the serum creatinine (>1.3 mg/dl), amount of proteinuria and the presence of interstitial fibrosis (> or =20%). The one factor which is a significant negative predictor of progression to ESRD is the achievement of a remission in proteinuria. Unfortunately, spontaneous remissions are rare in FSGS, occurring in < or =6% of patients only.

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1. Clinical features at the time of biopsy
   a. Nephrotic range proteinuria or massive proteinuria
   b. Elevated serum creatinine
   c. Black race
2. Histopathologic features at the time of biopsy
   a. Collapsing variant
   b. Tubulointerstitial fibrosis
3. Clinical features during the course of the FSGS
   a. Failure to achieve partial or complete remission.

Table 3. Risk factors for progressive loss of renal function in FSGS

Thomas DB et al. (2006) described that the morphological subtype identified on renal biopsy also provided useful prognostic information. The collapsing variant, the main variant seen in HIV-induced FSGS, is associated with a worse prognosis than the other forms. The tip variant has a better prognosis than the other forms.

9. Summary

Focal segmental glomerulosclerosis is still largely an idiopathic disease but in the recent past, more genetic mutations and secondary causes have been described. As more and more pathogenetic mechanisms involved in idiopathic FSGS are coming into light and more secondary causes of FSGS are described, the occurrence of true idiopathic FSGS diagnosis is

decreasing and thus the frequency of the latter is often overstated. Other than the genetic and secondary causes of FSGS, the treatment strategies are still not based upon multiple large randomized controlled trials, and in fact are based upon predominantly anecdotal experiences. Still there are more questions than the answers for the pathogenesis, classification, treatment and prognosis of this an almost a century old disease. This clincopathologic entity still remains a challenge for the nephrologists and transplant physicians.

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3

Membranoproliferative Glomerulonephritis

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1. Introduction

Membranoproliferative glomerulonephritis (MPGN) refers to glomerular pathology in which there is thickening of the capillary wall together with mesangial expansion. In this article we firstly review the pathological features of MPGN and discuss how advances in our understanding of the association between abnormalities in the regulation of complement and MPGN have revealed limitations in the historical pathological sub-division of MPGN. Secondly we review the clinical presentation of MPGN, its prognosis and therapeutic considerations.

2. Pathological features of MPGN

The name 'membranoproliferative glomerulonephritis' derives from the light microscopic glomerular histologic pattern. MPGN is synonymous with 'mesangiocapillary glomerulonephritis'. The glomeruli are large and hypercellular. The hypercellularity is typically uniform though-out the glomeruli. Mesangial hypercellularity and expansion of the mesangial matrix can accentuate the appearance of discrete lobules within the glomeruli. In some cases the hypercellularity includes infiltration of the glomerulus with neutrophils [1] and in severe cases monocytes have been detected in the glomerulus [2]. The degree of leukocyte infiltration shows some correlation with the degree of C3d deposition, possibly due to the chemotactic effects of complement split products. In patients who have undergone two biopsies, for example, when the abundance of C3d decreased in the second biopsy fewer leukocytes were observed [3]. The basement membranes of glomerular capillaries in MPGN are thickened. A characteristic change in the capillary wall is splitting of the glomerular basement membrane (GBM), termed 'tram-tracking' or 'double-contours'. This is due to the inter-position of the proliferated mesangial cells between the endothelial cells and the GBM. The inter-positioned mesangial cells generate new basement membrane material between the endothelial and mesangial cells, a process that is readily identifiable on electron microscopy.

Immunofluorescence studies of glomerular immunoglobulin and C3 in MPGN typically demonstrated granular deposition of these immune factors along the capillary loops. Staining for IgG is often fainter than it is for complement C3 and is sometimes absent [4 5].

An early study described three immunofluorescence patterns in MPGN: glomerular deposition of both immunoglobulin and C3 (66%), predominant deposition of C3 (21%) and deposition of C3 only (13%) [4]. The finding of immunoglobulin and complement is characteristic of immune complex-mediated glomerular inflammation and suggests that the MPGN is secondary to systemic disorders in which there is a propensity for immune-complexes to deposit or form within the kidney. The known conditions include diseases such as autoimmune disorders (e.g. systemic lupus erythematosus), malignancies and chronic infections. Hepatitis C, for example, is now recognized as a major cause of mixed cryoglobulinemia and MPGN [6]. Where a systemic disorder is identifiable the MPGN is referred to as secondary MPGN. In the absence of a clear aetiology MPGN is appropriately termed 'idiopathic or primary MPGN'.

The finding of glomerular complement deposition alone suggests activation of the complement system in the absence of immunoglobulin. This most commonly is a consequence of activation of the complement alternative pathway. Perhaps not surprisingly we now know that inherited and acquired disorders of the alternative pathway are associated with MPGN in which the histological features are characterized by predominant or isolated glomerular C3 deposition. The prototypic example of this is dense deposit disease. In the 1960s it was recognized that ribbon-like electron dense deposits are detectable within the lamina densa of the glomerular basement membrane in some patients with glomerulonephritis [7]. These intra-membranous deposits are the histological defining feature of dense deposit disease and this, rather than mesangial inter-positioning, produces thickening of the GBM. Dense deposit disease is rare: of children whose biopsies demonstrate an MPGN pattern by light microscopy, less than 20% have dense deposit disease [8 9]. Prominent C3 deposits are virtually always present in the glomeruli of patients with dense deposit disease. Granular C3 deposits are almost always present within the mesangium, although different patterns have also been observed [8], e.g. "ring-like" pattern of mesangial C3 staining. Glomerular C1q and/or immunoglobulins may be seen [10]. Our current understanding of dense deposit disease has recently been reviewed [11].

Since the MPGN in dense deposit disease was associated with distinct immunofluorescence studies (predominant or isolated glomerular C3 deposition) and GBM ultrastructural appearances (striking linear electron dense transformation of the lamina densa) an MPGN classification emerged which sub-divided MPGN initially into two groups: MPGN type I and MPGN type II. MPGN type I was characterized by immunoglobulin and C3 deposition and sub-endothelial electron dense GBM deposits [5]. MPGN type I contained both primary and secondary types. MPGN type II was used to describe dense deposit disease. Hence in the literature dense deposit disease was renamed MPGN type II. A further group, MPGN type III was subsequently added to describe MPGN where there were prominent subepithelial GBM deposits, possibly caused by immune-complexes similar to those found in membranous disease [12]. As in membranous disease, the deposits are associated with spikes along the GBM that can be detected by silver stain. A further MPGN type III variant was characterized by the presence of basement membrane ruptures on electron microscopy [13]. C3 deposition is invariably present in the glomeruli of patients with MPGN type III whilst immunoglobulin deposition is variable [13]. The traditional classification of MPGN is depicted in figure 1.

Subsequent studies have revealed limitations in this MPGN classification. Firstly, it is now recognized that patients with the dense deposit disease may present with many different patterns of glomerular injury by light microscopy. These patterns, in addition to MPGN,

include mesangial proliferative and crescentic lesions [10]. In fact more than half of the dense deposit disease biopsies did not show MPGN. The emerging consensus is that dense deposit disease is a distinct pathologic entity and should not be thought of as an MPGN variant [10 14]. Secondly, we now know that defects in complement regulation are strongly associated with glomerular inflammation in which there is isolated glomerular C3 deposition irrespective of whether the glomerular lesion is MPGN. We discuss the intimate association between complement and MPGN next.

	MEMBRANOPROLIFERATIVE GLOMERULONEPHRITIS		
	type I	**type II**	**type III**
Immunofluorescence:	complement C3 and IgG	complement C3	complement C3 variable IgG
GBM deposits:	sub-endothelial	intra-membranous	sub-endothelial & sub-epithelial

Fig. 1. The traditional classification of MPGN. Dense deposit disease was renamed MPGN type II in this classification. Immunofluorescence refers to glomerular staining for complement C3 and IgG. GBM – glomerular basement membrane.

3. Complement and MPGN

The complement system is an integral component of immunity. Its principal role is concerned with host defence against pathogens and it forms an important component of innate immunity. Complement also acts as natural adjuvant enhancing the B cell response to antigen and more recent data implicates an important role for complement in T cell responses [15]. To understand the relationship between MPGN and complement it is important to understand how complement is activated. Binding of antibody to antigen forms an immune complex and immune complexes are the triggers of the complement classical pathway. In immune-complex associated MPGN the classical pathway is activated and contributes to glomerular inflammation. The complement alternative pathway is continuously activated i.e. requires no specific activating trigger. Unlike the classical pathway, the alternative pathway is antibody-independent and, through this pathway, the key effector molecules of the pathway (C3 and C5) can be deposited on surfaces in the absence of immunoglobulin. The key negative regulator of the alternative pathway is an abundant plasma protein called complement factor H (CFH). Early investigators studying MPGN hypothesized that MPGN lesions in which complement components such as C3 were present in the absence of immunoglobulin, were mediated by alternative pathway activation. We now know that both inherited and acquired causes of alternative pathway regulation are associated with this type of MPGN. Dense deposit disease, in which

glomerular C3 deposition is typically seen with little or no immunoglobulin, is associated with genetic and acquired factors that enhance alternative pathway activation (reviewed in [11]). C3 nephritic factor was associated with dense deposit disease decades ago [14] and the 'factor' is now known to be an immunoglobulin which targets an enzyme complex within the alternative pathway. This autoantibody stabilizes the enzyme complex and enhances alternative pathway activation. Hence C3 nephritic factor is associated with over-activation of the alternative pathway. Consequently, through consumption, plasma C3 levels are typically low in individuals with C3 nephritic factor. Genetic factors include genetic deficiency of the alternative pathway regulatory protein, CFH (reviewed in [16]) and 'gain of function' mutations in the alternative pathway activation protein, complement C3 [17]. Genetic deficiency of CFH in pigs and gene-targeted CFH-deficient mice also results in spontaneous MPGN [18 19]. Acquired dysregulation of the alternative pathway due to neutralizing autoantibodies against CFH [20] has also been described. Recently, an autoantibody to factor B, an activation protein within the alternative pathway, has also been associated with MPGN [21]. In summary factors that increase alternative pathway activation have been associated with MPGN in which there is glomerular C3 with little or no immunoglobulin. It is clearly important to distinguish MPGN driven by these factors from MPGN associated with systemic immune complex disease or MPGN due to other aetiologies. Consequently, there has been much discussion on how to develop our current classification of MPGN.

4. C3 glomerulopathy – moving away from the traditional classification of MPGN

In order to identify individuals with inherited or acquired defects in complement regulation we proposed a classification called C3 glomerulopathy (Figure 2) [22]. C3 glomerulopathy defines glomerular pathology characterized by isolated or predominant glomerular C3 deposition in the absence of immunoglobulin irrespective of both the glomerular light microscopic appearances and the ultrastructural appearance of the GBM [22]. Whilst many patients with isolated glomerular C3 deposition and complement abnormalities develop MPGN some do not [23 24]. For example, Servais and colleagues described 19 cases of primary glomerulonephritis cases with isolated deposition of C3 in the absence of morphological GBM changes of dense deposit disease [24]. Thirteen cases had an MPGN pattern by light microscopy whilst the remaining 6 did not [24]. They used the term 'C3 glomerulonephritis'. Recently a familial C3 glomerulopathy associated with a mutation in a complement protein called complement factor H-related protein 5 (CFHR5) was characterised [23]. Affected individuals have renal biopsies consistent with C3 glomerulonephritis. Biopsies show mesangial C3 deposition and variable degrees of mesangial hypercellularity. Mesangial and sub-endothelial GBM electron dense deposits are typical and some develop MPGN. These patients were identified and specifically investigated for complement disorders because renal biopsies demonstrated glomerular C3 deposition in the absence of immunoglobulin. The discovery of CFHR5 nephropathy is fascinating and we direct the interested reader to [25]. The impetus to propose the term C3 glomerulopathy was to enable the rapid identification of patients with glomerular disease who ought to be investigated for complement abnormalities and who may benefit from complement modulating therapeutic strategies. The relationship between C3 glomerulopathy and MPGN is discussed in detail in reference [26].

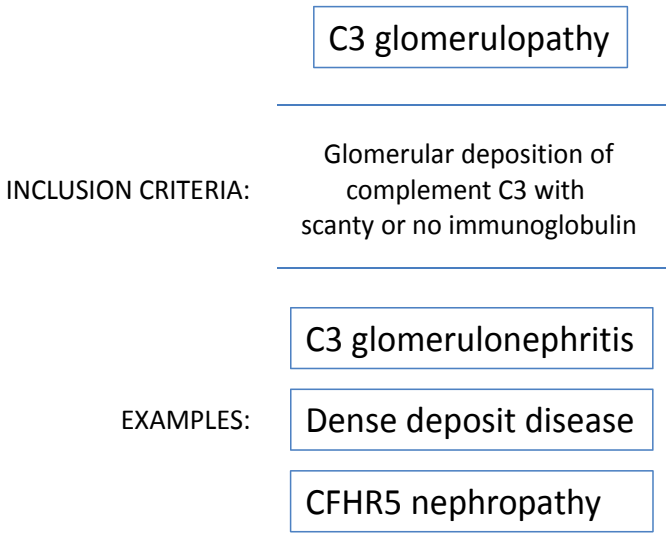

Fig. 2. Definition and examples of C3 glomerulopathy

In summary the traditional MPGN classification has become outdated due to advances in our understanding of complement-mediated glomerular inflammation and our knowledge of the histopathological spectrum of dense deposit disease. Nevertheless, in reviewing historical studies the use of the traditional MPGN sub-groups is unavoidable. In the next sections we have used the traditional sub-groups acknowledging that the reader will now be aware of the limitations of this classification. The recent descriptions of C3 glomerulonephritis and CFHR5 nephropathy are discussed separately.

5. Clinical features of MPGN

MPGN commonly presents with the nephritic syndrome (microscopic hematuria, non-nephrotic proteinuria, and renal insufficiency). However, up to one third of the patients present with relatively preserved renal function and the nephrotic syndrome [27]. The spectrum of clinical findings is generally the same for all of the subgroups considered in this review, but some differences exist and are discussed under individual headings below.

MPGN type I

MPGN type I may be more common in children than in adults [28]. It typically presents as a renal limited disease, although many patients have hypertension at the time of diagnosis [29]. Hypertension may be less common in children [29], particularly when the disease is detected early through screening of asymptomatic individuals. The majority of patients have microscopic hematuria, and some have macroscopic haematuria [29]. Cameron reported that 75% of their patients with MPGN type I had normal C3 levels at the time of disease onset [29]. In an analysis of 9 patients with MPGN type I, however, Ooi and colleagues reported depressed C3 levels for all of the patients on at least one occasion [30]. Approximately 30% of patients have a C3 nephritic factor [29 30]. C4 levels were low for some of the patients.

MPGN type II/Dense deposit disease

Dense deposit disease usually presents in children between the ages of 5 and 15 [14], although a recent series included more patients diagnosed in adulthood than in childhood (12). There appears to be a slight female to male preponderance [31]. The clinical presentation is similar to that of MPGN type I. MPGN type II usually presents with the nephritic syndrome [28], but proteinuria is usually present and is in the nephrotic range in approximately 50% of patients [31]. Interestingly, the renal disease is often preceded by an infection [31]. More than 80% of patients with dense deposit disease have hypocomplementemia at some point [29], and 100% of children with the disease had depressed C3 levels in the report of Nasr et al. [31]. Approximately 80% of patients with dense deposit disease have detectable C3 nephritic factor [32]. As described above, dense deposit disease is strongly associated with defects in regulation of the alternative pathway of complement. This underlying defect probably explains the association of dense deposit disease with acquired partial lipodystrophy [33] and with retinal drusen (deposits within Bruch's membrane of the retina) [34]. In acquired partial lipodystrophy, uncontrolled complement activation causes the loss of subcutaneous fat. The drusen resemble those seen in patients with age related macular degeneration and presumably also form as a result of defective complement regulation. However, only one patient with each of these co-morbidities was described in a recent report of 32 patients with dense deposit disease [31].

MPGN type III

MPGN type III usually presents with similar clinical findings to those seen in MPGN type I. The proportion of patients with hypocomplementaemia was comparable to that of MPGN type I [35].

C3 glomerulonephritis

C3 glomerulonephritis is a recently described entity so the full spectrum of its clinical manifestations is not yet known. Servais *et al.* reported a series of 19 patients [24]. Men and women were represented nearly equally, and the patients ranged in age from 7 years old to 70. Sethi *et al.* recently reported three more cases of C3 glomerulopathy [36], all of whom were adult males (aged 38-73). Approximately 30% of the patients in the two reports had a creatinine clearance below 60 ml/min. Most of the patients had proteinuria (eight of the 22 patients described had nephrotic range proteinuria) and 15 patients had hematuria [24 36]. A comprehensive evaluation of the complement system was performed in both reports. Nine of the 22 total patients had depressed C3 levels. Six patients in the first series had C3 nephritic factor, and one of the patients reported by Sethi *et al.* had C3 nephritic factor. Mutations in complement regulatory proteins were identified in six of the patients reported by Servais *et al.* [24]. The three patients reported by *Sethi et al.* all carried the Tyr402His allele of CFH which has been identified as a risk allele for dense deposit disease [36].

CFHR5 nephropathy

CFHR5 nephropathy is a familial form of C3 glomerulopathy that has to date only been described among individuals with Cypriot ancestry. It was characterized only recently [23 25]. Affected individuals all carry a mutation in CFHR5 and to date only heterozygous affected individuals have been identified. The biological role of CFHR5 is unknown although there is evidence that it interacts with complement deposited within the glomeruli in many different glomerular pathologies [37 38]. The mutation in CFHR5 nephropathy is an

internal duplication in exons 2 and 3 of the *CFHR5* gene. This results in a secreted abnormally large CFHR5 protein. The clinical course of CFHR5 nephropathy has been described in a comprehensive review of 91 patients from 16 pedigrees [39]. Affected patients have continuous microscopic haematuria and often develop macroscopic haematuria during periods of infection. Hypertension, proteinuria and end-stage renal failure are more common in men. In this report of affected individuals aged over 50 years, 80% of affected men developed chronic renal failure whilst 21% of affected women developed chronic renal failure [39]. The condition recurs in the transplanted kidney [40].

6. Prognostic considerations in MPGN

As highlighted in the above discussion, many of the patients who were previously identified simply as having MPGN are now recognized as having distinct disease processes. Consequently, older data regarding the prognosis of MPGN may have combined patients who would now be categorized differently. For example, early studies of MPGN may well have included patients with dense deposit disease due to defective complement regulation or patients with secondary MPGN caused by hepatitis C associated cryoglobulinemia.

MPGN type I

Studies of patients with MPGN type I have reported fairly wide variation in the long-term prognosis of the disease. The 10-year renal survival for children has been reported to be 60-80% [41 42]. The patients included in these studies were treated with corticosteroids and other immunosuppression. The prognosis may be improved by early detection, such as that afforded by screening of school children [43]. This improvement could be due to lead-time bias, however. Adverse prognostic features include nephrotic syndrome [32 44], an elevated creatinine at presentation or within the first year, and structural injury on the renal biopsy [44].

MPGN type II/Dense deposit disease

The prognosis of dense deposit disease may be worse than that for MPGN type I [8 9 29], although the small numbers of patients with each disease make it difficult to control for other variables. Spontaneous remissions of dense deposit disease are rare, and approximately 50% of patients will reach end-stage renal disease within 10 years [29]. Of the 27 patients in the report by Nasr *et al.* for whom follow-up was known, 25.9% had a complete response to therapy. There was no response in the remaining patients and 25.9% progressed to end-stage renal disease (duration of follow-up 2 months to 24 years) [31]. Age and the serum creatinine at biopsy were predictive of progression to end-stage renal disease. Only 7.1% of the adults had a complete response to therapy. Although uncontrolled complement activation is believed to be pathogenic in this disease, perturbations in C3 levels do not appear to correlate with clinical outcomes [29 31]. Recurrence in renal allografts is common [31 45].

C3 glomerulonephritis

During the period of follow-up (ranging from 0.4 – 34 years) in the series reported by Servais *et al.* [24], most of the patients had a decline in renal function. Three patients reached end stage renal disease, and another two patients had creatinine clearances below 15 mL/min. The patients described by Sethi et al. did not show a decline in renal function during the short period of follow-up (6 months to 3 years) [36].

CFHR5 nephropathy

As mentioned above the course of this condition is more severe in males. In affected individuals aged over 50 years the incidence of end-stage renal failure was 78% in men and 22% in women [39].

7. Therapeutic approaches to MPGN

Given the distinct mechanisms of glomerular injury between immune-complex-mediated MPGN and the primary complement-mediated MPGN groups, evidence of treatment efficacy in one group may not be applicable to the other. However, for both groups non-specific therapies may be beneficial at slowing the progression of renal disease. The blood pressure should be rigorously controlled, and ACE inhibitors or angiotensin receptor blockers are probably agents of choice [46]. Complications of the nephrotic syndrome, such as hyperlipidemia, should be treated.

MPGN type I

As this is a disease of immune-complex deposition, there is a rationale for treating this disease with immunosuppression. Unfortunately, there is not conclusive evidence that any of the common treatments are effective. Perhaps the best study to date was a randomized controlled trial of alternate day prednisone that included 41 children with MPGN type I [47]. The patients had high-grade proteinuria or renal impairment, and renal survival was better in the group that received steroids. Although this difference did not reach significance ($P = 0.07$), the authors concluded that this was due to the small number of patients. Other uncontrolled studies further support the finding that long-term treatment with corticosteroids may be effective at inducing disease remission [9 48 49]. One of these studies included patients with diffuse lesions on their biopsies, but who were detected early through school-based screening [49]. These patients were treated with alternate day steroids, and all of the patients but one was treated for at least four years. Of 19 patients evaluated, four patients had persistent mild proteinuria but only one patient had a disease relapse (successfully treated with a second course of steroids). Other case series have not shown improved outcomes in patients who received steroids. In one such study, however, the authors determined that patients who received steroids were more likely to have had the nephrotic syndrome [44]. They concluded, therefore, that steroids may, in fact, have been beneficial. After analyzing the available data, Levin concluded that corticosteroids are indicated for children with nephrotic syndrome or with renal insufficiency [50], but the optimal criteria by which patients should be stratified for treatment are still under debate [44].

Similarly, some studies have suggested a benefit of treatment with anti-platelet agents [51 52]. A randomized, controlled trial of aspirin and dipyridamole, for example, indicated that treatment with these agents was effective at preserving renal function [51]. However, a long-term follow-up study that examined renal survival in these patients from the time of diagnosis (not from the start of treatment) did not see a sustained benefit [53]. Another randomized trial of patients demonstrated that aspirin plus dipyridamole was effective at reducing proteinuria at 36 months [54]. The serum creatinines in both groups were unchanged, however, so the effect of this treatment on the progression of renal disease remains uncertain.

Several case series and case reports have described patients treated with other immunosuppressive agents, such as cyclophosphamide or calcineurin inhibitors [29 44 55 56].

The patients treated with these agents are probably selected because they have concerning prognostic factors. Thus, it is difficult to determine the efficacy of these agents. Case reports have also described patients with steroid-resistant MPGN type I who responded to mycophenolate mofetil [57 58]. Certainly more data is needed, but given the relative safety of this medication it is a reasonable choice for patients who do not respond to steroids.

Approximately 30-60% of patients with MPGN type I who undergo renal transplantation have a recurrence of the disease, and disease recurrence adversely affects graft survival [59 60]. Although there are anecdotal reports that increasing immunosuppression may be beneficial, there is no well established therapy for recurrent disease [60].

MPGN type II/Dense deposit disease

No clinical trials have been conducted in patients with dense deposit disease. Based upon what is known about the pathophysiology of the disease, the complement inhibitor eculizumab may be beneficial, and a clinical trial of this agent in dense deposit disease is currently underway. Eculizumab is a monoclonal antibody that blocks C5 activation and is currently licensed for treatment of anemia in paroxysmal nocturnal haemoglobinuria. It has been used successfully to treat atypical haemolytic uraemic syndrome and is likely to be licensed for this indication soon.

Plasma exchange may be effective at removing autoantibodies or dysfunctional complement components, while also enhancing CFH function through the infusion of plasma. Plasma exchange was reported to be effective in two affected sisters who had a factor H mutation and C3 nephritic factor [61].

The role of immunosuppressive agents in dense deposit disease is uncertain. Theoretically, immunosuppressive drugs may be beneficial in patients with evidence of autoantibodies, yet corticosteroids are not of clear benefit in this disease [14]. The patients reported by Nasr et al. included 18 patients who received immunosuppression [31]. The immunosuppression regimens included steroids in all patients. Two patients were also treated with mycophenolate mofetil and three received calcineurin inhibitors. A trend towards a benefit was seen in patients treated with immunosuppression but this did not reach significance. The greatest benefit was seen in those who received immunosuppression and a renin-angiotensin system inhibitor. Recently, a patient with fulminant disease was treated with high-dose corticosteroids, plasma exchange, and cyclophosphamide, and apparently responded to treatment [62]. This patient had a low C3 level, but did not have C3 nephritic factor or a complement mutation, so the mechanism by which the treatment benefited the patient is difficult to infer.

Based on existing data, the optimal treatment of patients with dense deposit disease is uncertain. A treatment algorithm incorporating complement testing and the above treatment options has been proposed [63]. Treatment may need to be initiated before genetic testing can be performed, however, and the presence or absence of C3 nephritic factor is probably not sufficiently accurate to guide therapy. Thus, the decision to use plasma exchange, standard immunosuppressive drugs, and/or eculizumab must ultimately be made based on the clinical severity of the disease.

Recurrence of MPGN type II is very common in patients who receive renal transplants, and some estimate the recurrence rate is 100% [60]. Graft survival at 5 years is approximately 50%, and the most common cause of graft loss is recurrent disease [64]. The impact of more aggressive (e.g. peri-transplant plasma exchange) or newer therapies (e.g eculizumab) remain unknown.

MPGN type III

Patients with MPGN type III do not seem to respond as well to corticosteroids as do those with type I disease as assessed by disease relapse and estimated GFR [35]. Thus, other than non-specfic therapies there is scant evidence to guide the treatment of these patients.

C3 glomerulonephritis

In the series by Servais et al., five of the patients were treated with steroids [24]. The authors reported that there was no clear effect of treatment on the disease outcomes. The patients reported by Sethi et al. were treated conservatively (no patients received immunosuppression), and no deterioration in renal function was seen during the period of follow-up [36]. Thus, based upon the available data there is little evidence to support immunosuppression in these patients.

CFHR5 nephropathy

The optimum treatment for CFHR5 nephropathy presently remains unknown. There are theoretical grounds to investigate the utility of eculizumab in this condition e.g. during disease flares. The relationship between renal decline and infective episodes in CFHR5 nephropathy implies that immunosuppressive strategies may be a potentially harmful approach.

8. Conclusions

MPGN is a fascinating glomerular pathology. We have made significant progress in understanding the role of complement in MPGN. There are limitations to the traditional histological classification. Dense deposit disease should not be referred to as MPGN type II since many patients with dense deposit disease do not have MPGN. C3 glomerulopathy is a new term which encompasses glomerular pathologies in which there is isolated or predominant deposition of glomerular C3. C3 glomerulopathy includes dense deposit disease and C3 glomerulonephritis. The most recent addition is CFHR5 nephropathy. Individuals with C3 glomerulopathy should be investigated for complement dysregulation and represent logical patient populations in which to explore the efficacy of complement modulating therapies.

9. References

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4

Primary IgA Nephropathy: An Update in 2011

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1. Introduction

IgA Nephropathy (IGAN) is also called mesangial IgA glomerulonephritis and was first described by Jean Berger, a French general pathologist, in 1968 in the journal d'Urologie-Néphrologie [1, 2]. Many reviews have been written on the subject [3, 4, 5].

2. Definition [3, 4, 5]

The definition is histopathologic with the characteristical **deposition** of the **immunoglobulin A** in the renal **mesangium**: these deposits are **predominant** (or codominant with other immunoglobulins, IgG and/or IgM), **granular**, **coarse** (with the "en mottes" aspect), **generalized** in the glomerulus and **diffuse** to all glomeruli. These deposits are evidenced usually by the technique of direct immunofluorescence on a semi-quantitative scale: traces (+/-), 1+, 2+, and ≥ 3+. There is a general agreement to accept **at least 1+ IgA deposits** for the definition.
By contrast, the lesions observed by light microscopy are often segmental and focal. The main lesions concern the mesangium with increased matrix and hypercellularity with in addition endocapillary proliferation. The most severe lesions are represented by obsolescent glomeruli ("pains à cacheter"), focal and segmental glomerulosclerosis (FSGS) with capsular adhesions, and extracapillary proliferation with the formation of cellular/hyalinized crescents.

3. The IgA nephritides [3, 4, 5]

The deposition of mesangial IgA has been observed in different types of glomerulonephritis leading to the classification of **Primary IgA nephropathy** (Berger's disease) versus the **Secondary IgA nephropathies**: associated to Schönlein-Henoch Purpura, to patent Alcoholic Liver Cirrhosis, to Systemic lupus Erythematosus, or to Ankylosing Spondylarthritis. Primary disease represents at least 80 % of the cases.

4. Epidemiology [3, 4, 5]

Primary IGAN is worldwide the most frequent glomerulonephritis; it accounts for about a quarter of the percutaneous renal biopsies performed on native kidneys. In France, the **incidence** is about 30 new cases per million inhabitants (pmp) and its **prevalence** is closed

to 1000 pmp according to the disease duration from onset to dialysis or to last follow-up (from few months to more than 5O years). All these numbers are of course dependent of the "politic" of renal biopsy: liberal indication or restricted to the most severe cases with already proteinuria over 1g/day or already some degree of renal insufficiency (Glomerular Filtration Rate <60 ml/mn/1.73 m² body surface area).
The disease is more frequent in men than in women, about 70% males, in most continents except in Asia. Age at onset ranges from 5 years to 75 with a peak frequency in adolescents and young adults.

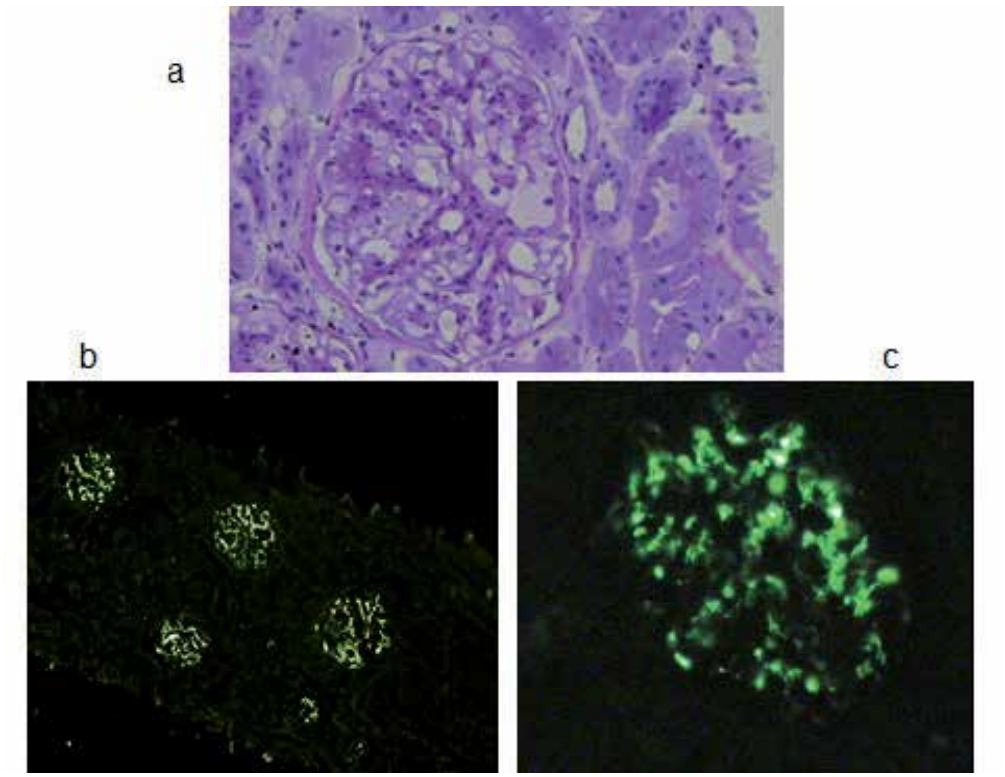

Fig. 1. HistoPathology of IgA Nephropathy:
(a) Light microscopy showing one glomerulus with segmental increased in mesangial matrix and mild hypercellularity.
(b) Immunofluorescent microscopy showing diffuse IgA deposits in all glomeruli.
(c) Immunofluorescent microscopy showing generalized mesangial IgA deposits within all the glomerulus surface/volume.

5. Mode of onset/initial presentation [3, 4, 5]

The onset of the disease can be **acute/subacute** in about 30 to 35 % of the patients with the classical **intra-infectious gross haematuria**: at time of an infectious episode of various origin (pharyngitis, bronchitis, or even intestinal or urinary infection), the patient is urinating blood with colored urine, more brown than red with a color of coffee or coca-cola; this is a total haematuria (sometimes with loin pain) which lasts from few hours to

few days; there is no clot and the characteristics of this gross haematuria is nephrological with the presence on urine cytology of typical red blood cell casts. After such an episode, the patient is usually presenting microscopic haematuria or could be in total remission until the next episode.
The discovery can be **chance proteinuria** or **chance microscopic haematuria** at time of systemic urine control for medical check-up at school, at different institutions or at work; the time of onset is therefore imprecise and we should refer to the last negative control if any.
The disease can be diagnosed later on with arterial hypertension (HT) and/or oedema and/or chronic kidney disease stage 3 or up (CKD-3+).

6. Progression of the disease [6-12]

Overall, IGAN is a **progressive disease** both clinically and pathologically. The **clinical progression** starts with urine abnormalities, followed by occurrence of HT, sometimes oedema related to massive proteinuria or nephrotic syndrome, and later on occurrence of CKD-3 through CKD-5 and ultimately renal death necessitating chronic dialysis. The progression is also **pathological** and this was demonstrated by repeated renal biopsies 5 years later [6, 13]: it was shown that the global optical score (GOS) progressed in the majority of the patients with increased glomerular but also vascular and tubular/interstitial indices.
We are taking as example our prospective cohort of primary IGAN patients [12] whose diagnostic biopsy was performed between January 1st 1990 and December 31st 1999 at our institution with loco-regional patients coming from the Saint-Etienne area, the IGAN-STET-CO. This cohort is composed of 332 patients (237 men, 71.4 %) with a mean age of 35.9 at onset, 41.4 at diagnosis and 48.8 years at dialysis/death or at last follow-up visit. The total exposure time was about 13 years. Overall, 32 patients needed dialysis, 13 died before reaching dialysis, and 45 (13.6 %) reached the primary composite outcome while 99 (29.8 %) presented the secondary outcome (CKD-3+).

7. Predictive risk factors and the absolute renal risk of dialysis/death: A new concept [12]

One very important goal was to sort out the major and independent risk factors (RF), present at diagnosis, able to predict accurately the ultimate final prognosis (dialysis or death as primary end-point). From the literature, we already know that the amount of proteinuria (g per day), the occurrence/presence of HT, and the severity of renal lesions on the initial biopsy were associated with progression [6-11]. Many other risk factors were also described such as gender, overweight/obesity [14], metabolic syndrome, age at onset [15], hypertriglyceridemia/hyperuricemia [16], and also different immunogenetic markers (HLA antigens, different cytokines polymorphisms [17, 18],…) but they were not consistent or controversial in different published cohorts. We recently confirmed [12] that these 3 major risk factors were sufficient to cover the whole prediction in our cohort. These risk factors were simplified, dichotomized for easier use and shown to be independent predictors in a multivariate model of Cox regression: - the most important is the **presence of HT (Yes or No)** defined according to WHO (≥ 140/90); - the next is the **presence of Proteinuria ≥ 1g/d (Yes or No);** and - the last is the scoring of the renal lesions; we have used our own **global**

optical score (GOS) developed 20 years ago and integrating all elementary lesions (glomerular from 0 to 6, vascular from 0 to 5, tubular from 0 to 4, and interstitial from 0 to 5) with a GOS up to 20. We set up by ROC analysis that the best cut-off for predicting dialysis was the **presence of GOS ≥ 8 (Yes or No).** These 3 RF turned out to have a similar weight in the prediction of dialysis/death by the different accuracy parameters and also by the Cox regression (β/SE ratio of the same magnitude).

In analogy to the absolute cardio-vascular risk (ACVR) of death or major CV events at 10 years [19, 20], we proposed the **Absolute Renal Risk of Dialysis/Death**; this ARR is calculated at diagnosis and is very simply the number of these RF present: 0, 1, 2 or 3. By Kaplan-Meier survival curves, we could calculate the cumulative rate of primary event at 10 and 20 years after onset (time zero); it was respectively 2 and 4 % for ARR=0; 2 and 9 % for ARR=1; 7 and 18 % for ARR=2; and 29 and 64% for ARR=3.

In addition, this ARR integrates gender (less RF for women), age at diagnosis (more RF for older patients), and also body mass index (more RF in overweight/obese patients). We could also use it as prospective with time zero set-up at diagnosis and not at onset; the cumulative incidence of dialysis/death 10 years after diagnosis is 4% for ARR=0, 8% for ARR=1, 18% for ARR=2, and 68% for ARR=3. It is remarkable to underline the similarity of the values obtained 20 y after onset and 10y after diagnosis.

Distribution of these Risk Factors at time of Diagnosis and at last follow-up (LFU): HT was present in 120 patients (36.1%) at diagnosis and in 164 (49.4%) at LFU; Proteinuria ≥1g/d was present in 100 patients (30.1%) at diagnosis and in only 61 (18.4%) at LFU; and GOS ≥ 8 was present in 120 patients (36.1%) at time of diagnosis. The distribution of the ARR was as follows at time of diagnosis: 151 patients (45.5%) with ARR=0, 69 (20.8%) with ARR=1, 65 (19.6%) with ARR=2, and 47 patients (14.1%) with ARR=3.

It should be stressed that this cohort is an adequately treated cohort with all RF targeted as soon as they were identified: perfect control of blood pressure (target <130/80) with all antihypertensive agents; persistent reduction of proteinuria with ACEI and ARBs; and prednisolone for severe renal lesions.

8. Pathological classification of IgA nephropathy

We have developed our own classification in 1990 [6, 13] with the Global Optical Score already described. During the past decades, the classifications of Haas [21] or Hass modified by Lee [22] were frequently used. The international Oxford classification was published in 2009 [23, 24] and retained only 4 parameters with significant clinical prediction: - mesangial hypercellularity (M score= 0 or 1); - endocapillary hypercellurarity (E score= 0 or 1); - segmental glomerulosclerosis (S score= 0 or 1); and - tubular atrophy/interstitial fibrosis (T score= 0 or 1 or 2). Overall, the MEST score ranges from 0 to 5. One limitation is that patients were only included if proteinuria was ≥ 1g/d in adults and there was no patients with ARR=0 who are in fact the majority of the patients; in addition patients with extracapillary GN (≥ 50% crescents) were also excluded; the two tails of the IGAN cohorts were therefore lacking!

9. Principles of treatment in IGAN

The treatment should in fact target all major risk factors when present: hypertension, proteinuria, and severe renal lesions.

The permanent control of HT is a major step; the goal is to lower BP ≤ 130/80. Sodium chloride restriction is recommended with 24 h urinary sodium below 100 mmol/d corresponding to a maximum of 6 g daily sodium chloride. All antihypertensive agents can be used: diuretics, beta blockers, calcium blockers, central-acting, ACE inhibitors, angiotensin-2-receptor blockers (ARBs), and more recently renin inhibitors. However two classes have demonstrated a better protection and should be used alone or in association: ACEI and ARBs [25, 26]. In our prospective cohort [12], we have demonstrated that survival without dialysis/death improved in patients with adequately controlled BP on long term [27].

The significant reduction of proteinuria is another major step [28]; the reduction can be obtained with the use of ACEI and ARBs which have a significant antiproteinuric effect. It is recommended to start with either one of these drugs, to titrate the dose to the effect, and to use the association in case of resistant proteinuria; the goal is to bring proteinuria ≤ 1g/d or ideally < 0.30 g/d. We have also demonstrated [12] that the permanent reduction of proteinuria is associated with a better survival on long-term.

The **treatment of severe renal lesions** on the biopsy; this could be achieved by Prednisone or Prednisolone treatment [29-31] which should be in theory able to reduce hypercellularity and cellular infiltration within the glomeruli; however in the trial, there was no repeated biopsies at the end of the steroid therapy. For the most severe cases with extracapillary GN (>50 % crescents), the association of high dose steroids and immunosuppressive agents as already proposed might be a good option.

The use of Fish Oil was limited with controversial results [32, 33].

We are still in the need of large randomized controlled trials with a long duration (5 years seems optimal) to draw definite conclusions on the treatment of IGAN.

10. Pathogenesis and patho-physiology of primary IgA nephropathy

Very significant progress has been made during the last decades. The key protein in this disease is the immunoglobulin A and more precisely the subgroup 1; in fact IgA1 is deposited in the mesangium but not IgA2 [34]. The major difference between IgA1 and IgA2 is the presence of an hinge region in IgA1 composed of 23 aminoacids with usually five sugar side chains linked to threonin or serin. There is now a consensus [35-39] about the fact that the main difference between IgA1 in Controls and in IGAN patients is the hypo-galactosylation of these glycosylated side chains. The normal complete sugar chain is O-linked to threonine or serin and composed of one molecule of N-acetyl galactosamine (GalNac) and one molecule of Galactose; in addition a molecule of sialic acid can be bound in terminal to Galactose or in lateral to GalNac. In IGAN, more side chains are truncated with loss of terminal Galactose with its terminal sialic acid and is referred to the Galactose-deficient IgA1 (deGal-IgA1). This deGal-IgA1 represents the specific autoantigen in this disease and is present in the serum, within the circulating immune complexes and in the mesangial deposits [40]. This loss in terminal galactose is associated with a down-regulation of the gene controlling the linkage of galactose to GalNac, C1GALT1 [41] and the description of specific polymorphism [42] raising the possibility of genetic predisposition [43].

The loss of terminal galactose unusually exposed the GalNac molecules, which become antigenic with elicitation of a specific antibody response [44, 45]: IgG and/or IgA anti O-GalNac, the specific auto-antibodies. It is now possible to measure the amount of circulating

autoantigen and autoantibodies in patients sera and to discriminate between Controls and IGAN patients [46, 47].
The mesangial deposition of deGal-IgA1 is dependent on physical characteristics of this molecule (more sticky) and the presence of transferrin receptor (CD71) which is able to bind IgA1 variants [48]. After binding, the deGal-IgA1 and the deposited immune complexes are able to activate the different mediators of inflammation both cellular [49] and in fluid phase.
There is also the CD89 system [50] that we personally consider as an amplification loop. This is a specific receptor for IgA1 variants, FcαRI, present on circulating monocytes but not in the mesangium; this receptor is able to bind immune complexes with further amplification and longer persistence in the circulation, and may play an additional pathogenic role.

11. Renal transplantation in patients with biopsy-proven IGAN on native kidneys

In cases which progressed to dialysis, renal transplantation should be a strong option and overall the results are similar to the other recipients matched for age and gender. However, there are two specific situations which have strong pathogenic implication.
First, a silent IgA nephropathy can be present on grafted kidney from apparently normal donors leading to the discovery of mesangial IgA deposits on graft biopsies performed early after transplantation; it was demonstrated in few cases that these deposits can regress and disappear demonstrating a contrario that the disease has a systemic (blood) transmission.
Second, the original disease may reappear (**recurrence**) on the normal grafted kidney after few years and despite immunosuppression [51-59]. The cumulative incidence of clinic-pathological recurrence is high reaching 35 % or more at 10 years post-transplant [56] and may lead to graft losses in up to 17 % at 10 y [57]. The factors associated to recurrence are not well understood: living donors, better HLA matching, short duration of the original disease, etc. There is yet no specific treatment for the recurrent disease; however in a retrospective study [56] we demonstrated that induction treatment with ATG seems able to reduce the incidence of recurrence in comparison to no induction or to induction with Basiliximab. A prospective randomized controlled trial comparing rabbit ATG to Basiliximab has already started, the PIRAT study: Prevention in IgA nephropathy recipients of full Recurrence After renal Transplantation according to induction immunosuppressive therapy: ATG versus Basiliximab. It seems now mandatory to carefully measure the serum levels of the auto-antigen and the auto-antibodies at time of grafting to check for any predicting value of these parameters for recurrence.

12. Conclusions

IgA nephropathy is a frequent disease whose individual prognosis can be totally different: no significant progression for 50 years versus progression to dialysis in few months or years. The clinical challenge is to accurately predict the long term individual prognosis at time of diagnosis (at time of the renal biopsy which is still mandatory for this purpose). We have made significant progress with our new concept: the **Absolute Renal Risk of Dialysis/Death** (ARR); it takes in account the presence or not of three independent, simplified and dichotomous risk factors: - **arterial hypertension**; - **proteinuria ≥1g/d**; and severe histopathological lesions appreciated by the **Global Optical Score ≥ 8** (range from 0 to 20). The cumulative incidence rate of primary event (dialysis or death) at 10 years post-

diagnosis, is 4 % for ARR=0; 8 % for ARR=1; 18 % for ARR=2; and 68 % for ARR=3 in our prospective cohort adequately treated/managed on long term.

The pathogenesis of the disease has also made significant progress: it is an **auto-immune disease** with a known **auto-antigen**, the Galactose-deficient IgA1, which can elicit a specific **auto-antibody** response, IgG and IgA anti-O-GalNac. There is formation of specific **immune complexes** which are **circulating** and then **deposited** in the mesangium with creation of the disease. These recent findings will have significant future applications in the diagnosis and in the treatment.

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5

IgA Nephropathy: Insights into Genetic Basis and Treatment Options

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In memoriam of Prof. Efstathios Alexopoulos

1. Introduction

IgA nephropathy (IgAN), is the most common primary glomerulonephritis worldwide. On light microscopy the picture can vary from slight mesangial hypertrophy to extra capillary proliferation of glomeruli, with sclerosis and interstitial fibrosis. On immunofluorescence staining of kidney sections the disease is characterized by mesangial deposits of IgA, predominantly polymeric IgA (pIgA) of the IgA1 subclass, and often co-deposition of complement factor C3, properdin and IgG. It is important however to realize that, although IgA mesangial deposits are necessary for the diagnosis of IgAN, the latter is not obligatory in every individual with mesangial IgA deposits. Thus, IgA deposits may also be seen in subjects with no evidence of renal disease [Suzuki et al, 2003; Waldherr et al, 1989] at an incidence that ranges from 3 to 16 percent. Furthermore, there are also a number of reports documenting IgA deposition in other forms of glomerulonephritis, particularly thin basement membrane disease, lupus nephritis, minimal change disease, and diabetic nephropathy, a finding which is most probably casual rather than causal.

IgAN occurs at any age, but most commonly the age of onset is in the second or third decade of life. Males are more often affected than females, with a male:female ratio of 2:1. Most patients with IgAN present microscopic hematuria with or without mild proteinuria. About 40% of patients have episodes of macroscopic hematuria. This is sometimes preceded by infections, most commonly upper respiratory tract infections, a phenomenon known as "synpharyngitic" hematuria. Other infections like gastrointestinal or urinary tract infections have also been reported to precede macroscopic hematuria. Proteinuria is common and can vary from mild proteinuria to nephrotic syndrome.

IgAN has been considered a benign disease for a long time, but nowadays it is clear that 30-40% of patients may develop renal failure with significant socioeconomical consequenses. In Western Europe and the United States of America 7-10% of the patients on renal replacement therapy suffer from IgAN. The severity of histological lesions, especially diffuse proliferative glomerulonephritis, marked capsular adhesions, fibrocellular crescents, glomerular hyalinosis and severe sclerosis, as well as tubulointerstitial damage correlate

with poor renal outcome [Schena, 1998]. Next to the gravity of histological lesions, unfavourable outcome is associated with persistent hematuria and proteinuria of more than 1g/day, decreased glomerular filtration rate (GFR) at the time of the diagnosis, and hypertension. Although several laboratory tests have been reported to correlate with clinical outcome, so far no reliable biomarker has been identified to predict outcome in IgAN. Recurrence of IgAN after renal transplantation is common. This finding, along with the observation that IgA depositions disappear from a kidney of an IgAN patient, after transplantation of this kidney to a non IgAN patient, are suggestive of a rather systemic disease.

2. Genetics of IgAN

The strongest evidence for the existence of genetic factors in the development and/or progression of IgAN comes from descriptions of familial IgAN, largely in white populations [Julian et al, 1985], and recent studies suggest that familial and sporadic IgAN may share a common pathogenic mechanism and similar outcomes [Izzi et al, 2006]. The genetic predisposition may be independent of environmental factors and may reflect an inherited susceptibility to develop mesangial glomerulonephritis.

IgAN does not exhibit classic single-gene Mendelian inheritance pattern [Frimat & Kessler, 2002]. The complex genetic pattern of IgAN is reflected by the multiple pathways involved in its immunopathogenesis, namely multiple discrete immunologic abnormalities related to the abnormal overproduction and release of mucosal pIgA1 in the systemic compartment and possibly other protein functional abnormalities related to a propensity for mesangial deposition of pIgA1. It is therefore probable that the disease-associated genetic variations at identified *IGAN* loci, instead of occuring in the form of "classic" nonsense/missense/splice site mutations and deletions/insertions that affect protein structure and function, may be rather of the type specific single-nucleotide polymorphism (SNP) alleles in non-coding regions or synonymous SNPs in coding regions. The latter function as *cis*-acting elements that alter the transcriptional activity of a disease gene and/or messenger RNA stability and, therefore, the expression level of the encoded protein. It is interesting that recent studies indicate that 30% to 50% of human genes with coding SNPs can present allelic variation in gene expression [Hoogendoorn et al, 2003; Lo et al, 2003]. From this point of view, the most comprehensive theory is that several genetic loci contribute significantly to the disease susceptibility that underlie the primary immunologic defects observed in IgAN. Each locus may occur at a different prevalence rate in different racial/ethnic groups. Variations at these major genetic loci may not be sufficient for the development and progression of IgAN and the contribution from a potentially large number of modifying genes with modest genetic effects but high prevalence is probably needed as well. The various allelic combinations of these loci may underlie the different disease phenotypes (disease development and progression, nephritic vs. nephrotic clinical presentation, histopathologic subclass, severity of disease, responsiveness of proteinuria to angiotensin-converting enzyme [ACE] inhibitors and/or angiotensin II receptor blockers [ARBs], etc.) observed in IgAN. For diseases with complex genetic pattern, it has been shown that the optimal analysis approach is the combination of linkage, association and sequence approaches. Until now, two basic approaches have been used in genetic studies of IgAN: a) genome-wide linkage analysis study, a methodology that has been used successfully to identify major disease/susceptibility loci, b) candidate-gene association study, mainly used to identify

altered genes with modest genetic effects but high prevalence. Recently, a genome-wide association study was carried out in cohorts of Chinese and European IgAN patients (A.G.Gharawi et al, 2011). Five loci (3 in the major histocompatibility complex at chromosome 6p21, a common deletion of CGHR1 and CFHR3 at chromosome 1q32 and one locus at chromosome 22q12) were identified. They explain 4-7% of the disease variance.

2.1 Genome-wide linkage analysis studies

Genome-wide linkage analysis is used successfully to identify major disease/susceptibility genes but has limited power to detect genes of modest effect. Linkage studies involve recruitment of families with multiple affected individuals. In a typical whole-genome linkage scan, up to 400 microsatellites, or equivalently approximately 10,000 SNP markers, equally spaced across the genome, are typed in families to interrogate marker cosegregation with a disease phenotype. The advantage of genome-wide linkage studies is that they do not require a priori assumption about disease pathogenesis. These studies are very sensitive to phenotype misspecification, however their power is limited to detecting rare genetic variants with a relatively large effect on the risk of disease.

Linkage studies of IgAN are faced with multiple challenges. Familial forms of IgAN are frequently underrecognized because the associated urinary abnormalities in affected family members are often mild or intermittent. Moreover, once familial disease is documented, systematic screening by renal biopsy cannot be justified among asymptomatic at-risk relatives, necessitating reliance on less accurate phenotypes, such as microscopic hematuria, to diagnose affection. Additionally, IgAN has been observed to co-occur in families with thin basement membrane disease (TBMD), an autosomal dominant disease caused by heterozygous mutations in the collagen type IV genes (COL4A3/COL4A4) [Frasca et al, 2004]. Short of kidney biopsy or direct sequencing of the very large collagen genes, TBMD cannot be reliably excluded among relatives of IgAN patients. Finally, because urinary abnormalities may manifest intermittently, one also cannot unequivocally classify at-risk relatives as unaffected, necessitating affected-only linkage analysis. The inability to classify affected and unaffected individuals accurately is commonly encountered in linkage studies of complex traits, leading to decreased study power. Increasing sample size by including additional families is also not necessarily helpful in these situations because the diagnosis of IgAN likely encompasses several disease subsets, such that expansion to larger sample size can paradoxically reduce analytic power due to increased heterogeneity [Durner et al, 1992; Cavalli-Sforza & King, 1986].

To date, four genome-wide linkage studies of familial IgAN have been reported [Gharavi et al, 2000; Bisceglia et al, 2006; Paterson et al, 2007, Feehally et al, 2010]. Families in these studies have all been ascertained via at least two cases with biopsy-documented IgAN, with additional family members diagnosed as affected based on clinical evidence (renal failure or multiple documentation of hematuria/proteinuria). In the first study, 30 families with two or more affected members were examined [Gharavi et al, 2000]; multipoint linkage analysis under the assumption of genetic heterogeneity yielded a peak LOD score of 5.6 on chromosome 6q22-23 (locus named IGAN1), with 60% of families linked. The remainder of families linked to chromosome 3p24-23 with a suggestive LOD of 2.8. This study demonstrated that IgAN is genetically heterogeneous but argued for the existence of a single locus with a major effect in some families. In the second genome-wide linkage study 22 Italian IgAN families were enrolled [Bisceglia et al, 2006] (see section 2.3). The third linkage

scan was based on a unique large pedigree with 14 affected relatives (two individuals with biopsy defined diagnosis and 12 with hematuria/proteinuria on urine dipstick) [Paterson et al, 2007]. Linkage to chromosome 2q36 was detected with a maximal multipoint LOD score of 3.47. Most reported linkage intervals did not contain obvious candidate genes, but the 2q36 locus encompasses the COL4A3 and COL4A4 genes, which are mutated in TBMD. Together with the high penetrance of hematuria, this finding suggests that affected individuals in the 2q36-linked family may belong to an IgAN subtype that overlaps with TBMD. Finally, the fourth genome-wide analysis, carried out in a cohort of IgAN patients selected from the UK Glomerulonephritis DNA Bank, the region of the MHC (major histocompatibility complex). The strongest association signal included a combination of DQ loci and HLA-B. This study suggests that HLA region contains some alleles that predispose to the disease in the UK population. In conclusion, four genome-wide linkage studies, carried out in four different IgAN patient populations, demonstrate different chromosomal traits linked to the disease.

None of the genes underlying these linkage loci has been identified until now. The underlying reasons are numerous, including the phenotyping difficulties discussed above; the presence of locus heterogeneity, which limits the ability to precisely map the disease interval and find additional linked families to refine loci; the contribution from non-coding susceptibility alleles (e.g. point mutations or structural genomic variants within intronic or promoter regions), which usually escape detection if mutational screening is confined to exonic regions. It is expected that the availability of inexpensive Next-Gen sequencing will enable comprehensive interrogation of linkage intervals, facilitating identification of disease-risk alleles.

In addition, future studies of this kind that aim at dissection of increasingly genetically homogeneous cohorts must consider the importance of defining distinct clinical subtypes of IgAN that may exist within the single pathologic ascertainment criterion currently used to diagnose IgAN: light microscopic evidence of mesangial deposits of IgA by immunofluorescence. As with all family-based genetic studies, there is a high degree of dependency on access to sufficient numbers of clinically well-phenotyped and genetically informative cohorts. To address the paucity of cohorts with biopsy-proven IgAN available for the conduct of linkage-based, association-based, and sequence-based approaches, the European IgAN consortium has published the details of its IgAN Biobank resource [Schena et al, 2005].

2.2 Candidate-gene association studies

Candidate-gene association studies examine polymorphisms in only specific genes that are selected based on a priori assumption about their involvement in the disease pathogenesis, and they are highly sensitive to population stratification, multiple testing, and reporting bias. As a result, most candidate-gene association studies in the literature have not been replicated [Ioannidis et al, 2001; Hsu SI et al, 2000; Hsu SI, 2001; Frimat & Kessler, 2002], an issue which questions the validity and the methodology used in these studies [Hsu & Feehally, 2008]. Not surprisingly, candidate-gene studies for IgAN have also been largely unrevealing. Many candidate genes have been proposed, but for most of them no solid a priori evidence for their involvement in IgAN existed, whereas most were studied in the context of IgAN progression rather than causality. Over the last 15 years, there were more than 120 candidate-gene association studies for IgAN published in the English literature and

indexed on PubMed (e.g., components of the renin-angiotensin-aldosterone pathway, mediators of inflammation and/or vascular tone, components of the mesangial matrix, and various receptors for polymeric IgA1 expressed in mesangial cells) [Kiryluk et al, 2010]. Of these, 39 (31%) studies examined genetic polymorphisms in association with susceptibility to IgAN, 40 (32%) examined an association with disease severity, progression, or complications, and 44 (35%) examined both susceptibility and risk of progression. Many candidate-gene association studies are lacking in functional genetics.

Approximately one third of all studies involved polymorphisms in the renin-angiotensin-aldosterone system (RAAS). A widely studied example of the dilemma of repeated non-replication of results is represented by genetic case-control association studies of the angiotensin I–converting enzyme (*ACE*) gene insertion/deletion (I/D) polymorphism in the development and/or progression of IgAN, as well as a whole host of common human diseases and conditions, including cardiovascular disease, complications of diabetes such as retinopathy and nephropathy, glomerular, tubulointerstitial, and renal cystic renal diseases, and even renal allograft survival [Hsu SI et al, 2000; Hsu SI, 2001; Schena et al, 2001]. The interest in studying the *ACE* I/D polymorphism is based on evidence for "biologic plausibility." Rigat and colleagues reported in 1990 that the *ACE* I/D polymorphism in intron 16 of the human *ACE* gene accounts for half of the variation in serum ACE levels in a white study cohort [Rigat et al, 1990], and this is due to the presence of a transcriptional repressor element in the I allele [Hunley et al, 1996].

There have been numerous population-based studies that either support or refute an association between the D allele and progression of renal disease in these conditions [Hsu SI et al, 2000; Hsu SI, 2001]. Recent meta-analyses have concluded that the D allele is not associated with renal disease progression in patients with IgAN or diabetic nephropathy [Schena et al, 2001; Kunz et al, 1998]. Despite more than a dozen generally small genetic case-control studies of the *ACE* I/D polymorphism in both white and Asian IgAN cohorts have been done, no definite conclusions can be drawn from them regarding the association between the D allele or DD genotype and development and/or progression of IgAN. Population-based genetic association studies of other genes encoding proteins in the RAAS such as angiotensinogen (AGT) and the angiotensin II type 1 receptor (ATR1), as well as renin (*REN*) and aldosterone synthase (CYP11b2), have also generated conflicting results, as have similar studies of the "expanded" RAAS that includes 11b-hydroxysteroid dehydrogenase type 2 (11bHSD2) and the mineralocorticoid receptor (MLR) [Poch E et al, 2001]. In general, the approach has been to genotype a single common polymorphism in each gene with the use of polymerase chain reaction/restriction fragment length polymorphism (PCR-RFLP). It is remarkable that to date, the role of the RAAS, whose components ACE and ATR1 are the targets of ACE inhibitors and angiotensin-II receptor blockers (ARB), respectively, has not been convincingly demonstrated by any genetic association study.

In general, most of these studies were of poor quality and severely underpowered and, therefore, negative findings were almost universally inconclusive. Overall, the average size of case–control cohorts per study was 182 cases and 171 controls. Many studies used ad hoc controls derived from unscreened blood donors who were poorly matched to the cases in terms of ancestry and geography. The potential impact of confounding by population stratification was ignored by the majority of studies, despite the fact that the tools for quantification of this problem have been developed. An additional matter of concern is the

lack of adequate correction (permutation testing) for multiple, non-independent tests which would be anticipated as long as several of these studies tested several hypotheses (multiple polymorphisms, multiple phenotypes, or multiple genetic models). Other major problems included inadequate or variable SNP coverage of candidate genomic areas, with several studies examining only a single polymorphism. Thus far, only one group attempted to survey the entire genome, yet the results have not been replicated, are inconclusive and difficult to interpret, as long as an underpowered cohort was studied with inadequate coverage of ~80,000 SNPs [Obara et al, 2003; Ohtsubo et al, 2005]. Moreover, 77% of all published candidate-gene studies reported positive findings, an observation that is likely explained by a combination of high rate of false positives and a strong publication bias, whereas the statistical effect of the study of the same patient cohorts for multiple polymorphisms has never been accounted for in the literature [Kiryluk et al, 2010]. Most findings were not reproduced in other populations. None of the above problems is unique to the field of IgAN and for these reasons, new general guidelines aimed at improving the design and execution of genetic association studies have recently been formulated [von Elm et al, 2007; Little et al, 2009].

In the post-genomic era, there has been a renewed interest in conducting genetic association studies, especially SNP-based, whole-genome association studies, to identify genetic variations associated with the development and/or progression of a number of common human diseases. This renewed interest reflects the important finding that linkage disequilibrium (LD), the phenomenon that particular alleles at nearby sites can co-occur on the same haplotype more often than expected by chance [Goldstein, 2001; Wahl et al, 2003] is highly structured into discrete blocks separated by hotspots for recombination. The haplotype block model for LD has important implications for the way in which genetic association studies should now be conducted, and may explain at least in part the problem of repeated non-replication of results that has plagued such studies in the past. Based on the haplotype block model of LD, the ACE I/D polymorphism is a single marker variant in the ACE gene, whereas in unknown yet whether the D allele defines a simple population of subjects at risk for disease or not. The lumping of subgroups defined by haplotypes that share the D allele may explain at least in part the basis for discrepant reports of genetic association with disease.

Nowadays the common SNP haplotype block model is considered essential for the credibility of a study [Couser, 2003] and genetic association studies, especially family based, that employ one or more methodologically valid approaches and satisfy the minimum rigorous conditions for a reliable genetic association study are viewed as studies with solid documentation. These include mainly studies employing biologic plausibility, haplotype relative risk analysis to identify statistically significant "at-risk haplotype[s]" associated with small *P* values, use of family-based methodologies, such as the transmission equilibrium test (TDT/sib-TDT) or the family-based association test (FBAT) to directly study trios/sib-trios and extended families or to verify the absence of significant population stratification bias (admixture) inherent in population-based case-control association studies, and the study of moderately large i.e., adequately powered] cohorts. To date, very few studies examining candidate genes have employed the family-based TDT study methodology and/or analysis of "at-risk" haplotypes, reflecting the emergence of the first studies to attempt to satisfy minimum criteria for a valid association study.

A family- and haplotype-based association study employing the TDT methodology has shown that 2093C and 2180T SNP variants in the 3′-untranslated region of the *Megsin* gene

were significantly more frequently transmitted from heterozygous parents to patients than expected in the extended TDT analysis (increased co-transmission in 232 Chinese families, $P < 0.001$). In addition, haplotype relative risk (HRR) analyses showed that these same SNP alleles were more often transmitted to patients (HRR = 1.568, $P < 0.014$ for the 2093C allele; HRR = 2.114, $P < 0.001$ for the 2180T allele) [Li YJ et al, 2004]. The same group using a similar approach recently reported that the *Megsin* 23167G SNP variant is associated with both susceptibility and progression of IgAN in 435 Chinese patients and their family members using TDT and HRR analyses [Takei et al, 2006]. The GG genotype was found to be associated with severe histologic lesions and disease progression. Megsin is a member of the serpin (serine proteinase inhibitor) superfamily that is upregulated in the context of mesangial proliferation and extracellular matrix expansion in IgAN, and therefore represents a strong candidate gene for susceptibility to IgAN. Lately, the gene encoding Cosmc, a C1Gal-T1 chaperone protein which also mediates IgA O-galactosylation was studied as a candidate gene involved in the pathogenesis of IgAN but no evidence for a role for Cosmc mutations was reported [Malycha et al, 2009].

2.3 IgAN consortium

The IgAN Biobank, coordinated by F.P. Schena, contains at minimum 72 multiplex extended pedigrees, 159 trios, and 1,068 cases and 1,040 matched controls. All subjects enrolled are white and belong to various geographic areas from Germany, Italy, and Greece [Schena et al, 2005]. Aiming at a genome wide linkage study, which has been considered the most promising approach to identify IgAN susceptibility genes, a group of investigators constituted the European IgAN Consortium which was initially funded by the European Union. DNA samples of IgAN patients and relatives belonging to 74 multiple extended pedigrees were collected. Moreover, 166 trios (affected sons or daughters and their healthy parents), 1,085 patients with biopsy-proven IgAN and 1,125 healthy subjects were included in the Biobank. In combination with linkage analysis, family based candidate gene association studies were also applied in an efford to discover responsible genes and overcome obstacles inherent the genetic analysis of complex traits such as IgAN.

Linkage Analysis Studies – The European IgAN Consortium performed the first genome-wide scan involving 22 Italian multiplex IgAN families [Bisceglia L et al, 2006]. A total of 186 individuals (59 affected and 127 unaffected) were genotyped and included in a two-stage linkage analysis. The regions 4q26–31 and 17q12–22 exhibited the strongest evidence of linkage by non-parametric analysis (best p values of 0.0025 and 0.0045, respectively). These localizations were also supported by multipoint parametric analysis where a peak LOD score of 1.83 (α=0.50) and of 2.56 (α=0.65), respectively, were obtained using the affected only dominant model, and by allowing for the presence of genetic heterogeneity. These regions became the second (IGAN2) and third (IGAN3) genetic locus candidates to contain causative and/or susceptibility genes for familial IgAN. Other regions did not reach the threshold of a suggestive or significant LOD score; however, the enrolment of additional IgAN families means that these chromosomal regions may be explored in the near future. The above results provide further evidence for genetic heterogeneity among IgAN families. Evidence of linkage to multiple chromosomal regions is consistent with both an oligo/polygenic and a multiple susceptibility gene model for familial IgAN with

small/moderate effects in determining the pathological phenotype. The analysis of the known genes located in these two novel loci (positional information procedure), carried out consulting the National Center for Biotechnology Information, identified some potential candidate genes such as the transient receptor potential channel 3 (TRPC3) gene, the interleukin-2 (IL-2) gene, and the IL-21 gene located in 4q26–31, which could be largely involved in the unbalanced Th1/Th2 immune response reported in IgAN patients. In addition, the histone deacetylase 5 (HD5) gene and the granulin (GRN) gene located on the 17q12–22 region, which could be involved in the immune-response deregulation, will also considered. Family-based association studies, evaluating the distribution of these candidate gene polymorphisms, are in progress.

Microarray Studies – Different high-throughput gene analysis techniques can be used for obtaining transcriptome profiling of renal diseases. Microarray analysis represents the best and the latest approach to gain information on global gene expression. Genome-wide linkage analyses have identified at least three locus candidates containing IgAN susceptibility genes, although no specific gene(s) have been discovered. Microarrays are now in use to fingerprint the pathological process.

A published study postulated that changes in gene expression patterns in circulating leukocytes of IgAN patients may correlate with renal disease activity [Preston et al, 2004]. The investigators identified 14 upregulated genes. The BTG2, NCUBE1, FLJ2948, SRPK1, LYZ, GIG2 and IL-8 genes correlated with serum creatinine levels and the PMAIP1, SRPK1, SSI-3, LYZ and PTGS2 genes correlated with higher values of creatinine clearance, thus implying that the latter group of genes may provide a protective effect, while the overexpression of other genes such as B3GNT5, AXUD1 and GIG-2 indicated a worse prognosis. This gene signature reflected kidney function and did not correlate with hematuria or proteinuria. The authors concluded that studies carried out on large populations of IgAN patients will be necessary to confirm that the leukocyte gene expression profile can be used as a marker for diagnosis and for predicting outcome. The European IgAN Consortium has recently organized a protocol for studying gene expression in peripheral blood mononuclear cells (PBMC) and their subpopulations from IgAN patients with different clinical and histological patterns. Cox et al [2010] conducted a whole-genome expression study to identify genes and pathways differently modulated in peripheral blood leukocytes of IgAN patients. Gene expression of leukocytes demonstrated the hyperactivity of two important pathways as the canonical WNT-β catenin and the PI3k/Akt pathways. The abnormal WNT signalling was also confirmed in IgAN patient's monocytes and to a less extent in B lymphocytes. Low gene expression of inversion and phosphatase and tensin homolog (PTEN) are responsible for the hyperactivation of these two pathways that enhance cell proliferation through lymphoid enhancer factor-1 (LEF-1) of which the gene is located within our previous described region 4q26-31 linked to IgAN [Bisceglia et al, 2006]. Finally, the hyperactivation of the PJ3k/Akt pathway is in linkage with the upregulation of the immunoproteasome in peripheral blood mononuclear cells of IgAN patients, reported by Coppo et al, [2009].

Expression profiling using serial analysis of gene expression (SAGE) and microarray techniques allows global description of expressed genes present in renal tissue. This is a high throughput genomics technology which enables the simultaneous determination of a large number of genes from tissue samples. Preston et al identified 13 upregulated genes in IgAN renal biopsy samples. The cluster analysis identified 3 clusters with 7, 12 and 1

involved gene, respectively [Preston et al, 2004]. The expression levels of these genes were then examined on expanded RNA samples from other renal biopsies, leukocyte samples and cultured primary cells. Data demonstrated the involvement of the genes GABP and STAT3 in cluster I, and gp330 (megalin), MBP45K, MEF2, Oct1 and GABX in cluster II. The use of laser-capture microdissection applied to renal biopsy samples in combination with differential gene expression analysis is expected to provide novel knowledge in the search for IgAN candidate genes.

Candidate genes association studies – The IgAN Consortium takes care of the collection of biological samples from large homogeneous cohorts of IgAN patients, their parents and their first degree relatives, and family-based association studies are preferred to analyze the role of some candidate genes. A family-based association study, including 53 patients, 45 complete trios, 4 incomplete trios and 36 discordant siblings, evaluated the impact of some Th1/Th2/Th3/TR-type lymphocyte and monocyte/macrophage cytokines on IgAN susceptibility [Schena FP et al, 2006]. Cytokine gene polymorphisms with a potential regulatory role on their production were investigated using the family-based association test (FBAT): IFNγ intron-1 CA-repeat at position 1349–1373; IL-13 –1055C/T; TGFβ 915G/C; IL-10 5′-proximal and distal microsatellites; TNFα –308G/A, –238G/A. The FBAT multi-allelic analysis showed an association between IFNγ polymorphism and susceptibility to IgAN ($p=0.03$). The bi-allelic analysis showed that the 13-CA repeat allele was preferentially transmitted to the affected individuals ($p=0.006$; Bonferroni $p=0.04$). The direct sequencing of IFNγ amplicons showed a strict association between the 13-CA repeat allele and the A variant of the +874T/A SNP (rs2430561) directly adjacent to the 5′ end of the microsatellite. The in vitro production of IFNγ evaluated in PBMC from 10 genotyped patients demonstrated a correlation between the +874A allele and a lower production of IFNγ ($p=0.028$). Notably, the +874A variant was associated with transcriptional downregulation of INFγ gene promoter activity, consistent with the known role of NF-κB in the transcriptional regulation of the INFγ gene.

The occurrence of the +874A variant is responsible for the low production of IFNγ and predisposes to a preferential Th2-mediated immune response. The predominance of this variant in individuals with IgAN may be responsible for the onset of the disease. This unbalanced Th2 cytokine production in response to upper respiratory tract infections may be a significant pathogenic factor in human IgAN. The prevalence of Th2 cytokines may also explain the abnormality in IgA1 glycosylation occurring in IgAN patients and the concomitant formation of circulating IgA1-IgG immune complexes. Hyperfunction of Th2 cells and cytokine polarity are linked to a more nephritogenic pattern of IgA1 glycosylation in the animal model, and the decreased glycosylation of IgA1 elicited by Th2 cytokines is blunted in vitro by the addition of IFNγ [Ebihara et al, 2008]. The core 1 β1,3-galactosyltransferase (C1Gal-T1) is suspected to be involved in the abnormal glycosylation process of IgA1 in IgAN. With the genetic characterization of the enzymes responsible for O-glycosylation of IgA1, it has been possible to study changes in the O-glycosylation of IgA1 at a genetic level. Most recently two groups [Pirulli et al, 2009; Li GS et al, 2007] have independently found that SNPs in the C1Gal-T1 gene are associated with a genetic susceptibility to IgAN in Chinese and Italian populations, albeit it is not clear how these polymorphisms relate to changes in the functional activity of C1Gal-T1. The C1Gal-T1 gene complete sequence analysis was performed in 284 IgAN patients and 234 healthy controls. A statistically significant association of the genotype 1365G/G with

susceptibility to IgAN (χ^2=17.58, p<0.0001, odds ratio 2.57 [95% CI: 1.64–4.04]), but not with the progression of the disease, was found [Pirulli et al, 2009]. The latter case-control association study demonstrates that the low expression of C1Gal-T1 seems to confer susceptibility to IgAN.

In conclusion, to date the Consortium has identified two loci (located on chromosomes 4q26–31 and 17q12–22), in addition to a previous study which described the first IgAN locus on chromosome 6q22–23. The functional mapping of genes involved in the disease proceeds from the identification of susceptibility loci identified by linkage analysis (step 1) to the isolation of candidate genes within gene disease-susceptibility loci, after obtaining information by microarray analysis carried out on peripheral leukocytes and renal tissue samples (step 2). Next steps will be the design of RNA interference agents against selected genes (step 3) and the application of systematically tested effect of RNA agents on functional cellular assay (step 4). The above combined high-throughput technologies will give information on the pathogenic mechanisms of IgAN. In addition, these data may indicate potential targets for screening, prevention and early diagnosis of the disease and more appropriate and effective treatment.

3. Treatment of IgAN

Treatment strategy for IgAN remains a controversial issue, even more as published randomized controlled trials (RCTs) on this topic are few and most studies are underpowered to provide definitive information. Furthermore, the disease heterogeneity, its clinical course along with the slow rate of GFR decline (1 to 3 mL/min per year) seen in many patients hinders the ability to perform adequate studies. An additional obstacle is the fact that a significant percentage of the patients have only minimal clinical presentation, such as isolated microhematuria, no or minimal proteinuria and normal GFR, and are often not biopsied or even identified. Still no treatment is known to modify mesangial deposition of IgA, which obviously reflects our incomplete knowledge of immunopathogenesis of IgAN [Barratt et al, 2007], and available treatment options are directed mostly at downstream immune and inflammatory events that may lead on to renal scarring. Therefore, as more pathogenetic details, the genetic substrate and heterogeneity of IgAN become increasingly understood, novel treatment strategies with solid therapeutic targets are anticipated, as long as the traditional therapies used until today seem symptomatic rather than etiologic. The discovery and establishment of novel biomarkers associated with the disease activity and outcome will provide the prognostic and therapeutic tool for more accurate and clear therapeutic targeting.

However, there seems to be a consensus regarding patient selection for the different therapeutic approaches. Patient selection for therapy is based in part upon the perceived risk of progressive kidney disease:

- Patients with isolated hematuria, no or minimal proteinuria (<500mg/day), a normal GFR and no signs of progressive disease, such as increasing proteinuria, blood pressure, and/or serum creatinine, are typically not treated.
- Patients with persistent proteinuria (>500 mg/day), normal or only slightly reduced GFR (>50mL/min) that is not declining rapidly, and only mild to moderate histologic findings on renal biopsy are managed with general interventions to slow progression with ACE-inhibitors or angiotensin receptor blockers (ARB) or with combination therapy of corticosteroid (6 months) and ACE-inhibitors forever.

- Patients with more severe or rapidly progressive disease (eg, proteinuria > 1g or proteinuria persisting despite ACE inhibitor/ARB therapy, rising serum creatinine, and/or renal biopsy with more severe histologic findings, but no significant chronic changes) may benefit from immunosuppressive therapy in addition to non-immunosuppressive interventions to slow disease progression.
- In the future, the Oxford histologic classification system, once validated, is anticipated to become a useful prognostic tool that could lead in the future our therapeutic choices [Working Group of the International IgAN Network and the Renal Pathology Society, 2010].

3.1 Monitoring disease activity

Up to date, there are no specific markers to identify continued immunologic activity. Instead, the clinical parameters typically used as the main therapeutic criterion are the urine sediment, protein excretion and the serum creatinine concentration. Persistent hematuria is generally a marker of persistent immunologic activity, but not necessarily of progressive disease. This finding may be a sign of a "smoldering" segmental necrotizing lesion, suggestive of "capillaritis." Hematuria alone does not require any form of therapy. Proteinuria, rather than hematuria alone, is a marker of more severe disease [Donadio et al, 2002]. Increasing proteinuria may be due to one of two factors: ongoing active disease; and secondary glomerular injury due to non-immunologic progression. It is often not possible to distinguish between these two possibilities, except for a rapid increase in protein excretion which is only seen with active disease. Protein excretion most often falls with ACE inhibitor/ARB therapy and the degree of proteinuria is, as described below, one of the end points of such therapy. Protein excretion also may fall spontaneously, particularly during recovery from an acute episode and perhaps in children, and following effective immunosuppressive therapy. Finally, serum creatinine, unless it is rapidly rising, permits an estimation of the GFR. As noted above, most patients with chronic IgAN have stable or slowly progressive disease. The rate of GFR decline is often as low as 1 to 3 mL/min per year, a change that will not detectably raise the serum creatinine above normal levels for many years [Rekola et al, 1991]. Thus, a stable and even normal serum creatinine does not necessarily indicate stable disease.

The establishment of accurate biomarkers is necessary for the optimal categorization and treatment of patients with IgAN. Over the last few years specific urine biomolecules have been proposed as probable biomarkers to be used in the prognosis and therapeutic strategy in patients with IgAN. Two recent studies identified urine epidermal growth factor and monocyte chemoattractant protein-1 as strong independent predictors of renal outcome in patients with IgAN [Torres et al, 2008; Stangou et al, 2009]. These and other biomolecules are being validated as probable biomarkers of IgAN in studies underway.

3.2 Non-immunosuppressive therapies

Three main non-immunosuppressive therapies are in use in IgAN [Barratt & Feehally, 2006; Appel & Waldman, 2006]:

- ACE inhibitors or ARB, to control blood pressure and to slow down progression of the renal disease.
- Statin therapy, for lipid-lowering in selected patients, to lower cardiovascular risk and possibly reduce disease progression.
- Fish oil (omega-3 fatty acids) may also be beneficial in certain cases.

3.2.1 Angiotensin inhibition

The progression of IgAN may be slowed by anti-hypertensive and anti-proteinuric therapy that can minimize secondary glomerular injury [Kanno et al, 2000]. The treatment goals with angiotensin inhibition are the same as those in other forms of proteinuric chronic kidney disease as described in the K/DOQI guidelines [K/DOQI, 2004]. ACE inhibitors and ARBs act by reducing the intraglomerular pressure and by directly improving the size-selective properties of the glomerular capillary wall, both of which contribute to reducing protein excretion [Remuzzi et al, 1991; Maschio et al, 1994].

Both observational studies [Cattran et al, 1994; Kanno et al, 2005] and small randomized trials [Maschio et al, 1994; Praga et al, 2003; Li PK et al, 2006] have provided suggestive evidence that ACE inhibitors or ARBs are more effective than other antihypertensive drugs in slowing the progressive decline in GFR in IgAN as they are in other forms of chronic proteinuric kidney disease. Praga et al in their randomized trial in 44 IgAN patients with proteinuria (≥0.5 g/day, mean 1.9 g/day) and a serum creatinine concentration ≤1.5 mg/dL at baseline, found a significant decrease in proteinuria in the enalapril group (1.9 g/day at baseline to 0.9 g/day at the last visit) and a significantly higher renal survival, defined as less than a 50 percent increase in the serum creatinine concentration, at 6 years of follow up [Praga et al, 2003]. More recently, Li et al in their double-blind randomized placebo-controlled HKVIN trial in 109 Chinese patients with protein excretion ≥1 g/day (mean ~2.0 g/day), found a better renal survival, defined as doubling of serum creatinine or ESRD, a significant improvement in proteinuria (33 % reduction in proteinuria) and a slower rate of decline in GFR (4.6 versus 6.9 mL/min per year) in the valsartan group compared to placebo [Li PK et al, 2006]. Similarly, the IgACE trial in 65 young patients with moderate proteinuria (between 1 and 3.5 grams/day per 1.73 m^2) and creatinine clearance >50 mL/min per 1.73 m^2 revealed a better renal survival (fewer patients with >30% decline in renal function) and significant improvement in proteinuria at 38 months of follow-up in the benazepril group compared to the placebo group [Coppo, 2007].

Normotensive patients who excrete less than 500 mg of protein per day are not typically treated with angiotensin inhibition. However, because most patients progress slowly over time, monitoring of the serum creatinine and protein excretion at yearly intervals is recommended. Angiotensin inhibition should be started if there is evidence of progressive disease and protein excretion above 500 mg/day.

The addition of an ARB to an ACE inhibitor in patients with IgAN seems to exert a further antiproteinuric effect [Russo et al, 1999, 2001], albeit there are no randomized trials showing that this regimen improves renal outcomes. This finding is consistent with meta-analyses of trials in different glomerular diseases, the largest of which found a significant 18 to 25% greater reduction in proteinuria with combined ACE inhibitors and ARBs compared to monotherapy [Kunz et al, 2008; Catapano et al, 2008]. The rationale for this combination therapy is the assumption that ARBs would counteract theAT1-mediated effect of residual angiotensin II formation by non-ACE enzymes like chymase, whereas ACE inhibitors would additionally increase the level of kinins. Furthermore, ACE inhibitors as well as ARBs would synergistically elevate the levels of angiotensin, which also might promote vasodilation. Finally, combining both drug classes might simply provide a higher degree of blockade of the classic renin-angiotensin system pathways [Alexopoulos, 2004]. However, any anticipated benefits from this combination should be weighted against possible adverse effects in individual patients; this is important especially given the findings from the ONTARGET trial in 25,620 patients with vascular

disease or diabetes, where an increase in adverse side effects (including a possible increase in mortality) in patients who received combination therapy with an ACE inhibitor and ARB was shown, compared to those who received monotherapy [ONTARGET Investigators, 2008; Mann et al, 2008].

3.2.2 Lipid-lowering therapy

Chronic kidney disease is associated with a marked increase in cardiovascular risk, and is now considered a coronary artery disease risk equivalent. Furthermore, lipid-lowering with statins has been associated with a slower rate of loss of GFR in patients in some patients with mild to moderate CKD, and there are indications for such a beneficial effect of statins in patients with IgAN as well [Kano et al, 2003]. Therefore, it seems rational that all patients with decreased kidney function and/or hypercholesterolemia should receive lipid-lowering therapy with a statin, with treatment goals similar to that in patients with underlying coronary heart disease.

3.2.3 Fish oil

The rationale for using fish oil (omega-3 fatty acids) in IgAN is based on the premise that they may limit the production or action of cytokines and eicosanoids evoked by the initial or by repeated immunologic renal injury, and the resulting production of mediators involved in renal damage [Donadio, 1991]. Randomized controlled trials evaluating fish oil in patients with IgAN have reported conflicting results [Donadio et al, 1994; Alexopoulos et al, 2004; Donadio et al, 1999, 2001; Bennett et al, 1989; Pettersson et al, 1994; Hogg et al, 2006; Ferraro et al, 2009]. In the largest and most well documented and conducted randomized trial in 106 patients with baseline creatinine clearance 80 mL/min and protein excretion of 2.5 to 3 g/day, Donadio et al found better patient and renal outcomes at 4 years, extended also at >6 years, in patients having received 12g of fish oil for 2 years, compared to patients having received a similar amount of olive oil [Donadio et al, 1994, 1999]. Similarly, in a controlled study of 14 IgAN patients and 14 controls, a low dose of fish oil (0.85 g eicosapentaenoic acid and 0.57 g phytohemaglutinin) was found effective in slowing renal progression in high-risk patients with IgAN and particularly those with advanced renal disease [Alexopoulos et al, 2004]. On the other hand, in the randomized controlled trial by the Southwest Pediatric Nephrology Study Group in 96 patients with IgAN, mean GFR >100 mL/min per 1.73 m^2 and proteinuria 1.4 to 2.2 g/day, no significant benefit in the renal outcome was found in patients assigned to omega-3 fatty acids (4 g/day) for two years compared to the patients assigned to either alternate day prednisone or placebo [Hogg et al, 2006]. On the basis of the existing data, fish oil can be tried in addition to ACE inhibitors or ARBs in patients with protein excretion >500 to 1000 mg/day, a gradual reduction in GFR, and mild to moderate histologic lesions [Alexopoulos E, 2004].

3.3 Immunosuppressive therapy

The optimal role of immunosuppressive therapy in IgAN is uncertain [Barratt & Feehally, 2006; Appel & Waldman, 2006]. A variety of regimens have been used, mostly consisting of corticosteroids alone or with other immunosuppressive drugs. The available studies are not conclusive since most are relatively small and have limited follow-up, and the results are sometimes conflicting [D'Amico, 1992; Alamartine et al, 1991; Alexopoulos, 2004; Strippoli et al, 2003; Laville & Alamartine, 2004; Ballardie, 2004; Julian, 2000; Dillon, 2001]. There is

rather consensus that mild, stable, or very slowly progressive IgAN should not be treated with corticosteroids or other immunosuppressive therapies, given the limited evidence of benefit and their known toxicity from chronic use [Floege & Eitner, 2005; Locatelli et al, 1999]. Corticosteroid or other immunosuppressive therapy should only be attempted in patients with clinical and histologic evidence of active inflammation (eg, hematuria and/or proliferative or necrotizing glomerular changes). Patients with chronic kidney disease with significant tubulointerstitial fibrosis and glomerulosclerosis are not likely to benefit from such therapy and are likely to be harmed from the side effects.

3.3.1 Corticosteroids

Current evidence regarding the potential benefit of corticosteroid therapy in IgAN are rather limited, as long as most of the studies performed are uncontrolled retrospective observations; moreover, the few available randomized controlled trials are rather small and in most of them the standard recommendations for proteinuria and blood pressure goals were not followed, limiting thus the applicability of the findings to current practice. Whatsoever, these studies point towards a beneficial effect of corticosteroid therapy (administered for 6 up to >24 months) in proteinuria and perhaps in renal survival [Floege & Eitner, 2005; Nolin & Courteau, 1999; Kobayashi Y et al, 1989, 1996; Pozzi et al, 1999, 2004; Tamoura et al, 2001; Katafuchi et al, 2003; Hotta et al, 2001; Moriyama et al, 2004; Lv et al, 2009, Manno et al, 2009], probably preferentially in individuals with preserved kidney function (eg, creatinine clearance above 50 mL/min) [Kobayashi et al, 1989, 1996; Pozzi et al, 1999, 2004]. Two randomised clinical trials demonstrated the benefit of the combination therapy with corticosteroids and ACE inhibitors on long-term follow-up in proteinuric IgAN patients. Lv et al (2009) evaluated the efficacy of the combination therapy versus ACE-inhibitors alone in a small number of IgAN patients with mild or moderate histologic lesions and with a follow-up period that was too short to evaluate the renal survival. Data demonstrated that the combination therapy reduced better the urinary protein excretion than the administration of ACE-inhibitors alone.

Even more, Manno et al enrolled 97 IgAN patients with moderate histologic lesion (see IgAN classification of F.P. Schena in Manno et al., 2007) daily proteinuria more than 1.0g and estimated GFR more than 50 ml/min/1.73m^2. Patients were randomly allocated to receive a 6-month course of oral prednisone plus ramipril or ramipril alone for the total duration of the follow-up (96 months). The combination of corticosteroids and ramipril provided less probability of renal disease progression because induced the decline of GFR and daily proteinuria. In an interesting recent meta-analysis of randomized and quasi-randomized controlled trials of corticosteroid treatment in IgAN against treatment without steroids, Zhou et al found that steroid therapy, especially long-term, is associated with a significant benefit in proteinuria and renal survival [Zhou et al, 2011].

In addition, the IgAN patients seemingly to benefit from prednisone therapy are those with nephrotic syndrome, little or no hematuria, preserved kidney function, minimal glomerular changes on light microscopy, and diffuse fusion of glomerular epithelial cell foot processes on electron microscopy. These histologic findings are characteristic of minimal change disease, and these patients behave accordingly, frequently developing a remission with corticosteroids and occasionally requiring cytotoxics for frequently relapsing proteinuria [Mustonen et al, 1983; Lai KN et al, 1986; Cheng et al, 1989]. Mesangial IgA deposits in these patients often disappear or are greatly reduced following steroid-induced remission [Cheng

et al, 1989]. Nephrotic syndrome can also occur with severe chronic IgAN and relatively advanced disease on renal biopsy. These patients do not seem to benefit from corticosteroid therapy alone [Mustonen et al, 1983; Lai KN et al, 1986].

3.3.2 Combined immunosuppressive therapy

Combined immunosuppressive therapy should only be attempted in patients with more severe active disease as defined by a more rapidly progressive clinical course and/or histologic evidence of severe active inflammation (eg, crescent formation). Early therapy is important because improvement is rare when the baseline serum creatinine concentration is greater than 3.0 mg/dL in the absence of crescentic glomerulonephritis [Alexopoulos E, 2004].

Corticosteroids plus azathioprine — Whether the addition of azathioprine provide any benefit to that of corticosteroids is still debatable. In a multicenter randomized trial by Pozzi et al in 207 patients with plasma creatinine ≤ 2.0 mg/dL and protein excretion >1.0 g/day, at a median follow-up of 4.9 years, there was no difference neither in renal survival time, defined as the time to 50% increase in plasma creatinine from baseline, nor in the decrease in proteinuria between patients who received corticosteroids alone or along with azathioprine (1.5 mg/kg /day for six months) [Pozzi et al, 2010]. However, in the above study, only a rather small percentage of patients were receiving either ACE inhibitors or ARBs, and even fewer patients were receiving statins. Most recently, Stangou et al published a randomized, yet underpowered study in 22 patients with IgAN and eGRF≥30mL/min, urine protein ≥1g/day, blood pressure <130/80mmHg, who failed to respond to previous treatment with renin-angiotensin system inhibitors and poly-unsaturated fatty acids administered for at least 6 months. During the 5th year after the diagnosis was made, the patients were randomized to receive either methylprednisolone alone, or methyl-prednisolone in combination with azathioprine, for 12months, while treatment with renin-angiotensin system inhibitors and poly-unsaturated fatty acids continued unchanged in both groups. Both, steroid treatment alone, or steroids in combination with azathioprine were found to be equally effective in reducing the severity of proteinuria and stabilizing renal function [Stangou et al, 2011].

Corticosteroids plus cyclophosphamide— There are evidence suggesting that patients with severe or progressive disease (eg, rising creatinine, nephrotic range proteinuria, and/or marked proliferation without crescents) who do not have significant chronic damage on kidney biopsy may benefit from combined immunosuppressive therapy with prednisone and cyclophosphamide. This regimen was evaluated in a study of Ballardie et al in 38 patients with IgAN and initially impaired renal function (baseline serum creatinine between 1.5 and 2.8 mg/dL, mean baseline protein excretion 4.0 to 4.5 g/day, no crescents on biopsy) that was declining at a relatively moderate rate (by at least 15% per year). Compared with the control group, the patients treated with combination therapy (prednisolone 40mg/day tapered to 10mg/day by two years plus low-dose cyclophosphamide for 3 months followed by low-dose azathioprine for at least 2 years) had a significant reduction in protein excretion during the first six months of therapy that persisted during follow-up (eg, reached 1.8 g/day in treatment group versus unchanged at 4.4 g/day in controls at one year). Renal survival was significantly higher in the treatment group at two (82 versus 68 percent) and five years (72 versus 6 percent). [Ballardie et al, 2002].

Uncontrolled reports in patients with crescentic, rapidly progressive glomerulonephritis suggest possible benefit from regimens similar to those used in idiopathic crescentic glomerulonephritis: intravenous pulse methylprednisolone followed by oral prednisone, intravenous or oral cyclophosphamide, and/or plasmapheresis [Welch et al, 1988; Lai KN et al, 1987a; Rocatello et al, 1995; McIntyre et al, 2001; Tumlin et al, 2003]. Corticosteroids may act in this setting by diminishing acute inflammatory injury rather than by correcting the abnormality in IgA production [Galla, 1995]. In a study by Rocatello et al, although a substantial clinical improvement was found with the administration of aggressive combination therapy (including pulse methylprednisolone, oral cyclophosphamide, and plasmapheresis) for 2 months in six patients with crescentic glomerulonephritis due to IgAN [Rocatello et al, 1995], yet cellular crescents failed to remit in repeat renal biopsy, whereas the disease continued to progress in half of the patients after therapy was discontinued. Limited data for a more prolonged course of aggressive immunosuppressive therapy (pulse methylprednisolone 15mg/kg/day for 3 days, followed by oral prednisolone 1 mg/kg/ day for 60 days gradually tapered, along with monthly iv cyclophosphamide (0.5 g/m^2) for 6 months) point towards a significant improvement in the serum creatinine and in protein excretion along with a significant reversion of cellular crescents and endocapillary proliferation [Tumlin et al, 2003].

These limited data suggest that patients with active crescentic glomerulonephritis who do not have significant chronic damage on kidney biopsy may benefit from therapy that initially includes intravenous cyclophosphamide. This is consistent with the benefit noted with a similar regimen in other forms of crescentic glomerulonephritis.

3.3.3 Other immunosuppressive agents

Cyclosporine — Cyclosporine has been investigated in small studies, and resulted in reduced proteinuria. In a recent study, Shin et al reported a significant benefit of cyclosporine therapy in proteinuria reduction and renal pathology regression in 14 children with IgAN and near normal creatinine clearance [Shin et al, 2010]. However, there are important issues of concern regarding its use in IgAN treatment, with most important the associated nephrotoxicity, which can lead to harmful effects on renal function [Lai KN et al, 1987b; Cattran, 1991], as well as the rapid disease relapse after drug discontinuation.

Mycophenolate mofetil — Small, prospective placebo-controlled randomized trials of mycophenolate mofetil (MMF) in which the patients were also treated with ACE inhibitors, have produced conflicting results, ranging from no benefit [Maes et al, 2004; Frisch et al, 2005] to a reduction in proteinuria and decrease in rate of decline of GFR [Tang et al, 2010]. A short course (< 6 months) of MMF in patients with persistent proteinuria (>1.5 g/day) and well-maintained renal function (serum creatinine <1.5 mg/dL) in addition to ACE inhibitor/ARB therapy may be considered in patients with well-preserved renal histology on biopsy. Current evidence does not support the use of MMF in patients with advanced disease (serum creatinine >2.5 to 3 mg/dL) [Cattran & Appel, 2011].

3.4 Other possible interventions

Tonsillectomy — Several retrospective studies have suggested that tonsillectomy, usually in combination with some immunosuppressive therapy, is associated with improved renal survival among patients with relatively mild renal injury [Hotta et al, 2001; Xie et al, 2003; Komatsu et al, 2008]. These non-randomized studies provide some evidence that

tonsillectomy may be effective in inducing remission of proteinuria and hematuria in patients with IgAN (ie, proteinuria >500 mg/day). However, there are no randomized trials of tonsillectomy in IgAN, the design of the above studies precludes any definitive conclusions regarding the overall efficacy of tonsillectomy in IgAN, while other studies reported negative results [Rasche et al, 1999].

Low antigen diet — The rationale for using a low antigen diet in IgAN, ie diet free of gluten, dairy products, eggs, and most meats, is that dietary macromolecules may be responsible for activating the mucosal IgA system. When given to 21 consecutive patients with IgAN, protein excretion fell markedly in all 12 patients whose baseline rate was more than 1g/day. In addition, repeat renal biopsy showed significant reductions in mesangial IgA and complement deposition and mesangial cellularity [Ferri et al, 1993]. However, the benefits in the above study have not been confirmed and a report using a gluten-free diet alone for several years was unable to document improvement in either proteinuria or renal function despite a reduction in the level of circulating IgA-containing immune complexes [Coppo et al, 1990].

Intravenous immune globulin — At least part of the rationale for intravenous immune globulin (IVIG) therapy in IgAN comes from the observation that a partial IgG deficiency, which could be corrected with IVIG, may predispose to infections that trigger flare-ups of the renal disease [Rostoker et al, 1989, 1994]. Despite the promising findings from two small studies with the administration of high-dose IVIG in severe IgAN, characterized by heavy proteinuria and a relatively rapid decline in GFR (reduction in protein excretion, prevention of GFR decline, decreased inflammatory activity and IgA deposition on repeat renal biopsy) [Rostoker et al, 1994; Rasche et al, 2006], these findings need to be confirmed by larger studies.

4. Conclusion

Genetic susceptibility for IgAN exhibits a complex genetic pattern. To date various groups and the European IgAN Consortium have identified several loci. The extensive genetic studies under way with the use of delicate, high-throughput technologies will give information on the pathogenic mechanisms of IgAN. In addition, these data may indicate potential targets for screening, prevention and early diagnosis of the disease and more appropriate and effective treatment.

Summarizing the most updated data, following are concise treatment guidelines:

- Patients with isolated hematuria, no or minimal proteinuria (<0.5g/day), and a normal GFR need no treatment. Such patients should be periodically monitored at 6 to 12 month intervals to assess disease progression that might warrant therapy.
- Patients with persistent proteinuria (>0.5 or >1 g/day), should be treated with angiotensin inhibition (ACE inhibitor or ARB), with a target of a minimum reduction in protein excretion of at least 50 to 60% from baseline values and a goal protein excretion of <0.5 or <1 g/day.
- All patients who meet criteria for angiotensin inhibition may also be considered as candidates to receive fish oil.
- Patients with persistent nephrotic syndrome and/or chronic kidney disease who have dyslipidemia should be treated with a statin, primarily for cardiovascular protection.
- Corticosteroid therapy for at least 6 months is indicated in the following cases:

a. In patients with acute onset of nephrotic syndrome and minimal changes beyond mesangial IgA deposits on renal biopsy (as in patients with minimal change disease).
b. In patients with moderate renal lesions (eg, hematuria with persistent proteinuria >1 g/day and/or GFR>50ml/min) in association with ACE inhibitors or ARBs.

- For patients with severe disease at baseline (defined as initial serum creatinine >1.5 mg/dL) or progressive disease (eg, increasing serum creatinine and/or protein excretion) who do not have significant chronic damage on kidney biopsy, therapy with oral prednisone and cyclophosphamide is recommended.

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6

Rapidly Progressive Glomerulonephritis

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1. Introduction

Rapidly Progressive Glomerulonephritis are a group of renal diseases which are still posing serious threat to human health and survival. They are all characterised by acute and rapid deterioration of renal function. Renal biopsy reveals extracapillary glomerulonephritis, most frequently circumferential and diffuse, and immunofluorescence findings continue to represent the most important clue toward precise diagnosis. In the last years, with the development of new technologies and more targeted animal models, several discoveries have been made, that can help in better understanding the pathogenesis and perspectively defining new molecular targets for novel therapies, which are still required to improve the prognosis of these patients.

2. Definition

Introduced for the first time by Ellis in 1942 (Ellis, 1942), the term Rapidly Progressive Glomerulonephritis (RPGN) clinically describes a heterogeneous group of glomerulonephritis characterised by worsening of kidney function that, if not adequately and timely treated, rapidly progresses to end stage renal disease. From the pathological point of view, these diseases are classified as extracapillary or crescentic glomerulonephritis, generally showing extracapillary proliferation in more than 50% of glomeruli. Besides renal biopsy, which is mandatory to make the diagnosis and guide therapeutic decisions, clinical symptoms, biochemical exams, and the observation of the urinary sediment are relevant to the diagnostic process.

Observation of the urinary sediment in the acute phase of disease allows to detect in the vast majority of cases marked erythrocytic cylindruria, mild to moderate leukocyturia, presence of tubular epithelial cells and tubular epithelial cell casts. Fatty casts and leukocyte casts can be detected in about one third of cases (Fogazzi, 2009). Progressive disappearance of these features follows successful therapeutic intervention, and their reappearance frequently precedes disease relapses, making the urinary sediment an important exam not only at diagnosis but also during the patient's follow-up.

Despite the amelioration of prognosis obtained with introduction of high doses of steroids, immunosuppressive agents, and plasma exchange, these diseases are still life-threatening and a high percentage of subjects have a poor renal outcome.

3. Classification

Along the years, different schemes for classifying RPGN have been proposed. Among them, the classification which is still largely accepted and mostly utilised was proposed by Couser (Couser, 1988), and defines disease groups on the basis of immunofluorescent findings. Clinical features and haematological exams are as well very important in reaching a precise diagnosis (Table 1).

Light microscopy	Necrotising extracapillary or pure extracapillary glomerulonephritis		
Immuno-fluorescence	Linear IgG staining	None or minimal deposits	Granular deposits
Hemato-chemical exams	Circulating anti-GBM antibodies	ANCA	Autoantibodies Complement components
Clinical features	Lung haemorrhage	Systemic symptoms	Systemic symptoms
Diagnosis	Anti-GBM disease Goodpasture's syndrome	Wegener's granulomatosis Microscopic polyangiitis Renal limited vasculitis Churg-Strauss syndrome	Post-streptococcal GN Post-infectious GN SLE nephritis IgA GN/HS syndrome Primary MPGN Other primary GN

Table 1. Classification of RPGN according to immunofluorescence findings

Linear deposition of IgG along the glomerular basement membrane associated to circulating anti-GBM antibodies allow the diagnosis of anti-GBM disease. If pulmonary haemorrhage is present, the diagnosis becomes of Goodpasture's Syndrome.

When immunofluorescence on renal biopsy material demonstrates absence of immune deposits or scanty immune deposition in the glomerulus, in association to the presence of circulating ANCA antibodies, a diagnosis of pauci-immune ANCA-associated renal vasculitis is made. In these cases, necrotising crescentic GN can be associated to clinical symptoms of systemic vasculitis. A prevalent involvement of the upper respiratory tract is highly suggestive for a diagnosis of Wegener's Granulomatosis, whereas the presence of only general systemic symptoms, such as fever, is highly suggestive of Renal Limited Vasculitis. Rarely presenting as RPGN, Churg-Strauss syndrome is diagnosed when asthma and increased circulating eosinophils are present.

Importantly, there is a percentage (10-30%, according to the literature) (Chen, 2009) of renal vasculitis which are negative for ANCA antibodies. A part from subjects with circulating AECA (anti-endothelial cell antibodies), that according to a recent study may be present in about 50% of cases (Cong, 2008), diagnosis is mainly based on clinical and biopsy findings and exclusion of other causes.

Among cases of RPGN with granular immune deposition in glomeruli, the most frequent are Post-Streptococcal/post-infectious nephritis and extracapillary GN observed in cases of Lupus Nephritis. In these diseases, besides clinical symptoms, diagnosis is made thanks to the presence of autoantibodies (ASLO and anti-DNAseB in PSGN, and ANA in SLE).

RPGN can also complicate any primary form of GN, most frequently MPGN and IgA nephropathy. A part from the immunofluorescence findings, diagnosis is also guided by clinical and biochemical exams, such as the evaluation of complement components for the diagnosis of MPGN, and the presence of purpura in cases of Henoch-Schoenlein syndrome.
It remains to be said, as a word of caution, that it is not infrequent to find a completely negative immunofluorescence or immunofluorescence findings particularly difficult to interpret in cases with very severe extracapillary proliferation or necrotic lesions, because of the consequent compression or destruction of the glomerular tuft.

4. Morphological findings

Though the common element characterising this group of diseases is the presence of extracapillary proliferation, morphological findings can be very diverse, according to the stage of the disease and also to the underlying disease, likely reflecting the different pathogenesis of glomerular lesions.

4.1 Anti-GBM nephritis and Goodpasture's Syndrome

By light microscopy, variable degrees of necrotising extracapillary lesions can be observed, which range from focal and segmental to global and diffuse (Fig 1).

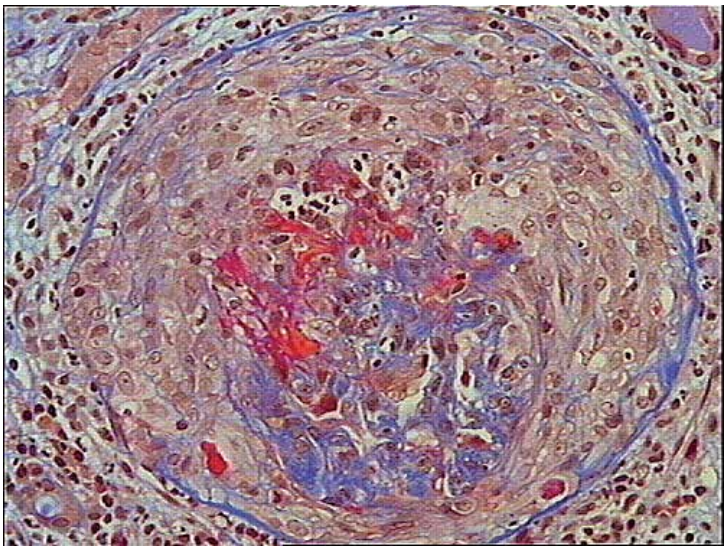

Fig. 1. Anti-GBM nephritis. A large area of necrosis of the glomerular tuft is surrounded by a circumferential crescent. Inflammatory cells surround the glomerulus.

Extracapillary lesions are composed by monocytes, epithelioid macrophages and epithelial cells. Glomeruli not involved by these lesions and the parts of the glomerulus not affected by necrosis can present normal features, but more commonly they show mild to moderate mesangial proliferation, some degree of mesangial matrix expansion, and increased leukocyte infiltration. Intraglomerular inflammatory cells are mostly monocyte-macrophages with variable numbers of T-lymphocytes (Ferrario, 1985; Bolton, 1987).
In about 50% of cases, multinucleated giant cells can be detected either in the crescent or in the periglomerular inflammatory infiltrate, forming the so-called granuloma-like lesions.

Generally well corresponding to the degree of glomerular damage, the tubulointerstitium shows variable extents of tubular atrophy, oedema, and interstitial inflammation. If the biopsy is timely performed, no interstitial fibrosis is observed.
Vascular lesions are not common, though necrotising arteritis and thrombotic microangiopathy have been reported occasionally in the literature (Dean, 1991; Stave, 1984).
Immunofluorescence is diagnostic, with the linear deposition of IgG along the glomerular basement membrane. This aspect can be best appreciated in glomeruli not particularly damaged, whereas it is more difficult to be seen when the glomerular capillary is largely destroyed by necrosis or compressed by extensive cellular crescents.
A combination of IgG and C3 can also be found, as well as a linear deposition of IgA or IgM (Gris, 1991; Peto, 2011) has been reported.
Linear IgG staining can be also detected along the Bowman's capsule, and along the tubular basement membranes. Additionally, the fibrinogen antiserum strongly stains the areas of necrosis in the tuft and within the crescents.

4.2 ANCA-associated renal vasculitis

Irrespective of diagnosis, identical renal microscopy features can be observed in Wegener's granulomatosis, microscopic polyangiitis, and renal limited vasculitis. Necrotising glomerulonephritis and extracapillary proliferation are the renal hallmark of these diseases, and can be found with variable degrees of association. Necrosis can be present alone in cases when renal biopsy is early performed, but more commonly is associated with segmental areas of extracapillary proliferation. Particularly compromised glomeruli show instead large areas of necrosis of the tuft and circumferential crescents, with frequent rupture of the Bowman's capsule and intense periglomerular leukocyte infiltration, so that the limit of the glomerular area is no more distinguishable (Fig 2), and the area has the aspect of a granulomatous reaction.

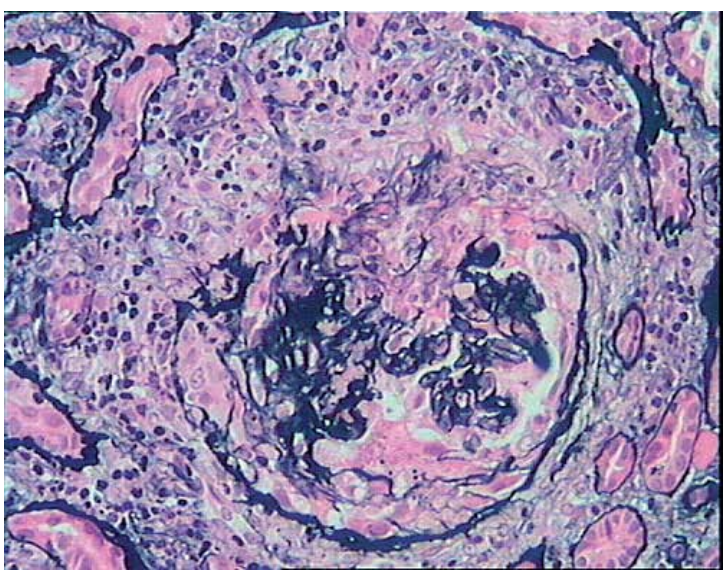

Fig. 2. ANCA-associated vasculitis. A large rupture of the Bowman's capsule can be observed.

Extracapillary and granuloma-like lesions are mainly made by inflammatory cells, mostly acutely activated monocyte-macrophages (Fig. 3, Fig 4) (Rastaldi, 1996; Rastaldi, 2000), whose entrance into the glomerulus seems to be facilitated by the de novo expression of the adhesion molecule VCAM-1 (Fig 5).

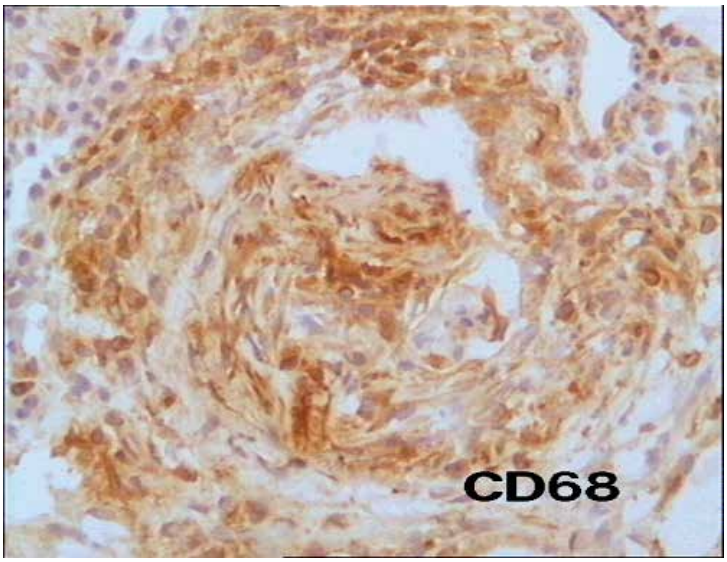

Fig. 3. ANCA-associated vasculitis. Glomerular damage and periglomerular granuloma-like reaction are mainly composed by monocyte-macrophages, as witnessed by the positivity for the marker CD68.

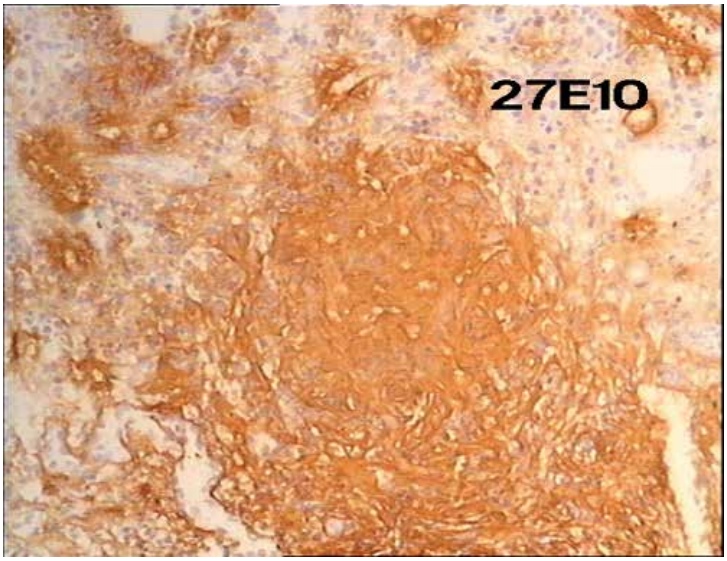

Fig. 4. ANCA-associated vasculitis. A vast glomerular granuloma-like reaction is strongly positive for the marker of acutely activated monocyte-macrophages 27E10.

The acute activation of cells composing the glomerular granuloma-like reaction differentiates this type of alteration from other kind of tissue granulomas, where acute macrophages have not been found (Bhardwaj, 1992).
Differently from those observed in other diseases, monocyte-macrophages present in renal vasculitis are proliferating cells, as we have shown by staining with antibodies against PCNA (Fig 6) and Ki67 (Rastaldi, 2000).

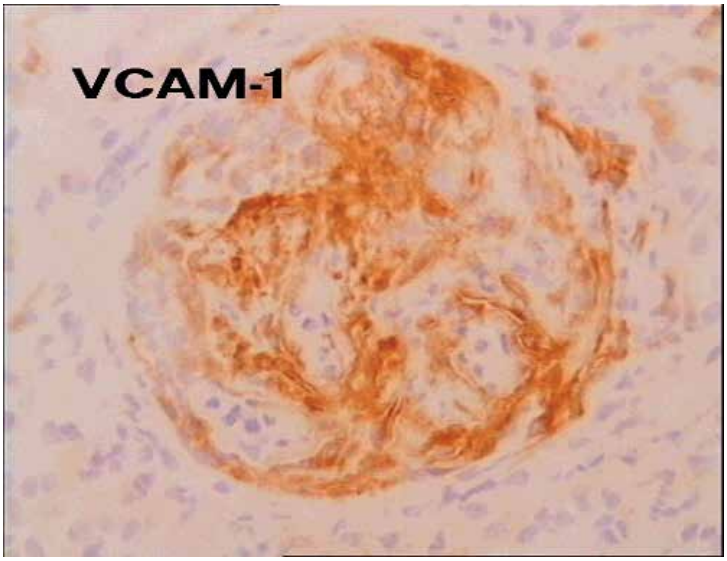

Fig. 5. ANCA-associated vasculitis. VCAM-1 de novo expression in damaged areas of the glomerulus.

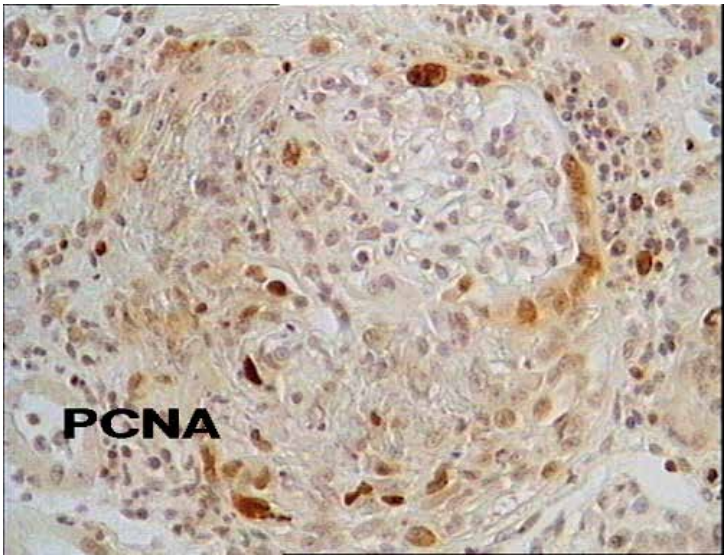

Fig. 6. ANCA-associated vasculitis. PCNA labels numerous cells in and around the glomerulus.

Depending on the timing of renal biopsy, glomeruli can be affected by active lesions or by more sclerotic alterations. It is not infrequent to observe both types of lesions in the same renal biopsy and even in the same glomerulus (Fig 7).

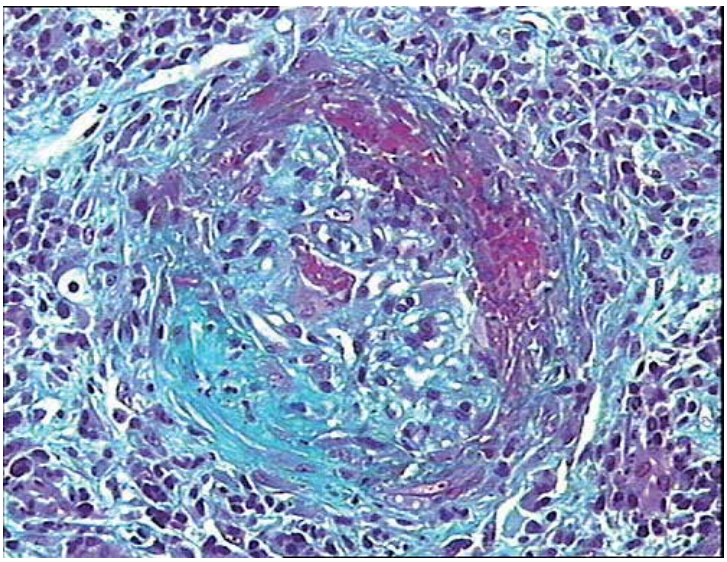

Fig. 7. ANCA-associated vasculitis. The glomerulus shows evident necrotic damage in the upper part of the crescent, whereas the lower part is already fibrous.

Besides periglomerular infiltrates, focal perivascular inflammatory cells are frequently detected in the interstitium, and a diffuse interstitial leukocyte infiltration is also present, whose degree well corresponds to the extent of glomerular damage. Interstitial cells are mainly monocyte-macrophages and T-lymphocytes.
Prevalence of eosinophils, in association to the clinical symptoms of asthma, and increased numbers of circulating eosinophils, stand for a diagnosis of Churg-Strauss syndrome.
By definition, in ANCA-associated renal vasculitis immune deposits are absent or few and scattered, hence the term pauci-immune glomerulonephritis. Instead, the fibrinogen antiserum strongly stains areas of necrosis of the tuft and fibrin deposits into the crescents.

4.3 Post-infectious glomerulonephritis

Either post-streptococcal and other post-infectious glomerulonephritis can present with a rapidly progressive course, which is indicative of a poor prognosis.
Several systemic infections, especially occult, such as infective endocarditis, infected atrio-ventricular shunts, visceral abscesses, and infected vascular prostheses, can be at the origin of RPGN. Blood levels of complement can be reduced.
By light microscopy necrotising lesions, but more frequently extracapillary damage without necrosis of the tuft are observed.
Especially in case of streptococcal infections, the presence of numerous intraglomerular granulocytes (so-called glomerular exudative lesions) (Fig 8) is useful for diagnostic purposes.

In post-streptococcal GN, granular IgG and C3 deposits are the most common finding. IgG, C3, and IgM deposits can be observed in other post-infectious GN, at various locations, but primarily subendothelial and mesangial.

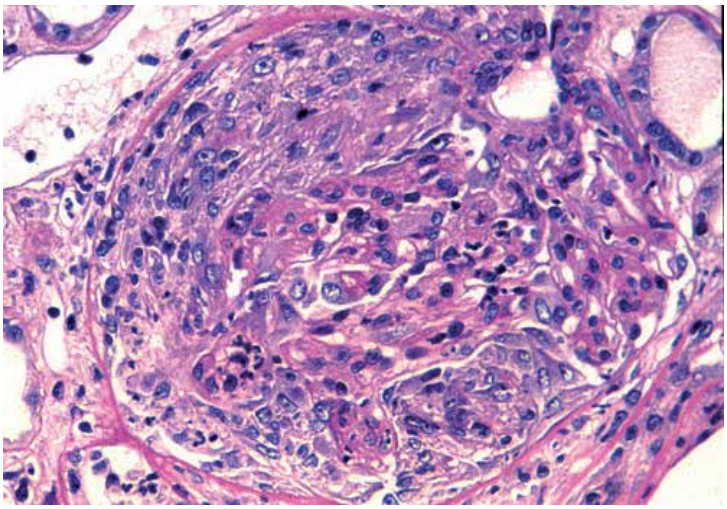

Fig. 8. Post-streptococcal GN. Numerous granulocytes can be observed in the glomerular tuft and in the crescent.

4.4 RPGN complicating primary and secondary glomerular diseases

Though rarely, any primary or secondary glomerular diseases can be complicated by a rapidly progressive course and display necrotising crescentic glomerulonephritis at light microscopy. Very recently, a report has shown for the first time the appearance of RPGN complicating the course of AL amyloidosis (Crosthwaite, 2010). Cases of association of primary or secondary glomerulonephritis and anti-GBM disease or renal vasculitis have also been published, as well as cases of association of anti-GBM disease and ANCA-positive renal vasculitis (Curioni, 2002; O'Connor, 2010). Diagnosis in these patients requires skilful and careful analysis of clinical features, renal biopsy findings, and hematochemical exams.

4.4.1 IgA nephropathy and Henoch-Schonlein purpura

Less than 10% of patients with primary IgA nephropathy or Henoch-Schonlein syndrome have been reported with a truly rapidly progressive course (Ferrario, 1997).

Clinical features of cutaneous purpura or abdominal and joint pain, accompanied by the finding of a small vessel leukocytoclastic vasculitis, most frequently detected in skin biopsies, help in making a diagnosis of systemic disease.

Focal segmental or global and diffuse necrotising and extracapillary lesions of the glomerulus can be found, or extracapillary lesions can be present without necrosis of the glomerular tuft (Fig 9), which always presents variable degrees of mesangial proliferation and expansion of the mesangial matrix.

Immunofluorescence shows prevailing IgA mesangial deposits, possibly in combination with IgG and C3 deposition, especially in the systemic disease.

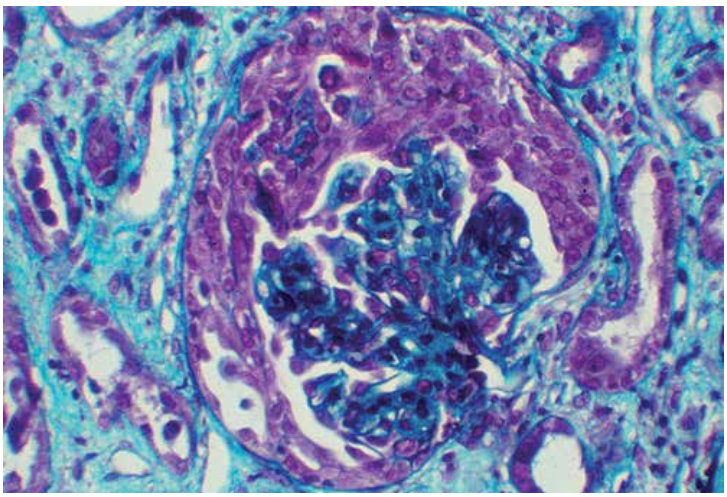

Fig. 9. Primary IgA nephropathy. A circumferential crescent surrounds a glomerulus affected by mesangial proliferation and mesangial expansion.

4.4.2 Systemic lupus erythematosus

Among the histological classes of SLE nephritis (Weening, 2004), RPGN is more frequently observed in classes III and IV. In these cases the occurrence of antineutrophil cytoplasmic antibodies is not uncommon and is thought to contribute to the development of necrotising and crescentic glomerular lesions (Sen, 2003).

Extensive extracapillary proliferation has been rarely reported (Fig 10), whereas segmental necrotising extracapillary alterations are a rather common finding, but not always translate in a RPGN clinical phenotype.

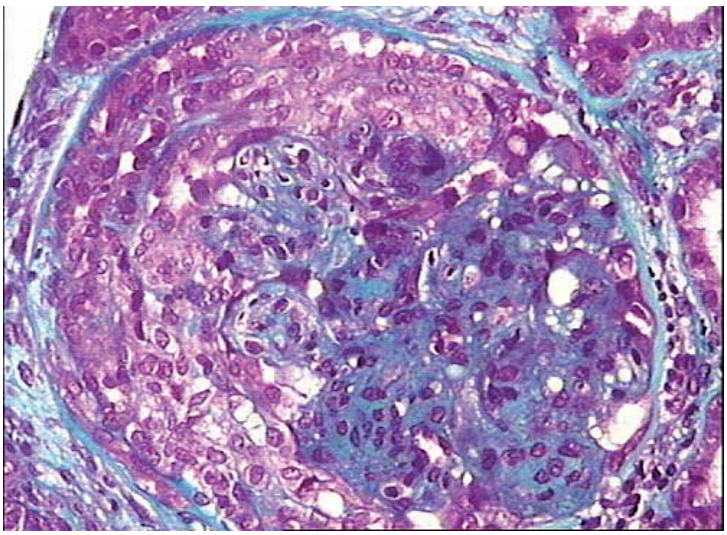

Fig. 10. Rapidly progressive class IV SLE nephritis. A circumferential crescent surrounds a glomerulus affected by intense intracapillary proliferation, mesangial expansion, and leukocyte infiltration.

Immunofluorescence has the typical findings of lupus nephritis, according to the class of disease, with frequent "full house" deposition, and fibrinogen positivity in necrotic areas and crescents.

5. Pathogenesis and experimental models

In recent years, thanks to the possibilities offered by molecular modelling, genetic studies, and the generation of novel animal models better reproducing human disease features, important advances have been made in understanding pathogenetic mechanisms underlying certain forms of RPGN, especially anti-GBM nephritis and ANCA-associated renal vasculitis.
Instead, it continues to be less clear why a rapidly progressive course can complicate virtually any type of primary and secondary glomerulonephritis.

5.1 Anti-GBM nephritis and Goodpasture's disease

The seminal discovery in understanding the pathogenesis of the disease was the identification of the antigen that causes production of pathogenic autoantibodies (Saus, 1988).
Thereafter, injection of the recombinant antigen, i.e. the noncollagenous domain (NC1) of the alpha3chain of collagen type IV, was shown to induce a severe glomerulonephritis in Wistar-Kyoto rats (Sado, 1998), hence proving a direct relationship between the self-antigen sustaining autoantibody production and the disease.
More recently, a second class of autoantibodies has been described, which are specific for the alpha5NC1 domain, occur in 70% of affected patients, and seem to be associated with a worse renal prognosis (Pedchenko, 2010).
In the normal glomerular basement membrane, the NC1 domain is assembled in alpha345NC1 hexamers, whose quaternary organisation has been shown in a three-dimensional model (Vanacore, 2008) as an ellipsoid-shaped structure composed by two NC1 trimers joined at the base by hydrophobic and hydrophilic interactions and reinforced by sulfilimine bonds. This crosslinked alpha345NC1 hexamer is inert to antibody binding. Anti-GBM antibodies in fact can bind only to dissociated monomer and dimer subunits that form after alteration of the hexamer and expose pathogenic neoepitopes. This explains why passive transfer of antibodies to the mouse, where hexamers in the GBM are completely crosslinked, does not result in glomerulonephritis (Luo, 2010).
The major epitopes within the alpha3 and alpha5 subunits have been identified as well, and named EA-alpha3, EA-alpha5, and EB-alpha3 (Netzer, 1999; Hellmark, 1999; Pedchenko, 2010).
Several questions, primarily regarding the causes of hexamer alteration that induce epitope exposure and antibody production, need to be answered. At present, the most accredited hypothesis is that environmental factors act in genetically predisposed subjects, leading to epitope alteration and antibody formation.
As for genetic predisposition, positive and negative associations with HLA molecules have been found, especially with the MHC class II HLA-DRB1*1501 allele (Yang, 2009), which is strongly associated to anti-GBM disease.
A number of experimental data are in favour of a role played by FcγR gene and the complement system, though their precise role in humans is still unclear.

Instead, several experimental models implicate T-cell mediated immunity in the pathogenesis of anti-GBM disease, which is based on the following findings. In rats, anti-GBM disease can be induced by injecting alpha3(IV)NC1-specific CD4+Tcells (Wu, 2002). Anti-CD8 monoclonal antibodies reduce disease severity and antigen-specific CD8+Tcell clones have been found in diseased patients (Reynolds, 2002). Invariant natural killer cells (iNKT) could have a role as well, because the disease has a worse course in iNKT cell-deficient mice (Mesnard, 2009). Finally, mice deficient in IL-23, which is important for the maintenance of Th17 cells, the CD4+Tcell subset producing IL17, are protected from anti-GBM disease (Ooi, 2009).

5.2 ANCA-associated renal vasculitis

The discovery of ANCAs (Falk, 1988) radically changed not only the diagnosis of small vessel vasculitis, but also introduced an important element for the study of the etiology and pathogenesis of this group of diseases. Major ANCA autoantigens are two proteins contained in azurophil granules of neutrophil granulocytes, MPO and PR3, which are mainly expressed during neutrophil development at the myeloblast and promyelocytic stage (Cowland, 1999). They are aberrantly expressed in mature neutrophils of ANCA patients, whereas are silenced in mature neutrophils of healthy subjects (Yang, 2004).

In vivo first evidence for a pathogenetic role of ANCA was demonstrated by injection of anti-MPO antibodies or anti-MPO lymphocytes, causing a pauci-immune focal necrotising extracapillary glomerulonephritis (Xiao, 2002). Subsequent research then showed that in this model neutrophil granulocytes are required, because mice depleted of neutrophils do not develop the disease, and disease worsening is obtained by priming neutrophils using a pro-inflammatory stimulus (Xiao, 2005). The model has been also useful in investigating the role of the alternative complement pathway, because the disease does not occur in C5 or Factor B null mice, but it fully develops in C4-KO animals (Xiao, 2007).

In an additional model, MPO-KO mice were first immunised with mouse MPO, determining production of anti-MPO antibodies. These mice having circulating anti-MPO antibodies were then irradiated and subsequently transplanted with MPO-wild type or MPO-KO bone marrow cells. A pauci-immune necrotising-crescentic glomerulonephritis developed only in mice engrafted with MPO-wild type cells, indicating the requirement for bone marrow derived cells in disease development (Schreiber, 2006).

5.3 Cells involved in crescent formation

Along the years, composition of glomerular extracapillary proliferation has been, and still remains, the object of intense investigation and discussion.

Though the exact mechanism/s of crescent formation remain elusive, novel animal models have recently added important information, that will lead to further clarification of the molecular pathways involved and the potential identification of possible novel therapeutic targets.

A word has first to be spent in stating that, morphologically speaking, extracapillary proliferation is a heterogeneous phenomenon. It has been shown by several investigators that presence or absence of necrosis of the glomerular capillary is relevant to the type of crescent. When necrosis is present, the crescent is more inflammatory, and mainly formed by monocyte-macrophages. In absence of tuft necrosis, the crescent has more epithelial and less inflammatory features.

If the presence of inflammatory cells and epithelioid macrophages has never been questioned either in animal models and in human disease, opposite data have been obtained when attempting to define the epithelial cell composition.

Until some years ago, both experimental and human studies aiming to study the cells contained in the crescents were mostly based on morphological findings and immunostaining. The conflicting results produced by these studies were due not only to the specific type of experimental model or of human disease under analysis, but especially to a dysregulated phenotype with loss of specific markers. In fact, both podocytes and parietal epithelial cells are likely to change their original resting phenotype once they start proliferating and filling the Bowman's space.

The advent of novel experimental models, though not generating unifying and conclusive data, is providing more convincing proofs of the participation of either podocytes or parietal epithelial cells, based on tagged expression of specific molecules.

Convincing evidence of podocyte contribution to crescent formation has been shown in a podocyte specific mouse model of Vhlh gene knockout (Ding, 2006). These mice showed rapidly progressive glomerulonephritis by 4 weeks of age and died by terminal renal failure after 3-4 weeks. Histology displayed a crescentic glomerulonephritis, and podocytes expressing tagged-ZO1 were found into the crescents. A part from showing podocyte participation in crescent formation, the model also identified a novel pathway potentially operating in extracapillary glomerulonephritis; deletion of Vhlh in fact resulted in stabilisation of hypoxia inducible factor-aplha (HIF1alpha) and consequent upregulation of target genes, among them the chemokine receptor CXCR4. Further, podocyte-specific expression of CXCR4 was sufficient to induce podocyte proliferation and crescent formation, and CXCR4 positivity was observed in glomeruli of human biopsies with necrotising extracapillary lesions, suggesting that the VHLH-HIF-CXCR4 pathway may have functional relevance also in humans.

The contribution of parietal epithelial cells to crescent formation has been recently shown in a mouse model where a construct containing 3 kb of the human podocalyxin (*hPODXL1*) 5′ flanking region and 0.3 kb of the rabbit *Podxl1* 5′ untranslated region were used to drive expression of rabbit podocalyxin, and transgene expression was detected exclusively within PECs but not in podocytes. In this model, injection of nephrotoxic serum caused extracapillary glomerulonephritis and cells within crescents could be clearly identified as of parietal origin (Smeets, 2009, a).

As a final consideration, recent work has demonstrated that the Bowman's capsule contains renal progenitors mainly located at the urinary pole of the glomerulus (Ronconi, 2009). If it is true that these cells are able to regenerate either tubular cells and podocytes, then their participation to crescent formation can be viewed as the pathological consequence of a tentative to repair glomerular damage in the course of inflammatory conditions (Smeets, 2009, b).

6. Conclusion

RPGN still constitute a threat for human health and survival. Despite numerous improvements in understanding the pathogenesis of these diseases, numerous questions still remain unanswered and will need clarification before providing targeted, pathway-based, novel therapeutics.

7. Acknowledgment

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Part 2

Infectious Glomerulopathies and Related Disorders

7

Post-Infectious Glomerulonephritis

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1. Introduction

This chapter will provide a comprehensive review of post-infectious glomerulonephritis focusing in particular on the changing epidemiology and long term outcome.
The immunological response of the kidney to an insult results in glomerulonephritis. The insult can result from a large number of conditions, both infectious and non-infectious. Regardless of the initial insult, the outcome is similar in terms of pathology and clinical symptoms. The most common and most-studied cause is post streptococcal Glomerulonephritis (PSGN). A list of causes is presented in Table 1.

INFECTIOUS
Bacterial: *Streptococcal (PSGN),* methicillin-resistant *Staphylococcus aureus* (MRSA), pneumococcal pneumonia, typhoid, secondary syphilis, meningococcemia, infective endocarditis, shunt nephritis, sepsis
Viral: Hepatitis B, infectious mononucleosis, mumps, measles, varicella, vaccinia, echovirus, parvovirus, and coxsackievirus
Parasitic: Malaria, toxoplasmosis
Fungal: cryptococccus imitis
NON INFECTIOUS
Primary glomerular diseases: Membranoproliferative GN (MPGN), IgA nephropathy, mesangial proliferative GN
M*ultisystem systemic diseases:* Systemic lupus erythematosus, vasculitis, Henoch-Schönlein purpura, Goodpasture syndrome, Wegener granulomatosis
Miscellaneous: Gullian-barre syndrome, pertussis-tetanus vaccine, serum sickness

Table 1. Causes of post-Infectious Glomerulonephritis

2. Burden of disease and changing epidemiology

Of all the bacterial pathogens, group A streptococcus (GAS) causes the widest range of illness in humans. These illnesses range from local infections of the skin and throat (impetigo and pharyngitis respectively) to invasive infections as well as the significant post-infectious immunological sequelae such as the well documented acute rheumatic fever and acute post-streptococcal glomerulonephritis and the lesser known PANDAS (Paediatric Autoimmune Neuropsychiatric Disorder Associated with Streptococcus) [1].

While global estimates of the burden of disease due to GAS infections are difficult to get, some estimates have been reported which are based on published population studies. The estimate of 500 000 deaths per year due to GAS makes it a major human Pathogen [2]. This minimal estimate places GAS infections as less common than HIV, *Mycobacterium tuberculosis*, *Plasmodium falciparum* and *Streptococcus pneumonia* but as common as rotavirus, measles, *Haemophilus influenzae* type b and hepatitis B as a cause of global mortality [2]. In addition there is the long-term morbidity associated with GAS infections.

The global burden of severe group A streptococcal disease is concentrated largely in developing countries and within disadvantaged populations living in developed countries such as Aboriginal Australians. The review of population based studies estimated the prevalence of severe GAS disease at a minimum of 18·1 million cases, with 1·78 million new cases each year [2]. The greatest burden was due to rheumatic heart disease, with a prevalence of at least 15·6 million cases, with 282 000 new cases and 233 000 deaths each year. The burden of invasive GAS diseases was found to be unexpectedly high, with at least 663 000 new cases and 163 000 deaths each year. In addition, there were more than 111 million prevalent cases of GAS pyoderma, and over 616 million incident cases per year of GAS pharyngitis [2]. The review estimated that over 470 000 cases of acute post-streptococcal glomerulonephritis occur annually, with approximately 5000 deaths (1% of total cases), 97% of which were in less developed countries [2].

The global incidence of acute PSGN was estimated at 472,000 cases per year, of which 456,000 (96.6%) occurred in less developed countries [3, 4]. A similar distribution of higher incident cases in less developed countries is also reported in a review of population based studies [2]. A review of 11 population-based studies documenting the incidence of acute PSGN in children from less developed countries or those that included substantial minority populations in more developed countries, estimated 24·3 cases per 100,000 person as the median PSGN incident rate [2]. The same review estimated an incidence in adults of 2 cases per 100,000 person-years for developing countries and 0.3 per 100,000 person-years in developed countries [2]. Due to the paucity of data in adults with PSGN, the estimate for developing countries was based on data from Kuwait and for developed countries on data from Italian Biopsy Registry and the most conservative estimates were reported [2]. Another recent study described a slightly higher incidence of 9.5-28.5 cases per 100,000 person-years in developing countries [5]. These rates represent only the clinical cases. When asymptomatic cases are screened for in household contacts and family members, asymptomatic disease is reported to be 4-19 times greater [5-7].

PSGN can occur sporadically or epidemically. The changing pattern of PSGN over the last few decades has been described in studies from Florida [8] and Singapore [9]. The overall incidence of PSGN has decreased over the last few decades [10]. The reasons for this decline have not been clearly delineated but possible reasons are the widespread use of antibiotics, changes in etiological pathogens, altered susceptibility of the host, better health care delivery and improved socioeconomic and nutritional conditions [8-10]. Nevertheless, epidemics and clusters of cases continue to appear in several regions of the world and sporadic cases of PSGN account for 21% (4.6–51.6%) of children admitted to the hospital with acute renal failure in developing countries [5]. Although epidemic PSGN has decreased dramatically and is almost unknown in the developed world, epidemics of PSGN continue to occur in the developing world, mainly in Africa, West Indies and the Middle East, as well as in Indigenous people living in the developed world [11]. Epidemics are described mainly in “closed” communities, clusters of densely populated

dwellings or areas with poor hygienic conditions, both urban and rural. These conditions are especially prevalent in Aboriginal peoples of Australia living in remote communities, in settings with a high burden of infectious disease and overcrowding [11, 12]. Sporadic cases of PSGN occur in the Northern Territory of Australia each year with outbreaks every 5-7 years [13]. PSGN in New Zealand occurs mostly in children of Pacific Island and Maori heritage (>85% of cases) [14]. Sporadic cases of PSGN also continue to be reported from all over the world.

The rates are higher in children than in adults, and PANDAS is described solely in the paediatric age group. PSGN primarily affects children, aged 2-12 years, with clinically detectable cases estimated to be 10% of children with pharyngitis and up to 25% of children with impetigo during epidemics [15, 16]. Children account for 50-90% of epidemic cases, with 5-10% occurring in people > 40 years and 10% in those below 2 years of age [5]. PSGN is uncommon below 3 years of age and rarely seen below 2 years [17]. This low incidence of PSGN is likely to be due to the decreased immunogenicity of children below 2 years of age, for although GAS pharyngitis is uncommon in children of this age, GAS skin infections are common. Decreased immunogenicity likely results in less robust immune complex formation thus leading to less PSGN [18].

Males have more symptomatic disease, but this difference is no longer present when symptomatic and asymptomatic cases are considered together [19]. Spontaneous recovery occurs in almost all patients, including those who develop renal insufficiency during the acute phase [16], with 1% of all paediatric patients developing renal insufficiency.

There are no large scale published studies of bacterial infections associated with GN other than streptococcal infection. These are limited to small cases series and individual cases reports. The most common of these are related to staphylococcal infections, both methicillin sensitive [20] and methicillin resistant [21, 22]. A case series of 10 cases, age range 21-65 years, of MRSA-associated glomerulonephritis reported polyclonal increases of IgA and IgG and massive T cell activation and suggested the role of the enterotoxin as a bacterial super-antigen initiating the immunological response leading to the glomerulonephritis [22]. The histopathologic findings on immunofluorescence in the patients with MRSA infection with nephritis resemble those seen in IgA nephropathy [22]. Nephritis associated with endocarditis and ventricular shunts is associated with staphylococcal infection [22]. A number of infections can cause nephritis as listed in table 1. Most have been reported as case reports, such as a report of nephritis following malaria due to *falciparum vivax* infection in a 7 year old girl [23], and a report of nephritis following pneumococcal pneumonia in an adult male [24].

Hepatitis-B-associated glomerulonephritis (HBGN) is a distinct entity occurring frequently in hepatitis-B-prevalent areas of the world. The disease affects both adults and children who are chronic hepatitis-B-virus (HBV) carriers with or without a history of overt liver disease. The diagnosis is established by serologic evidence of HBV antigens/antibodies, presence of an immune complex glomerulonephritis, immunohistochemical localization of 1 or more HBV antigens and pertinent clinical history [25]. With the high incidence of hepatitis B in Asia, this entity assumes a greater public health importance. A study from China reported 205 cases from a single hospital from September 1995 to November 2008 [26]. In this series, the peak incidence of HBV-GN was between 20 -40 years of age, with a 3:1 predominance of males. The most common clinic manifestation was nephrotic syndrome and the most common pathology was membranous nephropathy. Decreased renal function was present in 10% of cases. The degree of albuminuria correlated with the viral load [26].

Renal disease is not uncommon in those infected with HIV. The most common manifestation of HIV in the kidney is HIV-associated nephropathy (HIVAN). Immunotactoid glomerulonephritis is a rare disorder found in 0.06% of renal biopsies characterized by organized tubular immune complex deposits. This is seen more commonly Caucasians and tends to occur in an older age group. There are 6 reported cases of HIV associated immunotactoid glomerulonephritis [27].

3. Pathogenesis

The kidney has a limited number of ways of responding to injury. Similar pathological signs may be the end result of different processes, produced by different initiating mechanisms and different molecular pathways may perpetuate the injury process. The initiation and development of the inflammatory response of the kidney to infection are still poorly understood.

The pathognomonic feature of PSGN is the deposition of immune complexes in the glomerular basement membrane. A proposed sequence of events is that a nephritogenic antigen(s) leads to the activation of the complement pathway and/or activates plasmin or production of the circulating immune-complexes. These then lead to increased permeability of the glomerular basement membrane, which allows deposition of the immune complexes, and leakage of the protein and red blood cells. The nephritogenic antigen is responsible for the C3 deposition, the recruitment of immune cells, tissue destruction and IgG deposition which further aggravates tissue injury. Complement activation leads to the release of cytokines, such as C5a, which attracts phagocytes, and proliferation of intrinsic cells and formation of a membrane attack complex which also aggravate the process. The definitive nephritogenic antigen has not yet been defined, although a large number of streptococcal factors (M proteins) have been proposed as the triggering factor. M proteins are present on the pili of the organism and more than 100 have been identified so far. Nephritogenic M proteins are types 1, 2, 4, 3, 25, 49, and 12 following skin infections and types 47, 49, 55, 2, 60, and 57 following throat infections [28]. Infections with nephritogenic streptococci have considerable variability in their ability to cause nephritis. The reason for this variability is not known.

Pathology shows typical glomerular changes which include proliferation of mesangial, endothelial and epithelial cells, inflammatory exudate and deposition of C3 early in the disease process followed by deposition of IgG. This immune deposition has been classified into 3 patterns [29]. The "starry sky" pattern represents an irregular and finely granular deposit of C3 and IgG along the glomerular capillary walls and in the mesangium. This occurs early in the course of the disease and is also seen in subclinical cases [28, 29]. The "mesangial pattern" has mainly C3 and some IgG in the mesangium. The "garland pattern" shows dense deposits along the capillary walls, is commonly associated with severe proteinuria and a poor prognosis [28, 29].

The immunological response of the kidney to an insult results in glomerulonephritis. The causal factors that underlie acute GN can be broadly divided into infectious and noninfectious groups. The most common infectious cause of acute GN is infection by *Streptococcus* species (ie, group A, beta-hemolytic). Nonstreptococcal postinfectious GN may also result from infection by other bacteria, viruses, parasites, or fungi. Bacteria besides group A streptococci that can cause acute GN include diplococci, other streptococci, staphylococci, and mycobacteria. *Salmonella typhosa*, *Brucella suis*, *Treponema pallidum*, *Corynebacterium bovis*, and actinobacilli have also been identified.

In the absence of evidence of a recent group A beta-hemolytic streptococcal infection, infections with Cytomegalovirus (CMV), coxsackievirus, Epstein-Barr virus (EBV), hepatitis B virus (HBV) [30], rubella, rickettsiae (as in scrub typhus), and mumps virus may be accepted as causal organisms. Similarly, attributing glomerulonephritis to a parasitic or fungal etiology requires the exclusion of a streptococcal infection. Possible organisms are *Coccidioides immiti, Plasmodium malariae, Plasmodium falciparum*, *Schistosoma mansoni*, *Toxoplasma gondii*, filariasis, trichinosis, and trypanosomes. While hepatitis B infection has been well documented as a cause of renal involvement and glomerulonephritis [25, 26], acute GN is as a rare complication of hepatitis A [31].

Noninfectious causes of acute GN may be divided into primary renal diseases, systemic diseases, and miscellaneous conditions or agents. The primary renal diseases are membranoproliferative glomerulonephritis (MPGN), IgA nephropathy and mesangial proliferative glomerulonephritis. Multisystem systemic diseases that can cause acute GN are vasculitis such as Wegener granulomatosis, polyarteritis nodosa and hypersensitivity vasculitis, collagen-vascular diseases like systemic lupus erythematosus (SLE) which causes glomerulonephritis through renal deposition of immune complexes, Henoch-Schönlein purpura and Goodpasture syndrome. Miscellaneous noninfectious causes are Guillain-Barré syndrome, Diphtheria-pertussis-tetanus (DPT) vaccine and serum sickness. These are summarised in table 1.

It is important to identify the exact aetiology of the glomerulonephritis as the prognosis differs widely depending on the underlying cause. There are a number of clinical and laboratory features that may help to differentiate between or even point to a particular cause of glomerulonephritis. The latent period between infection and nephritis is helpful in differentiating PSGN from IgA nephropathy. In contrast to the latent period of 2-3 weeks seen in PSGN, the nephritis of IGA nephropathy may occur either at the same time or just 1-2 days after an upper respiratory infection. Similarly, patients with nephritis of chronic infection have an active infection at the time nephritis becomes evident. Despite the chronic nature of the underlying infection, the associated nephritis can present acutely. Circulating immune complexes play an important role in the pathogenesis of acute GN in these diseases.

The failure of the C3 levels to return to normal should prompt consideration of the possibility of MPGN or SLE as the underlying cause. MPGN is a chronic disease which can manifest with an acute nephritic picture. Gross haematuria is unusual in lupus nephritis. Other associated systemic findings may identify the underlying systemic disease, for example, vasculitic lesions of the lower extremities point to an underlying vasculitis as the cause of glomerulonephritis.

4. Clinical presentation

The typical presentation is the abrupt onset of acute nephritis occurring 1-3 weeks after a streptococcal throat infection and 3-6 weeks after skin infection [28]. The nephritis is characterised by the triad of oedema, gross haematuria, and hypertension. The classical presenting feature is the presence of "coca-cola" coloured urine which is characteristic of homogenous gross haematuria [32]. Other common features are facial puffiness and hypertension secondary to fluid overload and urinary abnormalities such as albuminuria and the presence of red cell casts. General features like malaise, weakness, and anorexia may occur in about half the patients and a minority complain of nausea and vomiting. Within a

week or so from onset of symptoms, most patients with PSGN begin to experience spontaneous resolution of fluid retention and hypertension and the urine abnormalities begin to subside. The low C3 levels begin to rise and normalise by 8 weeks. Normal urine findings are found by 12 weeks.

Microscopic haematuria is universally present. Of the triad of features, oedema is seen in 85% of cases and is often the presenting symptom, gross haematuria in 40% of cases (range 30-50%) and hypertension in 50-95% of hospitalised cases. Approximately 95% of clinical cases have at least 2 manifestations, and 40% have the full-blown acute nephritic syndrome. The puffiness of the face or eyelids is sudden, usually prominent upon awakening and tends to subside at the end of the day if the patient is active. The oedema is a result of a defect in renal excretion of salt and water leading to fluid overload. The severity of edema does not correlate well with the degree of renal impairment.

In most cases, urinary abnormalities clear by 12 weeks, although proteinuria may persist for 6 months to 3 years and microscopic haematuria from 1 year to 4 years after the onset of nephritis [33]. In some cases, generalized edema and other features of circulatory congestion, such as dyspnea, may be present.

Accompanying this clinical picture is laboratory evidence of streptococcal infection, typically increasing antistreptolysin-O titers (ASOT) or streptozyme titres following throat infections and anti-DNase B titers following skin infections. Complement levels are decreased; low C3 levels are found in almost all patients with acute PSGN and C4 levels may be slightly low. These low levels of C3 usually normalize within 8 weeks after the first sign of PSGN [34], although up to 12 weeks has been reported [33]. The typical accompanying histopathology is one of diffuse cellular proliferation in the glomerulus, an exudate containing neutrophils and monocytes and variable degrees of complement and immunoglobulin deposition. In most cases, hypertension subsides, renal function returns to normal and all urinary abnormalities eventually disappear [33].

There is, however, a wide variation in the clinical presentation as well in the histopathology associated with PSGN. At the severe end of the spectrum is a rapidly rising azotemia with a rapidly progressive nephritic picture associated with severe cell proliferation, massive exudates and crescent formation in biopsy specimens. This is seen in <5% of PSGN cases [19]. The severity of renal failure tends to be directly related to the degree of proliferation and crescent formation, and about 50% of these patients recover renal function [35]. This type of presentation is more common in the elderly. The mild end of the spectrum, represented by subclinical or asymptomatic glomerulonephritis, is more common. Diligent examination of people with acute, trivial or self-limited infections caused by a range of organisms including various bacteria, parasites or viruses reveal subclinical infection in the form of microscopic hematuria, proteinuria and pyuria. Histopathology reveals mesangial proliferation with mesangial deposits of C3 and IgG deposits [19]. Asymptomatic household contacts of PSGN cases show sub-clinical disease 4-5 times more commonly than the acute classical presentation [6, 36]. An older study puts the ratio of asymptomatic cases as high as 19:1 [7].

5. Typical findings on investigations

Urinalysis reveals haematuria in all patients and proteinuria (from trace to 2+ on dipstick testing) is usually present. Proteinuria may be in the nephrotic range and is usually

associated with more severe disease. Red blood cell casts are pathognomonic of acute glomerulonephritis. Occasionally other cellular casts and pyuria are present.
Serological evidence of an antecedent streptococcal infection is present in the form of raised ASOT (> 200 IU/ml) and increased anti-DNAse B (which is a better serological marker of preceding streptococcal skin infection). Bacteriological evidence of streptococcal disease may be present in throat or skin swabs.
Complement levels especially C3 are low at the onset of symptoms. C4 is usually within normal limits in post-streptococcal GN. Causes of nephritis with low complement besides PSGN are MPGN, SLE, cryoglobulinemia, Diabetes Mellitus and Hepatitis C Virus. These diseases should be screened for in case of either an atypical presentation or atypical clinical course of the glomerulonephritis.
Renal function tests, blood urea, sodium, potassium and serum creatinine may be elevated in the acute phase and reflect the decrease in the glomerular filtration rate that occurs at this time. These elevations are usually transient. Failure of renal function tests to normalize within several weeks or months suggests that the patient may not have PSGN and indicates the need to seek an alternative diagnosis by further investigation. The full blood count may show anaemia which is usually dilutional and will return to normal once the fluid overload resolves.
Renal ultrasound is not required to make a diagnosis of glomerulonephririts. Renal ultrasound images usually reveal normal-sized kidneys bilaterally. Renal imaging is often done to confirm that there are two kidneys and that they are structurally normal. It may be done as a prelude to a renal biopsy. PSGN is a clinical diagnosis and requires the detection of glomerulonephritis and evidence of preceding streptococcal infection. A renal biopsy is indicated in cases with an atypical presentation, an atypical course, persistence of clinical features, or a persisting low level of complement (C3) or abnormal renal function tests. Features that suggest a diagnosis other than PSGN in the early stages may also indicate a need for a biopsy. These include the absence of the latent period between streptococcal infection and acute glomerulonephritis, anuria, rapidly deteriorating renal function, normal serum complement levels, lack of rise in antistreptococcal antibodies, general symptoms of systemic disease and either persistent hypertension or lack of improvement in glomerular filtration rate for more than 2 weeks. In the recovery phase, persistent low C3 beyond 8 and definitely beyond 12 weeks from the onset of illness would indicate a need to look for alternate causes and for a renal biospsy.

6. Treatment

Treatment of PSGN remains largely supportive. Complete recovery occurs in over 90% of children, but only 60% of adults fully recover. The rest develop hypertension or renal impairment.
Treatment is directed towards monitoring the signs and symptoms, in particular facial puffiness and hypertension, the main cause of which is fluid overload. Treatment when it is required, is mainly directed towards managing the fluid overload, which is responsive to diuresis and sodium restriction. Effective diuresis reduces cardiac congestion and controls hypertension and in most cases no further treatment is required. Strict input-output monitoring is recommended during the acute phase.
As the hypertension is caused by fluid overload, loop diuretics are the first line treatment and may be adequate for control of hypertension. Furosemide in a dose of 40 mg either

orally or intravenously is given 12 hourly. Usually treatment is required for less than 48 hours. Sometimes other anti-hypertensive agents may be needed, especially when the blood pressure is very high and it is unsafe to wait for the effect of diuretic therapy. Nifedipine is then given every 4-6 hours in doses of 5-10 mg. Rarely parenteral hydralazine may be required. Captopril has shown to be effective [37, 38] but should be used in caution in the presence of renal failure and hyperkalemia. Occasionally acute renal failure requires dialysis. Pulmonary oedema may be complication of severe fluid overload and needs urgent treatment.

It is important to check serum complement levels 6-8 weeks after initial testing to make sure they have returned to normal. Blood pressure should be monitored every month for 6 months and then 6 monthly. Renal function tests and serum creatinine levels repeated every 3 months after the acute phase for 1 year and then yearly after that. Urine should be checked for hematuria and proteinuria every 3-6 months.

Aggressive therapy using pulse methyprednisolone has been used in adults with poor prognostic factors such as nephritic range proteinuria, cellular crescents on biopsy and renal insufficiency [39]. Plasmapharesis and pulse methyprednisolone was successfully used in a 6 year old girl with garland pattern PSGN [40]. Whether this would benefit all patients with poor prognosis has not been studied.

Penicillin treatment is given to treat any persisting streptococcal infection [41]. Methicillin resistant staphylococcus should be treated with appropriate antibiotics. Specific infections require treatment with specific antibiotics or antiviral agents. Treatment of the underlying infection may resolve the glomerulonephritis as well. A review of six trials (a total of 159 patients) of which five were specified as hepatitis B virus-associated membranous glomerulonephritis (HBV-MN) showed that antiviral therapy for hepatitis B infections including IFN and lamivudine is effective in leading to remission of proteinuria, HBeAg clearance, and HBV-DNA reduction in both children and adults [42].

7. Preventing spread

A case of PSGN has 2 or more of the following clinical manifestations: oedema, macroscopic hematuria or dipstick hematuria of ≥ 2, or diastolic blood pressure of >80mmHg if ≤ 13 years of age and >90 mmHg if >13 years of age, in the presence of a reduced complement level (C3) and evidence of streptococcal infection by either elevated ASO or anti-DNAse B titres or positive cultures of GAS from skin, if sores are present, or from the throat in the absence of skin sores.

There is evidence that outbreaks can be halted by treating all children with any evidence of skin sores with intra-muscular (IM) benzathine penicillin to stop the transmission of the bacteria in the community [43]. In experimental PSGN, the nephritic process is prevented if penicillin is given within 3 days of the streptococcal infection [41]. Prevention of epidemics requires the control of spread of skin sores and infected scabies [44]. Following the identification of a case(s), family and household members are screened for the presence of skin sores and scabies and tested for urinary abnormalities. Those with skin manifestations are treated with penicillin. Those with urinary abnormalities undergo complete investigation for PSGN including urea, electrolytes, C3, ASO, anti-DNAse B and cultures for streptococcal infection.

Prevention of epidemics of PSGN requires a community level control of skin sores and infected scabies. Promotion of regular washing, especially of children, will prevent spread.

Improvement in housing, especially reduction in overcrowding, will hinder spread of infectious disease. The significant decline in PSGN in Singapore children is attributed to an improvement in the socioeconomic status, the health care system and urbanization of the country [9].
Although research into the development of a vaccine is advanced and 3 GAS vaccines have been approved for phase 1 human trials [45], it is unlikely to be available in the near future.

8. Prognosis

In keeping with the clinico-pathological picture, the prognosis of PSGN is also extremely variable, and largely influenced by clinical presentation and histopathology. An episode of PSGN may result in complete recovery, progression of symptoms or progression to renal failure. Persistence of symptoms may represent either a slow recovery, limited injury without further progression or progression to renal failure.
The immediate prognosis is generally good. In general, children are believed to have an excellent prognosis with the majority showing complete recovery [5]. Fewer than 1% of children have elevated serum creatinine values after 10-15 years of follow-up. Adults have a poorer prognosis overall. Early mortality can be as high as 25% in the elderly who have congestive cardiac failure or azotemia in the early phase. In adults approximately 25% will progress to chronic renal failure. These are usually those with massive proteinuria which suggests a worse prognosis and often signifies the garland pattern of immune deposits on pathology.
It is difficult to predict the prognosis in an individual case particularly early in the disease. While a typical presentation and clinical course indicates a good prognosis and an atypical presentation, severe persistent hypertension and abnormal renal function tests, massive proteinuria and older age group suggest a poor prognosis, there is a lack of a clinical or biochemical marker that might differentiate those with a good prognosis from those with a poorer outcome. Neutrophil gelatinase-associated lipocalin (NGAL), is emerging as a promising biomarker of acute kidney injury [46, 47], but has not yet been evaluated in PSGN.
However, some studies have reported persistent urinary abnormalities [8, 11, 19, 48, 49] and subtle abnormalities in renal function, as defined by reduction in renal functional reserve, in patients who had recovered from PSGN without apparent sequelae [50]
Epidemic cases have a better prognosis than sporadic cases [14, 51, 52], but not always. An outbreak of PSGN in Brazil following an epidemic of *Streptococcus equi zooepidemicus* resulted in a high prevalence of renal abnormalities at a mean follow-up of 5.4 years [53, 54]. These were, however, mainly adult patients. A study of Iranian children has shown that even mild PSGN may result in impaired renal function and that a rising diastolic blood pressure may be an early sign of worsening renal function [55].
Elderly people have poorer outcomes as do those with co-morbidities, including diabetes, cardiovascular and liver diseases [5, 28]. In an Aboriginal population with high rates of ESRD, follow-up of children 6-18 years (mean 14.6 years) after epidemic PSGN showed that risk of overt proteinuria was 6 times (95% CI 2.2-16.9) greater than in healthy controls after adjustment of age, sex and birth weight [11, 56]. The Australian Aboriginal population is at considerably higher risk than the general Australian population of developing chronic diseases such as diabetes mellitus, cardiovascular and renal diseases. There is also a greater burden of infectious disease and adverse early life factors such as low birth and infant weights. It is proposed that in this high risk population with multiple adverse renal

influences, childhood PSGN might be a more important risk factor for ESRD than it would be in lower risk populations [11, 44, 57].

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8

S. pyogenes Infections and Its Sequelae

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1. Introduction

Suppurative streptococcal infections of the throat and the skin generate stimuli that lead Rheumatic fever (RF) in 1 to 5% of susceptible children. The disease manifests initially as polyarthritis, carditis/valvulitis, Sydenham`s chorea, erythema marginatum and/or subcutaneous nodules. Chronic renal disease can also occur.

RF occurs at an early phase of life (3 to 19 years of age); thus, heart damage (carditis) can appear in very young children. Rheumatic carditis usually presents as pancarditis, affecting the endocardium, myocardium and pericardium. Recurrent acute cardiac lesions frequently evolve into chronic rheumatic heart disease (RHD), of which valvular deformities are the most important sequelae; these deformities lead to mitral and aortic regurgitation and/or stenosis. Valve replacement surgery is usually the only treatment for chronic RHD patients and incurs high costs for both public and private health systems.

Here, we will present three cases of young RHD patients who underwent valve replacement and the autoimmune reactivity that triggered the heart-tissue rheumatic lesions.

Post-streptococcal glomerulonephritis (PSGN) is another immune sequelae that presents a latency period of one to three weeks after scarlet fever, streptococcal pharyngitis and purulent skin infections.

PSGN has become a rare disease, especially in adults in developed countries, due to an improved standard of living, earlier treatment of pharyngeal infections and widespread use of antibiotics (Rodriguez-Iturbe & Musser, 2008). Despite decades of research, the pathogenesis of PSGN remains obscure. It is still unclear whether or to what extent autoimmune reactions are involved, but several studies have shown that different streptococcal antigens are detectable by immunohistology in the diseased kidneys (Rodriguez-Iturbe & Batsford, 2007). These data are in favor of direct contributions of streptococcal nephritogenic factors to PSGN pathogenesis, although intact bacteria have never been found in affected kidneys. Two PSGN cases will also be presented.

2. Epidemiology

2.1 Acute rheumatic fever

The incidence of ARF in some developing countries exceeds 50 cases per 100,000 children (Carapetis et al., 2005). The worldwide incidence of RHD is at least 15.6 million cases and is responsible for around 233,000 deaths / year. However, these estimates are based on conservative assumptions, so the true disease burden is probably substantially higher (Carapetis et al., 2005). The incidence of ARF can vary from 0.7 to 508 per 100,000 children per year in different populations from several countries (Carapetis et al., 2005). In Brazil, according to the WHO epidemiological model and data from IBGE (Brazilian Institute of Geography and Statistics), the number of Streptococcal pharyngitis infections is around 10 million cases, which could lead to 30,000 new cases of RF, of which around 15,000 could develop cardiac lesions (Barbosa et al.,2009).

2.2 Post-streptococcal glomerulonephritis (PSGN)

PSGN has become a rare disease, especially in adults in developed countries, due to an improved standard of living, earlier treatment of pharyngeal infections and widespread use of antibiotics (Rodriguez-Iturbe & Musser, 2008). However, the occurrence of acute post-infection glomerulonephritis (APIGN) has emerged as a major risk in diabetic patients all over the world (Nars et al., 2008).

The global incidence of acute PSGN was estimated at 472,000 cases per year, of which 456,000 occurred in less-developed countries (Carapetis, 2005). In agreement with these data, the incidence of PSGN ranges from 9.5 to 28.5 new cases per 100,000 individuals per year in developing countries (Rodríguez-Iturbe, 2008).

3. Autoimmunity is the major mechanism leading to both diseases

3.1 Rheumatic fever and rheumatic heart disease

The autoimmune reactions in RF and RHD are controlled by several genes related to both the innate and adaptive immune responses (Guilherme et al., 2011). Briefly, in the last 50 years, several genetic markers from different populations have been studied, and the susceptibility of developing RF/RHD was first associated with some alleles of HLA (human leukocytes antigens) class II genes (DRB1, DQB and DQA), which are located on human chromosome 6. HLA alleles are involved in antigen recognition by T lymphocytes through the T cell receptor (TCR). Later, some studies showed that the TNF-α gene, located in the same region of this chromosome was also associated with the disease. The TNF-α gene encodes the inflammatory TNF alpha protein, which is involved in the inflammatory process mediating heart-tissue lesions in RHD. Several other associations have been established based on gene variability by studying single nucleotide polymorphisms (SNPs). These genes code for other proteins also involved with the immune response (innate and adaptive pathways) (see Diagram 1) (Guilherme et al., 2011).

3.1.1 Molecular mimicry

Molecular mimicry mediates cross-reactivity between streptococcal antigens and human proteins. Several autoantigens have been identified, including cardiac myosin epitopes, vimentin and other intracellular proteins.

Several streptococcal and human cross-reactive antibodies have been found in the sera of RF patients and immunized rabbits and mice over the last 50 years and have been recently

reviewed. Briefly, antibodies against N-acetyl β-D-glucosamine, a polysaccharide present in both the streptococcal cell wall and heart valvular tissue displayed cross reactivity against laminin, an extracellular matrix alpha-helical coiled-coil protein that surrounds heart cells and is also present in the valves (Cunningham, 2000; Guilherme et al., 2005).

Among human proteins, cardiac myosin and vimentin seem to be the major target antigens. By using affinity-purified anti-myosin antibodies, Cunningham´s group identified a five amino acid residue (Gln-Lys-Ser-Lys-Gln) epitope of the N-terminal M5 and M6 proteins as cross-reactive with cardiac myosin (Cunningham et al., 1989).

Cunningham´s group found that streptococcal and human cross-reactive antibodies upregulate the adhesion molecule VCAM-1 after binding to the endothelial surface, leading to inflammation, cellular infiltration and valve scarring (Gavin et al., 2000, Roberts et al, 2001). These data established the role of the heart–tissue cross-reactive antibodies (anti-cardiac myosin and laminin) in the early stages of inflammation and T cell infiltration in RHD lesions.

Studies performed in the last 25 years showed that CD4+ cells are the major effectors of autoimmune reactions in the heart tissue in RHD patients (Raizada et al., 1984; Kemeny et al.,1989; Guilherme et al., 1995). However, the role of T cells in the pathogenesis of RF and RHD was demonstrated through the analysis of heart-tissue infiltrating T cell clones (Guilherme et al., 1995). Immunodominant peptides of the M5 protein (residues 81-96 and 83-103) displayed cross-reactivity with valvular proteins and cardiac myosin peptides by molecular mimicry (Faé et al., 2006; Yoshinaga et al., 1995; Guilherme et al, 1995). These M5 epitopes were also preferentially recognized by peripheral T lymphocytes from RHD patients when compared with normal individuals, mainly in the context of HLA-DR7 (Guilherme et al., 2001). Analysis of the T cell receptors (TCR) of peripheral and intralesional T cells from RHD patients showed several antigen-driven oligoclonal T cell expansions at the site of heart-tissue lesions (Guilherme et al, 2000). These autoreactive cells are CD4+ and produce inflammatory cytokines (TNFα and IFNγ). IL-4+ cells are found in the myocardium; however, these cells are very scarce in the valve lesions of RHD patients. IL-4 is a Th2-type cytokine and plays a regulatory role in the inflammatory response mediated by Th1 cytokines. These findings indicate that the Th1/Th2 cytokine balance has a role in healing myocarditis, while the low numbers of IL-4-producing cells in the valves probably induced progressive and permanent valve damage (Guilherme et al, 2004).

Three cases of RHD patients (clinical, surgical data) will be presented. Histological and immunological data obtained from peripheral blood and T-cell lines and T cell clones derived from heart-tissue infiltrating T cells of these patients are summarized in Tables 1 and 2.

Patients	Mitral Valve					Myocardium (LA)	
	Inflammation	Rheumatic Activity	Neovasc	Fibrosis	Calcification	Inflammation	Rheumatic Activity
Case 1	(++)	AB-PR(+) VER(+)	(+)	(+)	(–)	AB-PR (+)	(+)
Case 2-	(+)	(–)	(–)	(++)	(–)	(–)	(–)
Case 3- 1st surgery						(+)	AB(–)
2nd surgery	(+)					(+)	(–)

LA-left atrium; AB-PR- Achoff Bodies in proliferative phase; Ver- verrucae, (-) negative;(+) mild; (++) moderate

Table 1. Histological data of Rheumatic Heart Disease patients

				Antigens recognized by T cell clones from myocardium and/or mitral valve		
	HLA Class II	Heart-tissue Infiltrating Cells/field $CD4^+$	$CD8^+$	M5 protein peptides	Myocardium derived- proteins	Valve derived proteins
Case # 1	15,7, 52, 53	9.4	3.3	M5 (81-96) M5 (83-103) M5 (163-177)	30-44 kDa; 24-30 kDa LMM25(1607-1624)	>150 kDa, 90-150 kDa; 65-90 kDa;43-65 kDa 30-43 KDa 90-150 kDa; 30-43 KDa
Case # 2	9, 11, 52,53	6.1	1.2	M5(11-25) M5 (81-96) M5 (83-103)		43-65 kDa 90-150 kDa, 43-65 kDa 90-150 kDa; 43-65 kDa
Case # 3	17, 13, 52,	4.5	0.9	M5 (83-103)	>150 kDa LMM10(1413-1430) LMM12(1439-1456)	90-150 kDa; 43-65 kDa; 30-43 KDa

Amino acid sequences of M5 protein were based on sequence published by Philips et al, 1981 and Manjula et al, 1985. M5 (81-96)-DKLKQQRDTLSTQKET; M5 (83-103)-LKQQRDTLSTQKETLEREVQN; M5-(163-177) ETIGTLKKILDETVK; cardiac myosin beta chain sequences published by Diederich et al, 1989: LMM 10 (1413-1430) CSSLEKTKHRLQNEIEDL; LMM12 (1439-1456) AAAAALDKKRNFDKILA; LMM25 (1607-1624) RSRNEALRVKKKMEGDLN, (Guilherme et al, 1995, Faé et al, 2006).

Table 2. T cells from heart-tissue of Rheumatic Heart Disease patients recognize streptococcal peptides and cardiac proteins.

Case # 1

Male 4 years old, presented mitral, aortic and tricuspid regurgitation, left ventricular diastolic diameter of 51 mm and systolic diameter of 34 mm, ejection fraction (LVEF) of 78%, left atrium (LA) of 40 mm, thickened pericardium.

At surgery, mitral valve prolapse was observed with very long strings and small tears of rope. Mitral annulus was dilated. A mitral valve replacement was done. Heart biopsy showed chronic valvulitis with areas of mucoid collagen degeneration and papillary muscles with Aschoff nodules in the granulomatous stage (Table 1).

Case # 2

Male 6 years old, presented clinical features of fever, polyarthritis and carditis with mitral valve involvement. On this occasion, patient showed evidence of inflammatory activity; Gallium 67 positive scintigraphy; endomyocardial biopsy suggestive of rheumatic carditis. A left ventricular diastolic diameter of 59 mm, left ventricular systolic diameter of 39 mm, ejection fraction (LVEF) of 71% and left atrium (LA) of 52 mm were observed. Two valve correction surgeries were performed. Pathological examination of the mitral valve showed sequelae of chronic valvulitis with intense fibrosis and mucoid degeneration.

Case #3

Male 10 years old, presented clinical features of fever, polyarthritis and carditis with progressive cardiac heart failure and mitral and aortic shortcomings as well as relapses of acute outbreak by irregular use of secondary prophylaxis with benzathine penicillin and progression to chronic atrial fibrillation, culminating in death at 18 years of life. Increased

left ventricular diastolic diameter (67/43 mm) and left atrial diameter of 62 mm, significant mitral regurgitation, aortic insufficiency and moderate impact tricuspid regurgitation with mild rebound were found. Subjected to two surgeries, first for mitral valve repair and prosthetic aortic and tricuspid valves, then for exchange of the mitral and aortic bioprostheses, and tricuspid valve repair.

3.2 Post Streptococcal Glomerulonephritis (PSGN)

Acute glomerulonephritis can occur sporadically or endemically as a result of infections of both the upper airways and skin by group A streptococcus strains.

Genetic susceptibility factors are likely involved with the development of the disease. HLA class II alleles (DR4 and DRB1* 03011) have been found to be associated with PSGN compared to healthy controls. Genetic association with endothelial nitric oxide synthase intron 4 a/b (eNOSa/b) defined by variable numbers of tandem repeats (VNTR) polymorphism was also described (Ahn & Ingulli, 2008).

The disease is mediated by immune complexes and complement pathway activation. Several theories seek to explain the formation of immune complexes in glomeruli. The most accepted one is that a streptococcal antigen, with affinity for the glomerular structures, can be deposited in the glomerulus, activating the host immune response and initiating development of immune complexes *in situ* (Rodriguez-Iturbe & Bastford, 2007).

Apparently, molecular mimicry between streptococcal antigens and glomerular proteins leads to tissue damage. Two antigens have been investigated as potential causes of PSGN: the plasmin receptor linked to nephritis (NAPlr), identified as glyceraldehyde 3-phosphate dehydrogenase, and a protein known as streptococcal pyrogenic exotoxin B (SpeB). Both are present in renal biopsies of patients with PSGN and are capable of activating the alternative pathway of the complement system. In addition, they are capable of promoting enhanced expression of adhesion molecules, facilitating inflammatory reactions mediated by cytokines (IL-6, TNF α, IL-8 and TGF ß). It seems that the nephritogenic properties of NAPlr and SpeB are related to the binding ability of plasmin, which facilitates the deposition of immune complexes (IgG and C3, properdin and C5) in the glomeruli and subsequent inflammation (Rodriguez-Iturbe & Bastford, 2007; Rodriguez-Iturbe & Musser, 2008).

Molecular mimicry, as mentioned above, leads to the recognition of streptococcal antigens and laminin, collagen and glomerular basement membrane (GBM). Sub-epithelial localization of immune complexes and complement factors in the injured glomeruli points towards a crucial role of the host immune system in tissue destruction.

As mentioned before, renal inflammation may result from a myriad of insults and is often characterized by the presence of infiltrating inflammatory leukocytes within the glomerulus or tubular interstitium. Accumulating evidence indicates that infiltrating leukocytes are the key to the induction of renal injury.

Two cases of PSGN are presented in which anti-streptolysin O (ASO) was positive, indicating a previous infection by *S. pyogenes.*

Case #1

Male 6 years old, presented swollen eyes followed by bilateral periorbital edema followed by progression of lower limb edema and increased abdominal size, decreased urine volume and urine darkness. Lab tests detected hematuria, increased serum levels of urea (63.0 mg/dl) and creatinine (1.2 mg/dl). Decreased levels of complement (16.1 mg/dl) and fractions C3 and C4 (both 11.7 mg/dl) were found. The patient also presented increased levels of ASO (1055 IU).

Case # 2

Male 15 years old, presented edema, hypertension, and gross hematuria and reported a skin abscess in the left leg 20 days before hospital admission. No previous signs of disease or significant co-morbidities were identified. Physical examination showed a 2+ lower edema, with no signs of current skin infections. Laboratory tests revealed 24 hr urine protein 2.4 g/day, serum creatinine 2.9 mg/dl, hematuria, and positive ASO (> 200 IU). Renal ultrasound showed normal kidneys. After introducing antibiotic and controlling edema and hypertension with diuretics and anti-hypertensive drugs, the patient was subjected to a renal biopsy that showed a diffuse proliferative pattern, with focal endocapillary and mesangial proliferation with no cellular crescents.

4. Frequencies of *S. pyogenes* strains collected at Clinical Hospital of the School of Medicine of the University of Sao Paulo

Diverse *S. pyogenes* strains are related with the development of RF/RHD or PSGN and are considered as rheumatogenic and nephritogenic, respectively.
We analyzed 177 samples obtained from diverse biological sources. Most samples were recovered from blood, throat and wound (Table 3).

Source	nº of cases	Strains identified									
		emm1	*emm87*	*emm22*	*emm12*	*emm77*	*emm6*	*emm75*	*emm89*	*st2904*	others
Throat	30	6	-	5	1	-	2	1	-	2	13
Blood	58	10	6	3	7	5	3	2	1	-	21
Wound	15	3	2	-	-	-	-	-	1	2	7
Sputum	8	2	-	1	-	2	1	-	-	-	2
Surgical wound	5	-	-	1	1	1	-	-	-	-	2
Ascitis	3	-	-	-	-	-	-	1	-	-	2
Catheter	3	-	-	1	-	-	-	-	-	-	2
Lymph node	3	1	2	-	-	-	-	-	-	-	-
Ocular discharge	3	2	-	-	-	-	-	-	-	-	1
Synovial fluid	2	2	-	-	-	-	-	-	-	-	-
Liquor	2	1	-	-	-	-	-	-	1	-	-
Others	11	3	2	-	-	1	-	1	1	-	3

Table 3. Distribution of *emm* types according to biological source.

The M1 type was more frequently observed. Figure 1 shows the frequencies of all strains analyzed. Our results are similar to those previously published (Table 4). It is interesting to note that studies done by Schulman et al., 2004 and Ma et al., 2009 showed variability of frequencies for some streptococcus strains over different periods, probably due to seasonal influence.

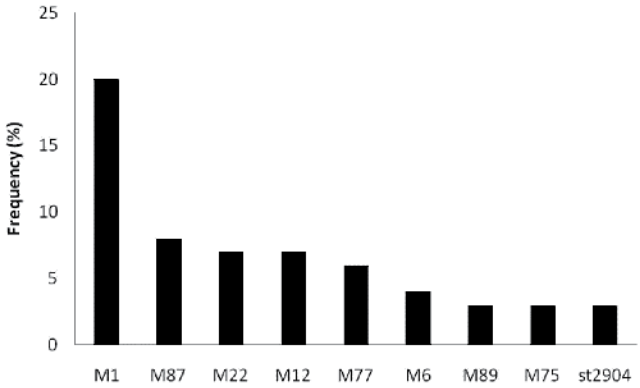

Fig. 1. Prevalence of the M types in a sample from São Paulo
Beta-hemolytic samples (177) obtained from diverse biological sites at the Clinical Hospital, University of Sao Paulo during the period of 2001-2008.

Country	Year	Nº isolates	Nº of *emm* types identified	Source	More frequent *emm* types	References
Germany	2003-2007	586	49	invasive	1, 28, 3, 12, 89, 4, 77, 6, 75, 11, 118, 2, 83	Imöhl et al., 2010
United States	1995-1999	1586	17	invasive	1, 28, 12, 3, 11, 4, 114, 89, 17, 77, 33	O´Brien et al., 2002
North America	1nd-2000-2001 2nd-2001-2002	1nd -975 2nd- 1076	1nd -29 2nd - 31	Non invasive	1nd-12, 1, 28, 4, 3, 2 2nd- 1, 12, 4, 28, 3, 2	Schulman et al., 2004
Barcelona	1999-2003	126	29	invasive and non invasive	1, 3, 4, 12, 28, 11, 77	Rivera et al., 2006
Sweden	1986-2001	92	28	invasive and non invasive	1, 2, 4, 8, 12, 28, 66, 75	Maripuu *et al.*, 2008
Australia	2001-2002	107	22	invasive and non invasive	1, 4, 12, 28, 75	Commons et al., 2008
Hungary	2004-2005	26	8	invasive	1, 80, 4, 28, 66, 81.1, 82, 84	Krucsó et al, 2007
China	1nd-1993-1994 2nd-2005-2006	1nd -137 2nd -222	1nd -24 2nd - 9	invasive and non invasive	1nd -3, 1, 4, 12, st1815, 6 2nd 12, 1	Ma et al., 2009
Denmark	2003-2004	278	29	invasive	28, 1, 3, 89, 12	Luca-Harari et al., 2008
Norway	2006-2007	262	29	invasive	28, 1, 82, 12, 4, 3, 87, 89, 6	Meisal et al., 2010

Two studies were reported for North America and China.

Table 4. Distribution of *emm* types around the world

S. pyogenes - **Infections**

Genetic Susceptible Untreated Children and Teenagers

Genetic markers	**Role**
• MBL, TLR2, FCN2, Fcγ RIIA alleles	Innate immunity
• HLA class II alleles	Adaptive immune response
• Cytokines genes: TNF-α, TGF ß1, IL-1Ra, IL-10	Mediators of inflammatory reactions (agonist or antagonist)

Peripheral Blood

- Streptococcal and human proteins: cross-reactions mediated by both antibodies and $CD4^+$T cells
- Inflammatory cytokines: IL-1, IL-6, IL-10, TNF- α, IFN- γ
- Circulating immune complexes

Heart Tissue (RHD)

- Anti-laminin and/or cardiac myosin antibodies upregulate the VCAM-1 molecule in the endothelium surface leading to inflammation, cellular infiltration and valve scarring
- Infiltrating T cells are predominantly $CD4^+$ (~80%)
- Antigen-driven oligoclonal T cells are expanded in the myocardium and valves
- Intralesional T cell clones recognize streptococcal M peptides and heart-tissue proteins and cardiac myosin peptides (LMM)
- High numbers of TNF-α and IFN-γ secreting mononuclear cells are mediators of myocardium and valvular inflammation
- Low numbers of mononuclear cells IL-4^+ in the valves probably lead to permanent and progressive valvular damage

Kidney (Glomerulonephritis)

- Streptococcal anti – SpeB crossreactive antibodies recognize NAPlr, laminin, collagen and the glomerular basement membrane (GBM) antigens
- Sub-epithelial deposition of immune complex and complement factors

Diagram 1. Major events leading autoimmune reactions on both RHD and Glomerulonephritis

5. Prospective vaccines against *S. pyogenes*

Many studies have focused on developing a vaccine against *S. pyogenes* in order to prevent infection and its complications. There are four anti-group A streptococci (GAS) vaccine candidates based on the M protein and eight more candidates based on other streptococcal antigens, including group A CHO, C5a peptidase (SCPA), cysteine protease (Spe B), binding proteins similar to fibronectin, opacity factor, lipoproteins, Spes (super antigens) and streptococcal pili (Steer et al, 2009).

We developed a vaccine epitope (StreptInCor) composed of 55 amino acid residues of the C-terminal portion of the M protein that encompasses both T and B cell protective epitopes

(Guilherme et al, 2006). The structural, chemical and biological properties of this peptide were evaluated, and we have shown that StreptInCor is a very stable molecule, an important property for a vaccine candidate (Guilherme et al, 2011). Furthermore, experiments with mice showed that this construct is immunogenic and safe (Guilherme et al, 2011).

6. Conclusions

The knowledge acquired in the last 25 years pointed out the molecular mimicry mechanism as one of the most important leading autoimmune reactions in RHD and PSGN. Although both diseases are triggered by *S. pyogenes,* RHD is mediated by both antibodies and T cells while PSGN is mainly due to immune complex deposition in the glomeruli.
Several streptococcal cross reactive autoantigens were identified in both diseases.
Many proteins and cardiac myosin epitopes were identified as putative cross-reactive autoantigens in RHD and collagen, glomerular basement membrane in PSGN. Laminin, another autoantigen is also involved in the cross reactivity in both diseases.
Diagram 1 illustrates the major events leading to RHDand PSGN.

7. Acknowledgements

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9

Insights from Genomics on Post-Infectious Streptococcal Glomerulonephritis

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1. Introduction

Acute post-streptococcal glomerulonephritis (APSGN) is one of the nonsuppurative sequelae that can occur following a group A streptococcal infection, the other common post-infection sequelae being rheumatic heart disease. Worldwide, it is estimated that approximately 470,000 cases of APSGN occur annually. Children and young adults most commonly are affected with males having twice the incidence as females. By the middle of the twentieth century, evidence was found that streptococcal skin infections were associated with APSGN, and these infections usually did not cause rheumatic fever, leading to the hypothesis that certain GAS strains were "rheumatogenic" while others were "nephritogenic." In contrast to the molecular and immunological details that have brought considerable insight into the pathogenesis of rheumatic heart disease, the bacterial and host factors that contribute to ASPGN remain poorly defined and at times controversial. Modern bacterial genome sequencing projects have now provided a rich genetic resource that includes complete sequences of nephritogenic group A streptococcus strain NZ131 (McShan et al., 2008) and nephritogenic group C streptococcus strain MGCS10565 (Beres et al., 2008) as well as other group A and C strains associated with rheumatic heart disease and other syndromes. A metagenomic analysis is presented and considers the contribution of genes previously associated with APSGN strains (such as streptokinase, protease SpeB, and M protein) as well as other potential genetic factors that may be found uniquely in these genomes including genes acquired by horizontal transfer and via mobile genetic elements. This analysis provides complementary information to the many published studies using nephritogenic *S. pyogenes* strain NZ131 and places them in a broader context, shedding light upon the genetic basis for the human disease caused by this and related streptococci.

2. Acute post-streptococcal glomerulonephritis

Streptococcus pyogenes (group A streptococcus) is a common bacterial pathogen of humans, causing a wide range of disease from uncomplicated pharyngitis to severe life-threatening infections like toxic shock syndrome or necrotizing fasciitis. Acute post-streptococcal glomerulonephritis (APSGN) is one of the two major post-infection sequelae that can follow acute streptococcal infections, the other being rheumatic heart disease. The typical causative agent of APSGN is the group A streptococcus although other Lancefield groups may occasionally trigger this disease. Worldwide, it is estimated that approximately 470,000

cases of APSGN occur annually (Carapetis et al., 2005). Children and young adults are the group that is most commonly presents with APSGN, and males have twice the incidence as females (Silva, 1998).

The link between group A streptococcal infections and the onset of nephritis was considered as early as 1917 by Ophuls (Ophuls, 1917). By the 1940s, a link had been found between streptococcal skin infections and the onset of APSGN, and since the associated strains usually did not cause rheumatic fever, the hypothesis was developed that certain *S. pyogenes* strains were "rheumatogenic" while others were "nephritogenic" (Futcher, 1940; Osman et al., 1933; Seegal and Earle, 1941). Additionally, it was observed that there were divergent seasonal patterns of peak incidence separating nephritogenic and rheumatogenic GAS, with skin infections and APSGN cases peaking in the late summer while throat infections and rheumatic fever had the highest incidence in October (Bisno et al., 1970). Cases of rheumatic fever were rare during the summer APSGN outbreaks, peaking instead during the autumn season. These observations lead to the development of the hypothesis that subpopulations of GAS exist that were adapted for colonization and infection of either the throat or the skin. These "throat specialists" and "skin specialists" were proposed to have specific sets of virulence factors that lead to the post-streptococcal sequelae of rheumatic heart disease or APSGN, respectively. This hypothesis has been refined over time to now define throat specialists, skin specialists and generalists using a classification scheme that relates the Mga regulon, the gene complement surrounding the major antiphagocytic protein M gene (*emm*), to preferred anatomical site of infection (Bessen et al., 1997; Enright et al., 2001; McGregor et al., 2004).

3. Comparative genomics of nephritogenic streptococcal strains

3.1 The nephritogenic streptococcal genomes

The role of streptococcal induced autoimmunity as the basis for the development of rheumatic heart disease has been supported by a number of detailed studies (Cunningham, 2000a; Cunningham et al., 1989; Ellis et al., 2010; Krisher and Cunningham, 1985). It is reasonable therefore to expect that a similar underlying source of antigenic cross-reactivity might be responsible for the development of APSGN, especially since the time of onset roughly follows the time required for the adaptive immune response. However, to date no definitive link has been found although a number of candidate streptococcal proteins have been proposed over the years, including streptokinase, protease SpeB, and the antiphagocytic M protein (reviewed by Cunningham (Cunningham, 2000b)). One of the goal's for genome sequencing of multiple streptococcal strains associated with different diseases was to use comparative genomics to gain insight into the genetic variations that underlie virulence. Several nephritogenic streptococcal isolates now have had their genome sequences determined, and this information will be crucial in understanding the pathogenic mechanisms underlying APSGN.

3.2 Physical chromosome characteristics

The genomes of nephritis-associated streptococcal isolates *S. pyogenes* NZ131 (group A) and *S. equi* subsp. *zooepidemicus* MGCS10565 (group C) were completely sequenced in independent efforts in 2008 (Beres et al., 2008; McShan et al., 2008). Both of these isolates were originally isolated from cases of human glomerulonephritis (Beres et al., 2008; McShan et al., 2008); additionally, NZ131 has been also studied intensively in over thirty published studies (McShan et al., 2008). Both genomes are single circular molecules of 1,815,783 bp and 2,024,171 bp for NZ131 and MGCS10565, respectively. Neither strain has been found to have naturally

occurring plasmids or other episomes. Strain NZ131 has 1,699 predicted open reading frames (ORFs) that use 1,548,919 bases so that 85.3% of the genomic DNA is used as coding sequences. The base composition of the ORFs is 39.18% G+C while the composition of the total genome is 38.57%; both values are similar to the composition seen in the other completed GAS genomes (McShan et al., 2008). The MGCS10565 genome has a 42.59% G+C content, and its genome has 1,961 predicted ORFs, which require 85% of the genome (Beres et al., 2008). The physical parameters of both genomes are typical for the family streptococcaceae.

3.3 Prophages and mobile genetic elements

Strikingly, strain NZ131 carries three prophages in its genome while MGCS10565 carries none. Prophages are always prominent features in the group A streptococcal genomes, sometimes accounting for 10% of the total DNA, and are well-known as vectors for carrying virulence genes such as superantigens or other bioactive proteins. While MGCS10565 has genes that are homologous to prophage integrases or regulatory proteins as well as virulence factors that are often associated with prophages (two DNases and a phospholipase A2), no organized prophage genome exists (Beres et al., 2008). This lack of prophages is in contrast to the *S. equi* subsp. *zooepidemicus* animal pathogen strain NC_012470 that was recently described as having four endogenous prophages (Holden et al., 2009).

The naturally competent streptococci have the *com*CDE operon that is thought to be essential in genetic transformation (Cvitkovitch, 2001). Although a previous study had not found this operon in *S. equi* subsp. *zooepidemicus* strain NCTC 4676 (Havarstein et al., 1997), it is present in MGSA10565 (Beres et al., 2008). The genomes of the naturally competent streptococci (including *S. pneumoniae* and *S. mutans* (Ajdic et al., 2002; Hoskins et al., 2001; Tettelin et al., 2001)) contain *com*CDE and lack prophages, and it is often suggested that frequent DNA transformation events may disrupt the genomes of prophages; thus, their typical absence. The presence of *com*CDE in MGCS10565 suggests that this isolate also may be naturally competent for DNA transformation, and this phenotype may be responsible for the absence of prophages (Beres et al., 2008). Balancing that viewpoint is the fact that these genes appear in the genome of *S. equi* subsp. *zooepidemicus* strain NC_120470, which does carry prophages. Thus, there may be other factors controlling competence in this species.

The prophages of NZ131 carry the virulence genes streptococcal pyrogenic exotoxin H (*spe*H), a streptodornase (*spd*3) and the paratox gene (McShan et al., 2008). Prophage-associated virulence factors have not been linked to APSGN in the literature, and comparison of these two genomes would tend to confirm that non-association. Rather, it would seem that if a common genetic trait exists that leads to APSGN, it would be found among the bacterial genes. Further, the absence of prophages in MGCS10565 and its potential to be competent suggests that if it has acquired genetic material from group A streptococci, it may have occurred via uptake of DNA from the environment rather by bacteriophage mediated transduction. This scenario suggests that genetic transfer may be somewhat of a one-way street, flowing from *S. pyogenes* to this and similar *S. zooepidemicus* strains since group A streptococci have not been demonstrated to be naturally competent. Therefore, the lack of prophages in MGCS10565 argues that group A streptococci, which probably use transduction as a means for horizontal transfer, would be somewhat genetically isolated from these group C streptococcal strains.

3.4 The nephritogenic strains and diversity

The NZ131 and MGCS10565 genomes are not collinear with respect to gene order, but a great number of genes are shared between the two. The genome map of NZ131 is shown in

Fig. 1 with gene homology comparisons to strains MGCS10565 (circle 6) and MGAS2096 (circle 5). Strain MGAS2096 is a group A streptococcus M12 serotype strain that was also isolated from a case of APSGN (Beres et al., 2006) and provides an inter-species reference.

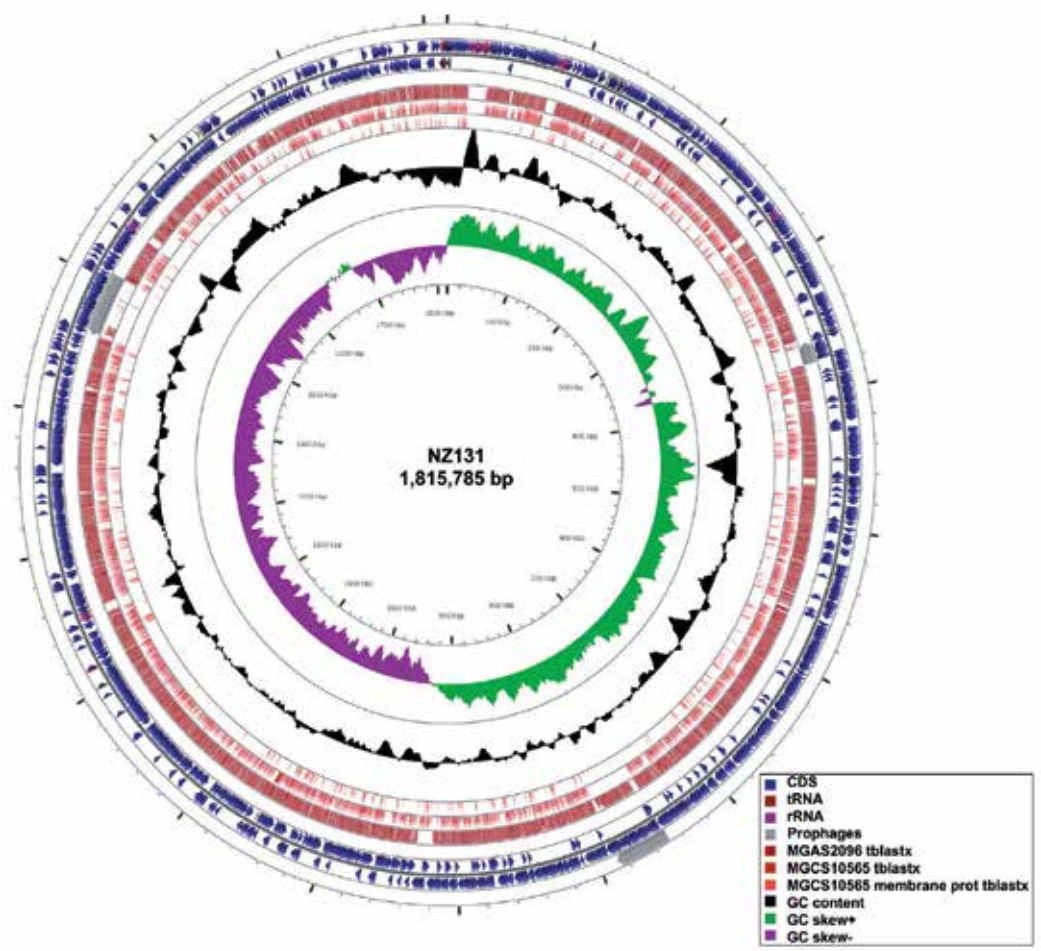

Fig. 1. **The genome map of M49 *S. pyogenes* strain NZ131.** The NZ131 genome map is shown with comparisons to the other two sequenced nephritogenic streptococcal genomes, *S. pyogenes* strain MGAS2096 and *S. equi* subsp. *zooepidemicus* strain MGCS10565. The outer circle and circle 4 indicate the positions of the three endogenous prophages in strain NZ131. The two circles they enclose (circles 2 and 3) show the location of the predicted NZ131 ORFs encoded by the two strands of DNA. The open reading frames were extracted from *S. pyogenes* strain MGAS2096 and *S. equi* subsp. *zooepidemicus* strain MGCS10565 and were compared to the genome of NZ131 using tblastx (circles 5 and 6). Circle 7 shows the specific tblastx hits of surface exposed proteins from MGCS10565. The innermost circles show the total G+C percentage of the NZ131 genomic DNA and the %G+C skew. The ribosomal RNA (rRNA) and tRNA (tRNA) genes were not included in the tblastx comparison. The figure was created using the online tools available at http://stothard.afns.ualberta.ca/cgview_server/index.html.

Overall, the main regions of divergence between the two *S. pyogenes* strains are in the endogenous prophages and other mobile genetic elements (MGE) carried by each (Fig. 1, circles 1 and 4), the genetic structures of the M protein gene region (Mga regulon) and streptococcal pilus region (circle 5), and by the presence the novel M49 and M82 specific NUDIX hydrolase operon in NZ131 (circle 5 (McShan et al., 2008)). NZ131 prophage NZ131.2 shares significant homology with a prophage from MGAS2096 (circle 5) but not including the crucial lysogeny module or virulence genes. Additionally, MGAS2096 has a deletion of a cluster of genes required for citrate metabolism (circle 5, about 925 kb on the map). To date, little evidence has been found to suggest that genes found on prophages or other MGE play a role in APSGN, and the lack of homology in these elements from the two group A streptococcal strains strengthens this idea. Thus, if a common mechanism for triggering APSGN exists, one would predict that bacterially encoded genes would be responsible, either in the form of unique nephritis-associated genes or gene alleles. A number of streptococcal proteins have been investigated as potential triggers of APSGN, and many of targets remain still largely unexplored. For example, circles 6 and 7 show the homology of MGCS10565 total genes and predicted membrane associated genes, respectively, to NZ131. While the homology is not as complete as was for the inter-species strain, many genes and particularly genes encoding cell-surface proteins are present in this intra-species streptococcus. Most of these proteins have not had their function or their potential immunogenicity identified. Thus, if genome comparisons tell us anything, it is that that many targets for future investigations remain. However, several genes have been considered to play a role in APSGN in previous work, and it is worthwhile to examine the variants found in each of these nephritogenic strains for possible shared features.

4. Genes associated with post-streptococcal glomerulonephritis

4.1 Streptokinase

Streptokinase is a plasminogen activator that is released as an extracellular protein by groups A, C, and G streptococci. It generates plasmin, which may promote bacterial spread through fibrinolysis and degradation of the extracellular matrix as well as induce inflammation via complement activation. This latter event may play a role in post-infection sequelae like APSGN (Nordstrand et al., 1999). It has been proposed that structural differences between alleles of streptokinase may be associated with diverse pathogenic outcomes, particularly in the variable βeta-domain (Lizano and Johnston, 2005). The role of streptokinase in the pathogenesis of APSGN has been supported by the use of a mouse model and derivatives of strain NZ131 with either nephritis- or non-nephritis-associated alleles of streptokinase (Nordstrand et al., 2000; Nordstrand et al., 1998).

The streptococcal streptokinase is composed of three domains, which are the highly conserved alpha and gamma domains and the variable βeta domain (Wang et al., 1998). The alpha and βeta domains are associated with plasminogen activation while the variable βeta domain is not required for this enzymatic activity (Lizano and Johnston, 2005). However, in the mouse model used by Nordstrand and her co-workers, the alleles that caused the onset of nephritis mapped their variations to the βeta domain (Nordstrand et al., 2000; Nordstrand et al., 1998). These studies, along with clinical observations (Johnston et al., 1992), have lead to a proposed role for streptokinase in APSGN, possibly in mediating complement deposition in the kidney.

One of the striking observations from the genome of the nephritis-associated group C MGCS10565 strain is the divergence of the encoded streptokinase when compared to those encoded by other group C and group A strains, whether nephritis-associated or not. Figure 2 shows a clustalw alignment of the amino acids from the streptokinase βeta regions from the group A genome strains M1, M2, M3, M4, M6, M12, M18, M28, and M49 as well as the group C strains H46 and GGS 124 (*S. dysgalactiae* subsp. *equisimilis*) and H70 and MGCS10565 (*S. equi* subsp. *zooepidemicus*). The βeta region from the two *zooepidemicus* strains is quite divergent from the other streptokinase proteins from both groups A and C subsp. *equisimilis* strains. Considerable divergence is also observed in the alpha and gamma domains (not shown). The βeta domain is required for docking to plasminogen via a kringle binding hairpin loop (Dhar et al., 2002; Wang et al., 1998), and the variation seen in this region suggests that a number of primary sequences are able to generate the needed secondary and tertiary protein structures.

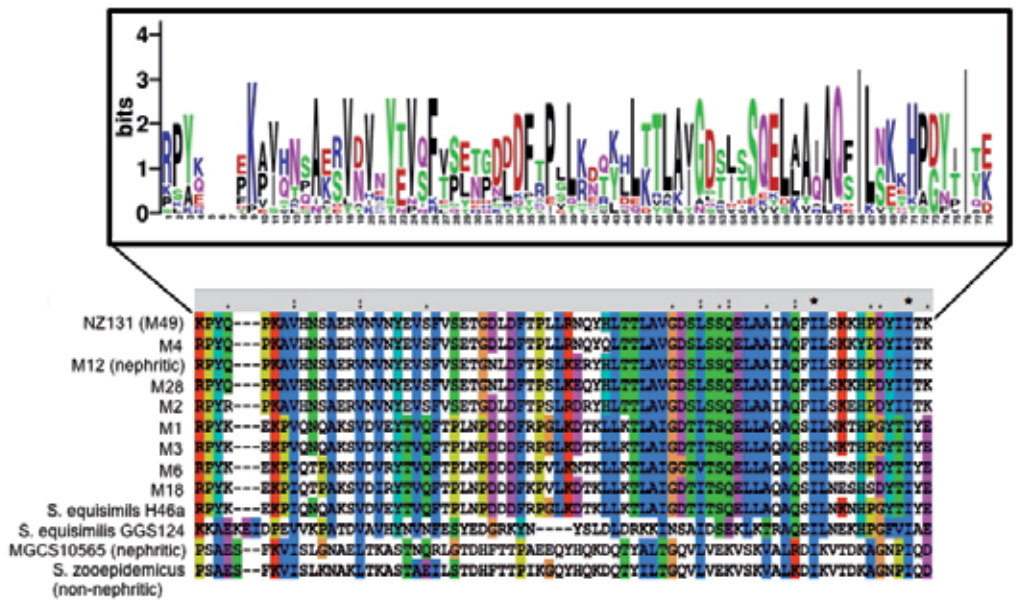

Fig. 2. **Analysis of the streptokinase gene *ska* βeta-region.** The variable βeta-region from the *ska* genes from GAS genome strains (NZ131 (M49, APSGN), MGAS10750 (M4), MGAS2096 (M12, APSGN), MGAS6180 (M28), MGAS10270 (M2), SF370 (M1), MGAS315 (M3), MGAS10394 (M6), and MGAS8232 (M18)) and GCS strains (*S. equisimilis* H46a, *S. equisimilis* GGS124, MGCS10565 (APSGN), and the non-nephritogenic *S. zooepidemicus* H70) was aligned using CLUSTALW (Thompson et al., 1994) and the resulting consensus analyzed using WebLogo (Crooks et al., 2004). The non-nephritogenic M12 *S. pyogenes* strain MGAS9429 was omitted from this analysis since its sequence is 100% identical to that of the nephritis-associated M12 strain MGAS2096. The primary descriptions of each of these genomes are found in the literature (Banks et al., 2004; Beres et al., 2006; Beres et al., 2008; Beres et al., 2002; Ferretti et al., 2001; Green et al., 2005; Holden et al., 2009; Holden et al., 2007; McShan et al., 2008; Shimomura et al., 2011; Smoot et al., 2002)

Further, it should be noted that a number of conserved neutral amino acid residues are positioned in the βeta domain, suggesting an essential contribution to function. Inspection of the primary sequence of the streptokinase proteins in this group shows additional conserved sequences in the two nephritogenic group A streptococcal isolates (NZ131 and MGAS2096) but not found in the nephritogenic group C strain. Protein folding of this domain may create similar structures in both groups that might contribute to APSGN, but such information is not available yet. Interestingly, the streptokinase gene from the non-nephritis M12 serotype strain MGAS9429 is 100% identical to the nephritogenic M12 strain. It is also possible that the mechanisms of pathogenesis that trigger nephritis following group A or group C infection may be different. Clearly, more detailed studies need to be done to address the possible role of streptokinase in APSGN.

4.2 Protease SpeB

Zabriskie and co-workers first observed that strains were associated with APSGN produced an extracellular protein not seen in non-nephritis strains, which was subsequently found to be bound to plasmin (Poon-King et al., 1993; Villarreal et al., 1979). This plasmin binding protein was discovered to be the major extracellular protease SpeB, and analysis of patient sera showed significantly higher levers of anti-SpeB antibodies in APSGN patients as compared to rheumatic fever patients or controls (Cu et al., 1998; Poon-King et al., 1993). Recent studies using a mouse tissue cage model have demonstrated that SpeB expression is inhibited in *S. pyogenes* strains that are maintained in the animal for extended periods, and this inhibition is inversely related to the increased expression of phage-encoded pyogenic exotoxins and DNases (Aziz et al., 2004). The strains studied were not associated with nephritis, suggesting that group A streptococci which cause APSGN may differentially express this protein when compared to non-nephritogenic strains. The lack of an identifiable homolog to SpeB in the group C strain MGCS10565 argues against the necessity of it in either the onset or progression of APSGN. However, the molecular mechanisms that underlie APSGN in the group C species may be different in some aspects from group A nephritogenic strains, and thus the absence of this protein in MGCS10565 may not be informative about group A APSGN disease. Further studies on this problem are clearly warranted.

4.3 Surface associated proteins

4.3.1 The Mga regulon genes

The molecular mimicry that underlies the onset of rheumatic heart disease results from the immune response to a prominent surface antigen of group A streptococci, the M protein. Therefore, such immunological cross-reactivity may be expected to contribute to APSGN, especially considering the time course of onset of symptoms. Particular M protein serotypes have been associated with APSGN, with M types 2, 49, 42, 56, 57, and 60 being associated with skin infections and APSGN while M types 1, 4, 12, and 25 being associated with throat infections and APSGN (Bessen et al., 1997; Bessen et al., 1996; Bisno, 1995; Enright et al., 2001; Kalia et al., 2002; Silva, 1998). As reported in the original description of the MGCS10565 genome, this group C contains a major deletion in much of the Mga regulon, including the genes for protease SpeB and the serum opacity factor (*sof*), although orthologs of many of these proteins including the M protein gene (*emm*) are found elsewhere in this genome (Beres et al., 2008). The genome of MGCS10565 revealed a large number of predicted extracellular collagen-like proteins, but none of these provided direct evidence of linkage to the nephritis-associated group A streptococcal serotypes. The linkage of serotype

and disease in the streptococcal, while clearly observed, remains somewhat unclear from the genetic level in terms of gene linkage or evolutionary co-selection.

4.3.2 Glyceraldehyde phosphate dehydrogenase (GAPDH)

Surface associated glyceraldehyde phosphate dehydrogenase (GAPDH) from nephritogenic strains of group A streptococci have been implicated in APSGN, with the molecule often detected in renal biopsies and patient sera having elevated anti-GAPDH antibody titers (Lange et al., 1976; Yamakami et al., 2000; Yoshizawa et al., 1992; Yoshizawa et al., 2004). This protein, which also has been referred to in the literature as the nephritis associated plasmin receptor, shows >85% homology between *S. zooepidemicus* MGCS10565 and the group A genome strains, which are virtually identical in amino acid sequence (Beres et al., 2008). Thus, it seems that if GAPDH plays a role in APSGN it is not a specific trigger for the disease. Indeed, other investigators have failed to find an association between GAPDH and APSGN (Batsford et al., 2005), so its role remains somewhat in question.

4.3.3 Enolase

Another *S. pyogenes* surface associated protein that has been implicated in APSGN is enolase, which is a major plasminogen binding protein and may be involved in triggering APSGN (Fontan et al., 2000). The phylogenetic analysis of the enolase genes from the *S. pyogenes*, *S. zooepidemicus*, *S. equisimilis* subsp. *dysgalactiae*, *S. pneumoniae*, and *S. mutans* genomes is presented in Fig. 3. The two *S. zooepidemicus* genes form a separate branch that is quite distinct from the *S. pyogenes* genes and encode proteins that are 100% identical.

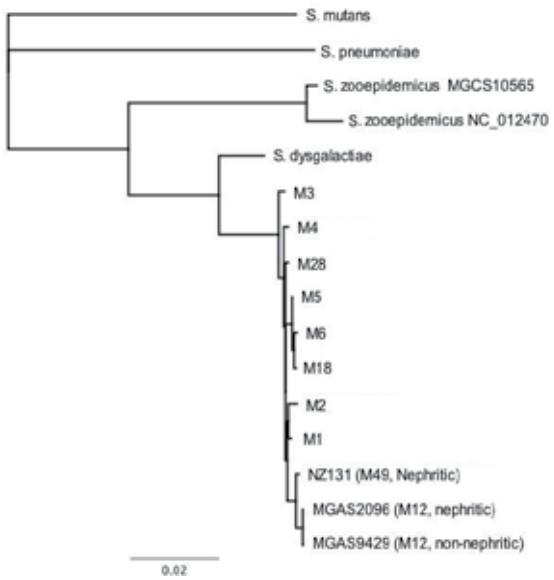

Fig. 3. **Phylogenetic analysis of streptococcal enolase genes.** The enolase genes from the group A streptococcal genomes (see Fig. 2 legend), now including the non-nephritogenic M12 strain MGAS2096, along with the genes from *S. zooepidemicus* strains MGCS10565 (nephritogenic) and NC_012470 (non-nephritogenic), *S. dysgalactiae* subsp. *equisimilis* GGS_124, *S. pneumoniae* TIGR4, and *S. mutans* UA159 were compared and a phylogenetic tree constructed using Geneious (Drummond et al., 2010).

Interestingly, the two nephritogenic group A streptococcal strains, NZ131 and MGAS2096, occupy a separate branch from the other *S. pyogenes* strains. However, this branch also includes the other M12 genome strain (MGAS9429) that was not nephritis associated. It may be that these closely related alleles of enolase play a role in the onset or development of APSGN in *S. pyogenes*, but the presence a non-nephritogenic strain argues that other factors must be required. Further, it would seem that enolase is probably not a common factor between groups A and C streptococci in the onset of APSGN.

4.3.4 Streptococcal pilus genes

Recent studies have demonstrated that group A streptococci and other Gram positive bacteria produce long, pili-like appendages that mediate binding to human fibronectin or collagen (Kang et al., 2007; Mora et al., 2005). These regions are identified by the presence of the genes encoding the pilus subunit proteins and their associated C sortases. A transcriptional regulator is included in the gene cluster of group A streptococci, and strains may be subdivided into two groups based upon whether the pilus region carries *rof*A or *nra* regulator genes. A recent classification scheme has described this region in *S. pyogenes* as belonging to one of six FCT groups (Kratovac et al., 2007). Strain NZ131 is a member of the *nra* group (FCT-3) that also includes the M3, M5, and M18 genome strains.

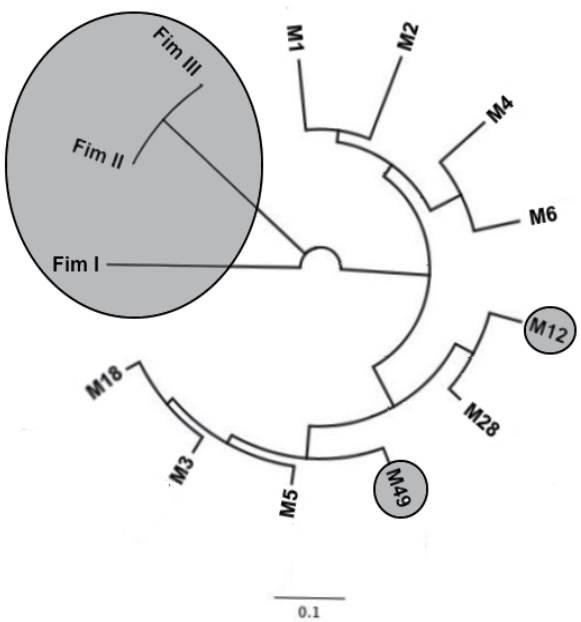

Fig. 4. **Phylogeny of the streptococcus pili regions.** The phylogenetic tree of the streptococcal pilus (fibronectin binding) regions from the *S. pyogenes* genome strains (see Fig. 2 legend) and from MGCS10565 is shown. The regions from the *S. pyogenes* genomes are indicated by the M protein serotype, and the nephritogenic group A streptococcal strains (NZ131 and MGCS2096) and group C strain (MGCS10565) are shaded.

Group C strain has three potential pilus (fibronectin binding protein) regions (Beres et al., 2008), which is in contrast to the one pilus (T antigen) cluster found in the group A

streptococci (McShan et al., 2008). Two of the group C pilus regions have homology to the pilus regions from the M2 and M6 genomes but little match that genome location in either the NZ131 or MGAS2096 chromosomes. Sortases are Gram-positive transpeptidases that anchor pili and other surface proteins to the cell wall (Hae Joo Kang and Baker, 2011; Janulczyk and Rasmussen, 2001). Strain NZ131 encodes an alternate sortase in this region (*src*C2, Spy49_0116). Interestingly, the *rof*A gene in MGCS10565 is not included in this gene cluster, being encoded elsewhere on the genome. When a phylogenetic tree of the pilus regions from the *S. pyogenes* and *S. equi* subsp. *zooepidemicus* genomes is constructed (Figure 4), the three pili regions (FimI – FimIII) form a distinct out-group from the *S. pyogenes* regions. The regions from the two nephritogenic group A streptococcal strains, NZ131 and MGAS2096, also are not closely related to each other, and the diversity of this region again argues that this is not the genetic location where are located unique factors that define nephritogenic strains of streptococci.

5. Conclusions

Comparison of the genomes of nephritogenic strain NZ131 and MGCS10565 reveals many similarities and differences that reflect related genera but distinct species. It is difficult, however, to make a convincing argument that an obvious shared trait is responsible for APSGN in both strains; rather, it is the differences that seem the most obvious when comparing potential virulence mechanisms.

One striking finding from genome comparison is the level of diversity between the group A streptococcal genomes and the genome of MGCS10565. Indeed, no common link to APSGN is immediately evident from the examination of the potential genetic sources from previous studies to the genomes. Beres et al. (Beres et al., 2008), after considering the diversity between the group A and group C genomes, tended to discount potential roles for streptokinase and protease SpeB in APSGN. Indeed, these proteins may not be key in the onset or progression of the disease although there are a number of studies supporting both. However, this conclusion presumes that the mechanism of pathogenesis of APSGN is the same in both species, which it well may not be. Many diarrheal diseases present with similar symptoms even though the underlying bacterial infection may be quite different, and it is possible that streptococcal nephritis caused by different species may result from a different series of molecular events. Further, even if there is a common molecular trigger for APSGN, it may be also true that for some bacterial factors that may amplify or promote the disease the phenotype may be more important through their enzymatic activity than their antigenicity. Thus, the important message from genomics at this time is that many aspects of APSGN remain to be explored so to uncover the roles played by virulence factors both known and yet to be characterized. Additional genome sequences from nephritogenic streptococci would help increase our understanding. The genomic information that is already available provides a rich resource for future studies as well as providing a framework for understanding the previous efforts in this field of investigation.

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10

Atypical Clinical Manifestations of Acute Poststreptococcal Glomerulonephritis

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1. Introduction

Acute poststreptococcal glomerulonephritis (APSGN) is one of the most common and important renal diseases resulting from a prior infection with group A β-hemolytic streptococcus (GAS) (Ash and Ingulli, 2008). Typical clinical features of the disease include an acute onset with gross hematuria, edema, hypertension and moderate proteinuria (acute nephritic syndrome) 1 to 2 weeks after an antecedent streptococcal pharyngitis or 3 to 6 weeks after a streptococcal pyoderma (Ahn & Ingulli 2008; Rodriguez-Iturbe & Mezzano, 2009). Gross hematuria usually disappears after a few days, while edema and hypertension subside in 5 to 10 days (Rodriguez-Iturbe & Mezzano, 2009). Although the incidence of APSGN appears to be decreasing in industrialized countries, more than 472,000 cases with APSGN are estimated to occur each year worldwide, with 97% of them occurring in developing countries (Carapetis et al., 2005; Eison et al., 2010).

APSGN occurs most commonly in children, 5 to 12 years old (Ahn & Ingulli, 2008), although 5 to 10 percent of the patients are more than 40 years old (Yoshizawa, 2000). The immediate and long-term prognoses of APSGN are excellent for children, assuming it is diagnosed in a timely fashion (Kasahara et al., 2001, Rodriguez-Iturbe & Musser, 2008). In contrast, adult patients with APSGN show markedly worse prognoses both in the acute phase and in the long-term (Rodriguez-Iturbe & Musser, 2008).

The most popular theory of the pathogenic mechanism of APSGN has been the immune-complex theory, which involves the glomerular deposition of nephritogenic streptococcal antigen and subsequent formation of immune complexes *in situ* and/or the deposition of circulating antigen-antibody complexes (Oda et al., 2010). Two antigens have been actively investigated as the potential causes of APSGN (Rodriguez-Iturbe & Musser, 2008): the nephritis-associated plasmin receptor (NAPlr) also known as streptococcal glyceraldehyde-3-phosphate dehydrogenase (Yamakami et al., 2000; Yoshizawa et al., 2004), and a cationic cysteine proteinase known as streptococcal pyogenic exotoxin B (SPEB) (Batsford et al., 2005).

Patients with APSGN sometimes exhibit atypical or unusual clinical manifestations, which may lead to diagnostic delay or misdiagnosis of the disorder (Eison et al. 2011; Pais et al. 2008). Recognition of these unusual manifestations in cases of APSGN is important in order to assure that the patient receives adequate treatment. In this chapter, I review the atypical clinical manifestations of APSGN.

2. Atypical manifestations of APSGN

Atypical manifestations of APSGN can be classified as the following: co-occurrence of immune-mediated diseases; non immune-mediated complications; and unusual clinical presentations or courses (Table 1).

Immune-mediated diseases
Acute rheumatic fever (ARF)
Poststreptococcal reactive arthritis (PSRA)
Vasculitis
Immune thrombocytopenic purpura (ITP)
Autoimmune hemolytic anemia (AIHA)
Diffuse alveolar hemorrhage (DAH)
Uveitis
Non immune-mediated complications
Posterior reversible encephalopathy syndrome (PRES)
Thrombotic microangiopathy (TMA)
Gallbladder wall thickening
Unusual clinical presentations or courses
Minimal urinary abnormalities
Recurrence

Table 1. Atypical manifestations of acute poststreptococcal glomerulonephritis

2.1 Immune-mediated diseases

Immune-mediated diseases most likely result from immune-complex formation between streptococcal antigens and their associated antibodies, and include acute rheumatic fever, poststreptococcal reactive arthritis, vasculitis, immune thrombocytopenic purpura, autoimmune hemolytic anemia, diffuse alveolar hemorrhage and uveitis.

2.1.1 Acute rheumatic fever

Acute rheumatic fever (ARF) is an autoimmune disease that follows infection by GAS and is characterized by inflammation of several tissues that gives rise to typical clinical characteristics (the so-called Jones criteria) including carditis/valvulitis, arthritis, chorea, erythema marginatum, and subcutaneous nodules (Steer & Carapetis, 2009). ARF is rare in developed countries, but it remains common in developing countries and some poor, mainly indigenous populations of wealthy countries (Steer & Carapetis, 2009; Carapetis et al., 2005).

Although both ARF and APSGN develop following GAS infection, the two diseases have different epidemiology, immunology and bacteriology, and simultaneous occurrence of them in the same patient is rare (Lin et al., 2007). Since Gibney et al. first reported a patient with co-occurrence of ARF and histologically proven APSGN (Gibney et al., 1981), seventeen patients with concurrent ARF and APSGN have been reported (Akasheh et al., 1995; Ben-Dov et al., 1985; Castillejos et al., 1985; Imanaka et al., 1995; Kakkera et al., 1998; Kujala et al., 1989; Kula et al., 2003; Kwong et al., 1987; Lin et al., 2003; Mastell et al., 1990; Öner, et al., 1993; Said et al., 1986; Sieck et al., 1992; Sinha et al., 2007).

Fourteen patients were children and ten were male. Eight patients initially presented with ARF preceding APSGN, 3 patients suffered from ARF following the development of APSGN and both ARF and APSGN simultaneously occurred in 6 patients. Although many patients showed carditis (16 out of 17 patients) and polyarthritis (13 out of 17 patients), the remaining characteristics (erythema marginatum, chorea and subcutaneous nodules) developed in only 4 patients, 1 patient and 1 patient, respectively.
Although it remains unclear why simultaneous occurrence of APSGN and ARF is so rare, one explanation may be that only few streptococcal strains have both nephritogenic and rheumatogenic antigenic features (Lin et al., 2003).

2.1.2 Poststreptococcal reactive arthritis

Poststreptococcal reactive arthritis (PSRA) is defined as acute arthritis of more than 1 joint following an episode of GAS infection in a patient whose illness does not fulfill the Jones criteria for the diagnosis of ARF (Barash et al., 2008; Gerber M, 2007). It remains controversial whether or not PRSA and ARF are distinct entities or not (Gerber M, 2007). Mackie and Keat reviewed 188 published cases of PSRA and concluded that PSRA was a heterogenous group of clinical entities (Mackie & Keat, 2004). However, two recent studies suggested that PSRA and ARF were separate disease entities on the basis of the differences in clinical presentation and disease course (Barash et al., 2008; van der Helm-van Mil, 2010). Compared to patients with ARF, patients with PSRA are older, respond poorer to salicylates and have non-migratory and persistent arthritis (Tokura et al., 2008; van der Helm-van Mil, 2010). The precise pathogenic mechanism underlying the development of PSRA is unclear, production of antistreptococcal antibodies that cross-react with human epitopes causing inflammation and tissue damage is a likely pathogenic mechanism for PSRA, as has been proposed for ARF (Niewold & Ghosh, 2003).
Simultaneous occurrence of PSRA and APSGN is rare, with only 3 cases having been reported (Niewold & Ghosh, 2003; Sugimoto et al., 2008; Tokura et al., 2008). Niewold & Ghosh described a 44-year-old man who developed severe PSRA and APSGN after a subclinical streptococcal infection (Niewold & Ghosh, 2003). Tokura et al. reported a 16-year-old man who presented with simultaneous occurrence of APSGN and PSRA with symmetric persistent tenosynovitis in hands and feet (Tokura et al., 2008). Sugimoto et al. described a 61-year-old man who exhibited PSRA and APSGN with marked renal interstitial inflammation after bacterial endophthalmitis due to *Streptococcus pyogenes* (Sugimoto et al., 2008). Arthritis of all the patients improved with corticosteroid therapies without any sequellae.

2.1.3 Vasculitis

Vasculitis is not a well-recognized condition associated with GAS infection, but there have been several reports of Henoch-Schönlein purpura (HSP) (al-Sheyyab et al., 1999) or Henoch-Schönlein purpura with nephritis (HSPN) (Masuda et al., 2003), cutaneous leukocytoclastic vasculitis (Chalkias et al, 2010), vasculitic neuropathy (Traverso et al., 1997), polyarteritis nodosa (PN) (David et al., 1993) and unclassified systemic vasculitis (Lucas & Moxham, 1978). Although the precise pathogenic role of GAS infection contributing to the development of vasculitis remains unclear, an immune complex-mediated mechanism triggered by GAS infection has been postulated (Ritt M. et al., 2006).
Vasculitides including cutaneous vasculitis mimicking HSP, cerebral vasculitis, PN, necrotizing vasculitis and Wegener's granulomatosis have been described to occur in patients with APSGN.

HSP is an IgA immune complex-mediated systemic leukocytoclastic vasculitis of small vessels that primarily affects the skin, gastrointestinal tract, joints and kidney (Dedeoglu & Sundel, 2007; Robson & Leung , 1994). Respiratory infections with GAS preceding the onset of HSP have been reported in up to one-third of cases (Dedeoglu & Sundel, 2007). APSGN patients simultaneously presenting with HSP or vasculitis mimicking HSP are rare and only five patients have been reported. Goodyer et al. described two boys with histologically proven APSGN, in whom cutaneous vasculitis and abdominal symptoms mimicked HSP (Goody et al., 1978). Onisawa et al. reported a patient with concurrent APSGN and cutaneous leukocytoclastic vasculitis without IgA deposition (Onisawa et al., 1989). Maruyama et al. presented a patient with congenital complement 9 deficiency exhibiting biopsy-proven APSGN and clinical symptoms mimicking HSP (Maruyama et al., 1995). Matsukura et al. also described a 20-month-old girl with biopsy-proven APSGN, who presented with cutaneous vasculitis mimicking HSP (Matsukura et al., 2003). All patients recovered completely without any sequellae.

Central nervous system abnormalities of APSGN are usually secondary to acute severe hypertension, electrolyte disturbances or uremia, but can also be attributed to cerebral vasculitis (Dursun et al., 2008; Ritt et al., 2006). To date, five cases of cerebral vasculitis associated with APSGN have been reported (Dursun et al., 2008; Kaplan et al., 1993; Ritt et al., 2006; Rovang et al., 1997; Wong & Morris, 2001), in which 4 patients were children. Clinical features of cerebral vasculitis in APSGN include severe headache with nausea and vomiting, transient focal neurological signs, visual disturbances, and seizures. Although the computed tomography of the brain may not detect abnormalities, the magnetic resonance imaging of the brain often demonstrated multiple supratentorial areas of abnormal signal intensity in the white and adjacent grey matter (Dursun et al., 2008). All patients underwent corticosteroid therapy (three of them commenced on methylprednisolone pulse therapy) and recovered without any neurological sequellae.

PN is a necrotizing vasculitis affecting the medium-sized muscular arteries (Dillon et al., 2010). Five patients with co-occurrence with PN and APSGN have been described. Fordham et al. reported three young adult patients with both PN and APSGN, two of whom died secondary to multi-system organ failure (Fordham et al., 1964). Blau et al. described two children with PN who also had serological and clinical evidence of APSGN (Blau et al., 1977).

Although extremely rare, necrotizing vasculitis other than PN (Bodaghi et al., 1987; Ingelfinger et al., 1977) and Wegener's granulomatosis (Garrett et al., 1993) has also been reported in patients with APSGN.

2.1.4 Immune thrombocytopenic purpura

Immune thrombocytopenic purpura (ITP) is an immune-mediated acquired disorder in which antiplatelet antibodies cause accelerated destruction of platelets, resulting in thrombocytopenia and an increase risk of bleeding (Psaila & Bussel, 2007). Childhood ITP often occurs following an infection with viruses such as varicella zoster, rubella, Epstein-Barr, influenza, or human immunodeficiency virus (Tasic & Polenakovic, 2003), but may also be preceded by a bacterial infection (Muguruma et al., 2000). Recently, a number of studies have suggested an association between *Helicobacter pylori* and ITP (Cooper & Bussel, 2006).

Since Kaplan and Esseltine first reported ITP in two patients with APSGN (Kaplan & Esseltine, 1978), five cases of ITP in patients with APSGN have been reported (Muguruma et al., 2000; Rizkallah et al., 1984; Tasic & Polenakov, 2003). All patients were children (4 to 7 years of age) who underwent corticosteroid therapy and fully recovered from APSGN and

ITP. One patient exhibited a marked increase in platelet-associated immunoglobulin G level (Muguruma et al., 2000). Although the precise pathogenic mechanism for the development of ITP in patients with APSGN is unclear, Muguruma et al. speculated about the pathogenesis of associated diseases through production of autoantibodies cross-reactive against GAS and against platelets (Muguruma et al., 2000).

2.1.5 Autoimmune hemolytic anemia

Anemia is common in APSGN and traditionally it has been attributed solely to volume overload (Eison et al., 2011). However, autoimmune hemolytic anemia (AIHA) has recently been reported in patients with APSGN. AIHA is a clinical condition in which IgG and/or IgM antibodies bind to red blood cells (RBC) surface antigens and initiate RBC destruction via the complement system and the reticuloendothelial system (Gehrs & Friedberg, 2002). Subtypes include warm AIHA, cold AIHA, mixed-type AIHA and drug-induced immune hemolytic anemia. Furthermore, cold AIHA has been categorized into cold agglutinin syndrome (CAS) and paroxysmal cold hemoglobinuria (PCH). Infectious agents associated with CAS or PCH include *Mycoplasma pneumonia*, Epstein-Barr virus, adenovirus, cytomegalovirus, influenza viruses, human immunodeficiency virus, measles, mumps, *Escherichia coli*, *Listeria monocytogenes, Haemophilus influenza* and *Treponema pallidum* (Gehrs & Friedberg, 2002).

Greenbaum et al. described three children with both APSGN and cold AIHA, two of whom had an anti-I autoantibody and thereby were diagnosed with CAS (Greenbaum et al., 2003). Two patients were transfused and all patients recovered from AIHA and APSGN. Cachat et al. presented a 10-year-old child with concurrent cold AIHA and APSGN, who developed anuric acute renal failure and profound anemia (Cachat et al., 2003). The patient responded well to corticosteroid therapy and had a full renal and hematological recovery.

2.1.6 Diffuse alveolar hemorrhage

Diffuse alveolar hemorrhage (DAH) is sometimes accompanied by glomerulonephritis, and is often referred to as pulmonary-renal syndrome (Papiris et al., 2007). Pulmonary-renal syndromes include Goodpasture's syndrome, antineutrophil cytoplasmic antibody-associated vasculitis, immune complex-associated glomerulopathy and thrombotic microangiopathy (Papiris et al., 2007).

DAH associated with APSGN is extremely rare and only three patients have been reported. Chugh et al. described a 38-year-old-male with concurrent DAH and crescentic APSGN who progressed to end-stage renal failure (Chugh et al., 1981). Gilboa et al. reported a 12-year-old girl who exhibited a noncrescentic APSGN and DAH (Gilboa et al., 1993). Sung et al. recently described a 59-year-old-woman with APSGN and DAH (Sung et al., 2007). DAH in all three patients subsided after intravenous corticosteroid therapies. The pathogenic mechanism of DAH in APSGN remains unclear.

2.1.7 Uveitis

Uveitis is believed to be an immunological response to exogenous and endogenous antigens (Leiba et al., 1998) and can occur following GAS infection, so-called "poststreptococcal uveitis". Since Cokingtin & Han reported the first case of poststreptococcal uveitis in 1991 (Cokingtin & Han, 1991), several patient reports and case series with poststreptococcal uveitis have been presented (Leiba et al., 1998, Ur Rehman et al., 2006).

Feldon et al. recently reported the first case of a child with concomitant APSGN and uveitis, whose uveitis subsided within a few days with topical corticosteroid and mydriatic treatment (Feldon et al., 2010).

2.2 Non immune-mediated complications

Non immune-mediated complications of APSGN include posterior reversible encephalopathy syndrome, thrombotic microangiopathy and gallbladder wall thickening.

2.2.1 Posterior reversible encephalopathy syndrome

Posterior reversible encephalopathy syndrome (PRES), also known as reversible posterior leukoencephalopathy syndrome (RPLS), is a recently described brain disorder associated with findings on neuroimaging that suggest white-matter edema, mostly in the posterior parietal-temporal-occipital regions of the brain (Hinchey et al., 1996). However, radiological lesions in PRES are rarely isolated to these areas, and often involve the cortex, frontal lobes, basal ganglia and brainstem (Fugate et al., 2010) (Fig. 1).

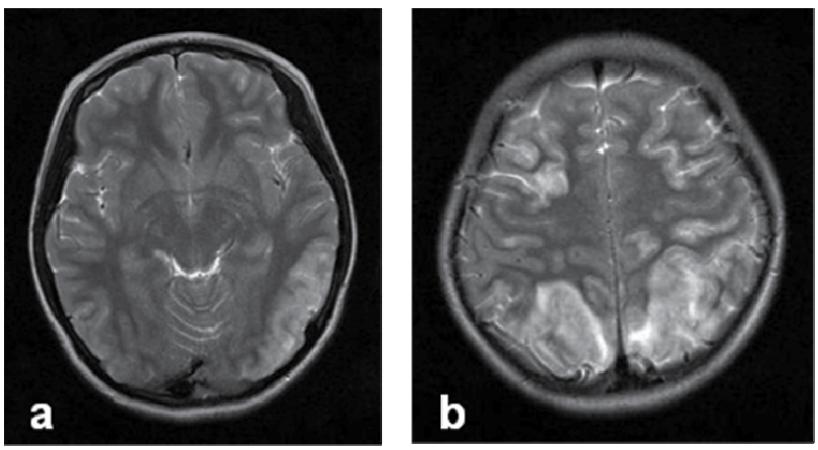

Fig. 1. Magnetic resonance images (MRI) of the brain (T2-weighted image) showing increased intensity in the cortex and subcortical white matter of left occipital (a), bilateral parietal and right frontal lobes (b), consistent with PRES.

The clinical characteristics of the disease include headache, decreased alertness, altered mental functioning, seizures, and visual loss including cortical blindness (Hinchey et al., 1996). The syndrome has been associated with acute hypertension, preeclampsia or eclampsia, glomerulonephritis, sepsis, autoimmune disorders and immunosuppressive or chemotherapeutic treatments (Bartynski, 2008a; Hinchey et al., 1996). Although the underlying pathophysiology of PRES remains elusive, three theories have been proposed: 1) hypertension-induced breakdown in cerebral auto-regulation; 2) cerebrovascular endothelial dysfunction and; 3) vasoconstriction and hypoperfusion with subsequent ischemia and vasogenic edema (Bartynski, 2008b; Fugate et al., 2010). The preferential involvement of the posterior brain in PRES may be caused by its relative paucity of sympathetic innervation in comparison to the anterior circulation (Froehlich et al., 1999). The outcome of PRES is generally favorable, but delay in initiating the appropriate treatment may result in permanent damage to the brain (Fux et al., 2006; Garg, 2001).

While it is estimated that PRES occurs in 5% to 10% of children hospitalized with acute glomerulonephritis of all etiologies, the prevalence of PRES associated with APSGN is unknown (Froehlich et al., 1999). PRES caused by hypertension has been reported in 7 children (from 7 to 15 years of age) with APSGN (Froehlich et al., 1999; Fux et al., 2006; Guputa et al., 2010; Nordby, 1997; Özcakar et al., 2004; Soylu et al., 2001). Six patients complained of headache, 5 exhibited decreased alertness and seizures, and 3 had altered mental functioning and visual loss. All patients exhibited abnormal findings of the brain MRI or CT in the white matter of the parietal and occipital lobes, and recovered without any neurological sequellae following adequate treatment of the associated hypertension.
One patient with APSGN suffered from PRES without severe hypertension (Nordby, 1997). The most important factor in development of pediatric hypertensive PRES is the rapidity of blood pressure elevation and the degree of elevation relative to the patient's baseline pressure (Froehlich et al., 1999). It has been suggested that blood pressures more than 30% above normal for age should alert clinicians to the possibility of hypertensive PRES (Nordby, 1997).

2.2.2 Thrombotic microangiopathy

Thrombotic microangiopathy (TMA) is a pathological term used to describe occlusive microvascular thrombus formation and is most commonly associated with hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) (Keir & Coward, 2010). Pathological features of TMA include vessel wall thickening, swelling and detachment of the endothelial cell from the basement membrane, accumulation of material in the subendothelial space, intraluminal platelet thrombosis, partial or complete vessel luminal obstruction and fragmentation of red blood cells (Keir & Coward, 2010). HUS is defined as the triad of microangiopathic hemolytic anemia, thrombocytopenia, and acute renal injury (Copelovitch & Kaplan, 2008). TTP is characterized by the pentad of microangiopathic hemolytic anemia, thrombocytopenia, fever, acute renal injury, and neurological abnormalities (Copelovitch & Kaplan, 2008).
TMA has been reported in 8 patients with APSGN, consisting of 5 children and 3 adults (Duvic et al., 2000; Izumi, et al., 2005; Laube, et al., 2001; Medani et al., 1987; Proesmans, 1996; Siebels et al., 1995; Tan et al., 1998). All patients exhibited severe hypertension. Hemodialysis or peritoneal dialysis was required in 2 patients. Renal biopsy showed histological features of both APSGN and TMA in 3 patients, and revealed characteristics of APSGN without features of TMA in 5 patients. The outcome in all patients was excellent.
The precise pathogenesis of TMA in patients with APSGN is unclear, although two causes have been postulated: severe hypertension and streptococcal neuraminidase (Duvic et al., 2000; Laube et al., 2001; Izumi et al., 2005). HUS has been reported as a complication of severe hypertension, regardless of the cause (Broyer, 1995). If severe hypertension is transient, histological lesions of TMA are absent. When hypertension becomes malignant, renal histological lesions show features of TMA (Duvic et al., 2000). Another possible cause of TMA in APSGN is alteration of vascular endothelial cells by streptococcal neuraminidase. Circulating neuraminidase causes exposure of the cryptic T-antigen on cell surfaces, to which most people possess a naturally occurring antibody. Therefore antigen-antibody interaction may damage the vascular endothelial cells leading to the clinical manifestations of HUS (Izumi et al., 2005).

2.2.3 Gallbladder wall thickening

Thickening of the gallbladder wall is the most common findings in acute cholecystitis, but it has also been reported in patients with kidney diseases including pyelonephritis (Talarico & Rubens, 1990) and chronic kidney failure (van Breda Vriesman et al., 2007). Only one child with APSGN and gallbladder wall thickening has been reported (Watanabe & Baba, 2009). Although the pathogenesis of this complication is unclear, elevated systemic venous pressure or subclinical vasculitis may have caused edema of the gallbladder wall (Watanabe & Baba, 2009).

2.3 Unusual clinical presentations or courses

Unusual clinical presentations or courses of APSGN include acute nephritic syndrome with minimal urinary abnormalities, and recurrence of the disease.

2.3.1 Minimal urinary abnormalities

Patients with APSGN usually exhibit hematuria and proteinuria. However, Blumberg and Feldman first reported two children with biopsy-proven APSGN without any urinary abnormalities (Blumberg & Feldman, 1962). Thereafter, several authors described biopsy-proven APSGN patients with minimal or no urinary abnormalities including 13 children and 4 adult patients (Albert et al., 1966; Cohen & Levitt, 1963; Dunn, 1967; Fujinaga et al., 2007; Goorno et al., 1967; Grossman et al., 1973; Hoyer et al., 1967; Kandall et al., 1969; Kobayashi et al., 1971). All patients exhibited edema and hypertension, and seven showed pulmonary edema or congestion. Hypertensive encephalopathy occurred in one patient (Hoyer et al., 1967) and acute rheumatic fever developed in another patient (Cohen & Levitt, 1963). All patients recovered completely without any sequellae. The mechanism for the elaboration on normal or minimal urinary abnormalities during the course of APSGN is unclear (Fujinaga et al., 2007; Kandall et al., 1969).

2.3.2 Recurrence

Recurrence of APSGN is a well-recognized, but relatively rare phenomenon, probably due to the relatively limited number of nephritogenic strains of streptococci and the acquisition of protective immunity against a nephritogenic streptococcal antigen after an initial episode of APSGN (Watanabe & Yoshizawa, 2001). Ramberg first mentioned this condition and reported eleven patients with the recurrent attacks out of 152 patients with APSGN (Ramberg, 1947). Thereafter, several clinical studies of APSGN have suggested an incidence of recurrent APSGN that ranges from 0.7% to 7.0% (Baldwin D et al., 1974; Bernstein et al., 1960; Dodge W. et al., 1968; Sanjad et al., 1977; Roy et al., 1969). In addition, a few case reports of recurrent APSGN have been described (Casquero et al., 2006; Derakhshan 2002; Kim et al., 1979; Rosenberg et al., 1984; Velhote et al., 1986; Watanabe & Yoshizawa, 2001). Clinical features and outcomes were well-described in 35 patients including 22 children. Most patients suffered from one recurrent episode, but one patient exhibited 2 recurrent attacks of APSGN (Velhote et al., 1986). Twenty-nine patients recovered completely, whilst 4 patients continued to have some urinary abnormalities and two patients progressed to end-stage renal failure.

Although the exact mechanism leading to recurrence of APSGN has not yet been determined, three possible explanations have been postulated: the suppression of immune response against nephritogenic streptococcal strains due to early antibiotic therapy (Roy et

al., 1969; Sanjad et al., 1977); an absence of natural immune responses against nephritogenic streptococcal components without antibiotic therapy (Watanabe & Yoshizawa, 2001), and; a failure to exclude microbial agents through the digestive and respiratory tract due to IgA deficiency (Casquero et al., 2006).

Sanjado et al. suggested that reinfection with the same type of *Streptococcus* would occur if the patient lacked antibodies against that particular type, and penicillin therapy given in the first ten days after a streptococcal infection suppressed the formation of type-specific immunity conferring antibodies, which might increase the chances of re-infection with the same nephritogenic strain responsible for the initial episode of APSGN (Sanjad et al., 1977).

Recently, Yoshizawa et al. identified a new nephritogenic streptococcal antigen and termed it nephritis-associated plasmin receptor (NAPlr) (Yamakami et al., 2000; Yoshizawa et al., 2004). They demonstrated that most patients with APSGN had high titers of long-lasting antibody against NAPlr and that it was present in glomeruli in 100% of patients with APSGN early in the disease (Yoshizawa et al., 2004). Watanabe and Yoshizawa described an 8-year-old boy with recurrent ASPGN who did not have serum antibodies against NAPlr, even though NAPlr was detected in glomeruli of an early kidney biopsy specimen from the patient during the second attack of APSGN. These results indicated that recurrence of APSGN in some patients might be caused by an absence of a natural immune response to NAPlr (Watanabe & Yoshizawa, 2001).

Recently, Casquero et al. published a patient with selective IgA deficiency who experienced two episodes of APSGN (Casquero et al., 2006), suggesting that a failure of IgA defenses might also predispose to streptococcal re-infection and cause recurrent APSGN.

3. Conclusions

Patients with APSGN sometimes exhibit atypical or unusual clinical manifestations, which are divided into 3 categories: immune-mediated diseases (ARF, PSRA, vasculitis, ITP, AIHA, DAH and uveitis), non-immune mediated conditions (PRES, TMA and gallbladder wall thickening), and unusual clinical presentations or courses (minimal urinary abnormalities and recurrence).

Immune-mediated diseases seem to result from immune-complex formation between streptococcal antigens and their associated antibodies. Hypertension contributes to the development of PRES and TMA, while fluid retention results in PRES and gallbladder wall thickening. Recurrence of APSGN may be the consequence of suppressed immune responses against nephritogenic streptococcal strains caused by early antibiotic therapy, by the absence of natural immune responses against NAPlr, or by selective IgA deficiency.

Because atypical or unusual manifestations of APSGN may lead to diagnostic delays or misdiagnosis of the disorder, recognition of them is important in order to assure that the patient receives adequate treatment.

4. References

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11

Hepatitis C Virus Associated Glomerulonephritis

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1. Introduction

Approximately 170 million persons worldwide are infected with the hepatitis C (HCV) virus. The incidence of glomerulonephritis in HCV-infected patients is unknown due to a lack of large-scale cross sectional surveys however subclinical renal involvement is believed to be highly prevalent among patients with HCV hepatitis. The most common HCV-associated glomerulonephritis is membranoproliferative glomerulonephritis (MPGN) type 1 with or without cryoglobulinaemia. MPGN typically presents several years, and often decades, after initial infection with HCV. Most patients have laboratory evidence of hypocomplementaemia, circulating rheumatoid factors, and cryoglobulinaemia. Other uncommon forms of glomerular disease that have been reported to be associated with HCV infection include membranous nephropathy, IgA nephropathy, focal segmental glomerulosclerosis, fibrillary glomerulonephritis/immunotactoid glomerulopathy, pauci-immune glomerulonephritis, and thrombotic microangiopathy.

The principal clinical manifestations of glomerular disease in HCV patients are the presence of proteinuria and microscopic haematuria with or without impaired kidney function. The clinical course of these HCV-associated glomerulopathies is generally characterised by remission and relapsing phases. The overall prognosis for HCV-associated glomerulonephritis remains poor, not only because of renal disease progression but because of the high incidence of cardiovascular disease, infection and hepatic failure.

The exact pathogenic sequence of injury that results in glomerulonephritis is not known. The prevailing theory is that glomerular injury results from deposition of circulating immune complexes that contain HCV antigens and anti-HCV antibody. Involvement of the innate immune system in HCV-associated MPGN has been suggested with demonstration of upregulation of Toll-like receptor 3.

In establishing a link between HCV infection and the immune response targeting the glomerulus, antiviral, plasma exchange and immunosuppressive therapies have been used in patients. The use of antiviral therapy in HCV-positive patients with glomerulonephritis is targeted at eliminating the virus and reducing the generation of HCV-related antibodies and immune complexes. The data to support antiviral treatment for HCV-associated glomerulonephritis is limited, however interferon therapy may be superior to

immunosuppressive agents in HCV-associated cryoglobulinaemic glomerulonephritis in lowering proteinuria. Agents such as rituximab have also been shown to be efficacious in the treatment of HCV-associated cryoglobulinaemic glomerulonephritis.

This chapter provides the reader with an overview of hepatitis C-associated glomerulonephritis that covers epidemiology, clinical manifestations, natural history, immunopathophysiology, and a review of the evidence underpinning current therapeutic approaches.

Hepatitis C virus (HCV) is a leading cause of chronic liver disease in the world. The World Health Organization estimates that there are 170 million individuals with HCV infection and an incidence of 3–4 million new cases per year (WHO, 2000). HCV infection leads to chronic liver disease, but also to extra-hepatic manifestations. These include mixed cryoglobulinaemia, lymphoproliferative disorders and renal disease. HCV infection has been reported in association with distinct histological patterns of glomerulonephritis.

In this review, we will canvass the epidemiology, clinical manifestations, natural history, immunopathophysiology, and current therapies of HCV-associated glomerulonephritis, as well as cover issues around renal transplantation.

2. Immunopathogenesis

HCV is a single-stranded enveloped RNA virus. Its genome codes for a nucleocapsid core protein, envelope proteins, and a number of non-structural proteins. Glomerular injury due to HCV occurs as a result of the direct interaction of viral RNA and proteins with glomerular cells, as well as indirectly through immunological mediators such as immune complex deposition.

The adaptive immune response to HCV infection includes both cellular and humoral pathways. Unlike the overt cytotoxic cellular T-cell response to infected hepatocytes in the liver, the mechanism of injury in glomerulonephritis appears predominantly due to circulating immune complex deposition. This humoral response to HCV infection includes the production of various antibodies against HCV protein antigens. To evade this, HCV displays antigenic variability resulting from lack of proofreading activity in the HCV RNA polymerase. Hypervariable regions are present particularly in the envelope protein E2 sequence. Combined with a high replication rate, this antigenic variability allows mutant virus strains to escape antibody binding. Thus in chronic HCV infection, HCV RNA persists in serum despite the presence of anti-HCV antibodies. The chronic simultaneous presence of both HCV antigens and anti-HCV antibodies is a fertile setting for the formation of circulating immune complexes. In addition, HCV-induced liver injury decreases the hepatic clearance of circulating immune complexes, prolonging their survival in the circulation.

Often the anti-HCV antibodies have the properties of cryoglobulins, that is they precipitate at low temperatures. Serum cryoglobulins are present at low levels in up to 50% of chronic HCV infected patients, however symptomatic cryoglobulinaemia occurs in 1% or less of patients and usually only after years of chronic infection (Meyers et al., 2003). In HCV infection, the cryoglobulins are almost always of mixed type, with type 2 (monoclonal rheumatoid factor usually IgM kappa) more common than type 3 (polyclonal rheumatoid factor) (Miller and Howell, 2000). Possibly, IgM directed against epitopes of the HCV envelope cross-reacts with IgG, forming the rheumatoid factor (Alpers and Kowalewska, 2007). Thus, the cryoglobulins in HCV infection are composed of HCV RNA and/or

proteins, complexed with anti-HCV IgG, in turn complexed to IgM rheumatoid factor. The concentration of HCV RNA in cryoprecipitates has been found to be around 1,000 times higher than in serum (Kamar et al., 2008). While symptomatic cryoglobulinaemia is relatively rare in HCV infected patients, conversely the vast majority (over 80%) of patients with mixed cryoglobulins have evidence of HCV infection (Kamar et al., 2008).

Circulating immune complexes, including cryoglobulins, deposit in glomeruli along the capillary walls and in the mesangium. IgM kappa rheumatoid factor has a particular affinity for cellular fibronectin in the mesangial matrix (Perico et al., 2009). While immunoglobulins are readily and routinely identified in glomerular tissues, the demonstration of HCV antigens in glomeruli is controversial and limited to relatively small numbers of studies. For example, Sansonno and colleagues (2005) used laser capture microdissection combined with PCR to identify HCV RNA in glomeruli, as well as immunohistochemistry to identify HCV core protein in glomeruli. Virus-like particles have been identified by electron microscopy in renal biopsies of patients with HCV infection (Sabry, 2002).

While much work has focused on humoral immunity and immune complex deposition, there has also been research on the innate immune response of the glomerulus to HCV. This innate immune response to microbes involves Toll-like receptors (TLRs), transmembrane proteins that recognise microbial antigens. Dolganiuc and colleagues (2004), using a variety of human and mouse cell types, found that HCV core protein and HCV non-structural protein 3 act via Toll-like receptor 2 (TLR2) to trigger inflammatory pathways. In the kidney, it is also hypothesized that HCV RNA is recognised by Toll-like receptor 3 (TLR3) expressed on mesangial cells. Using a microdissection technique, increased TLR3 mRNA expression has been demonstrated in glomeruli with HCV-associated glomerulonephritis, compared to glomeruli with non-HCV-associated glomerulonephritis (Wornle et al., 2006). Recognition of HCV by TLR3 with subsequent intracellular signalling activates mesangial cells to produce pro-inflammatory cytokines and growth factors. The cytokines involved include TNFalpha and the chemokine IP-10 (interferon gamma inducible protein-10), both of which are upregulated in HCV-associated glomerulonephritis (Merkle et al., 2011).

The innate immune response, together with the trapping and deposition of immune complexes, both contribute to generate activation of local inflammatory and complement cascades in glomeruli. These cascades induce glomerular cell proliferation and matrix production as well as the recruitment of inflammatory cells, which can be seen morphologically as the characteristic histological patterns of glomerulonephritis. While some morphological patterns are characteristic of HCV, none is specific, and the diagnosis of HCV-associated glomerulonephritis relies on correlation with clinical findings as well as the presence of serum anti-HCV antibody and HCV RNA. Evidence of HCV RNA and/or core protein in glomeruli has been identified irrespective of the histological pattern of glomerulonephritis (Sansonno et al., 2005).

Membranoproliferative glomerulonephritis (MPGN) type 1 with or without cryoglobulinaemia is the most characteristic pattern, comprising the large majority (roughly 80%) of all HCV-associated glomerulonephritis. Conversely, HCV is perhaps the most important cause of secondary MPGN. Histologically, the glomeruli have a membranoproliferative pattern with accentuated lobularity, mesangial hypercellularity, and mesangial interpositioning causing double contouring of the capillary walls. The glomeruli may have prominent inflammatory cell infiltration, particularly of monocytes (Alpers and Kowaleska, 2007). See Figure 1

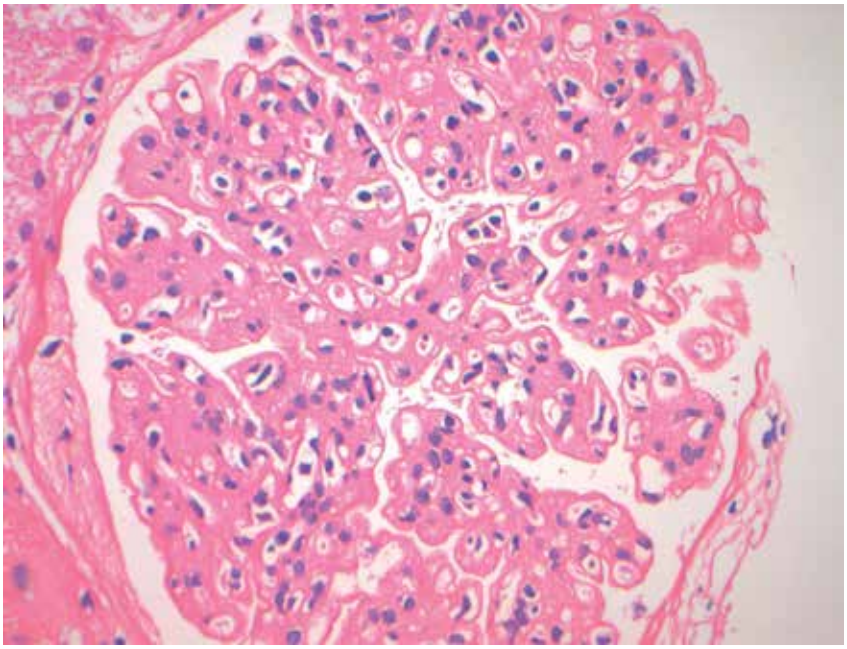

Fig. 1a. Membranoproliferative glomerulonephritis in a HCV-infected patient, with accentuated lobularity, mesangial hypercellularity and intracapillary mononuclear inflammatory cells (H&E, 400x).

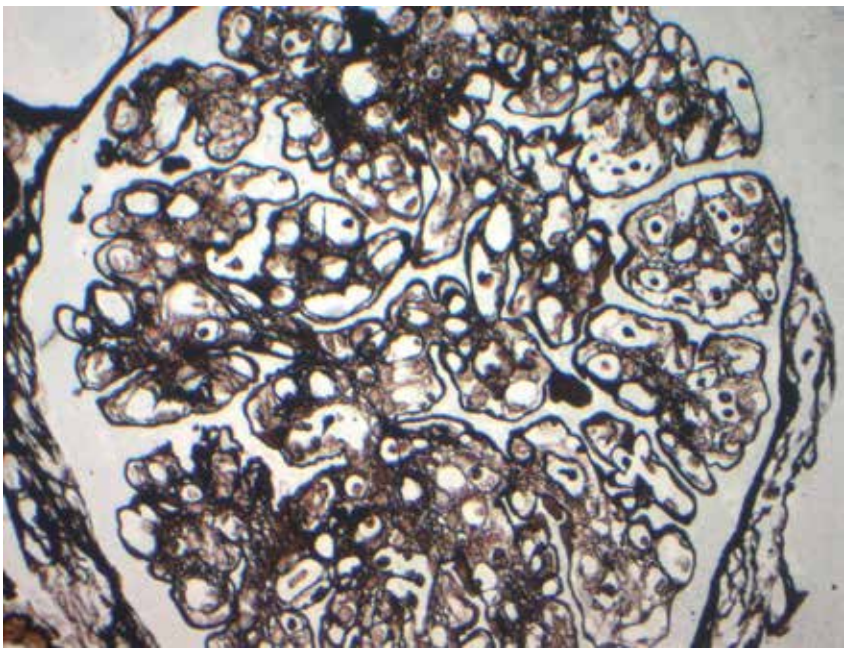

Fig. 1b. Double contouring of the capillary basement membranes is more evident on silver staining (Modified Wilder's silver stain, 400x).

Immunofluorescence staining highlights granular deposits of C3 and immunoglobulins (usually IgG and IgM) along capillary walls and in the mesangium, and electron microscopy confirms electron-dense immune complex deposits in subendothelial and mesangial locations. Cryoglobulin deposits themselves also produce a membranoproliferative pattern of injury. In these cases with cryoglobulinaemia, the deposits themselves may be seen histologically as eosinophilic glomerular capillary 'hyaline thrombi'. See Figure 2

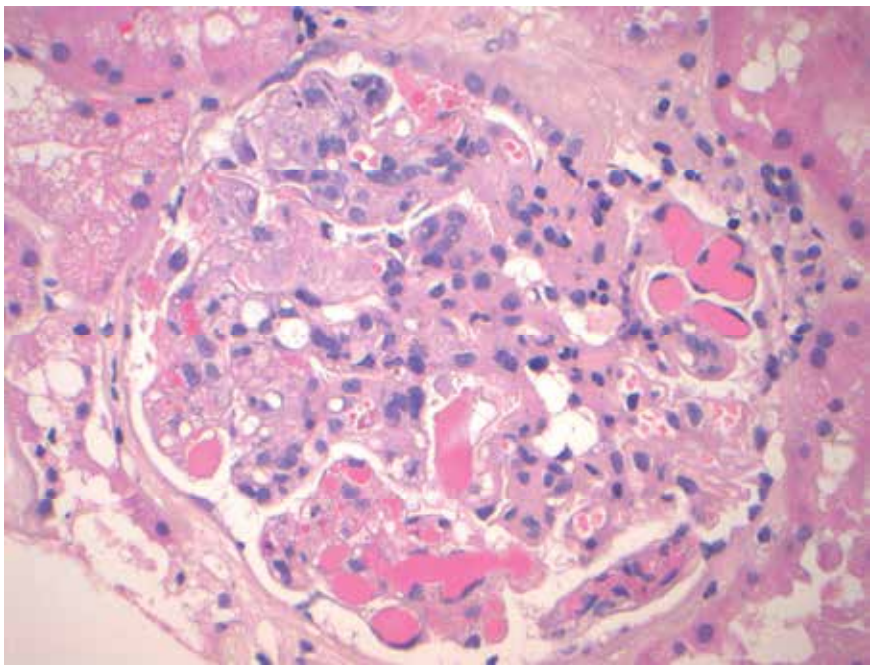

Fig. 2. Cryoglobulinaemic glomerulonephritis, with prominent intracapillary hyaline thrombi (H&E, 400x).

In a minority of cases, vasculitis of small vessels can also be seen on biopsy. Immunofluorescence staining reflects the composition of the usually mixed-type cryoglobulin precipitate, with strong staining for IgG, IgM and C3 as well as frequent kappa predominance. The cryoglobulin deposits by electron microscopy have variable morphology, but classically are seen as microtubular and/or annular organised structures (Iskanda and Herrera, 2002) . See Figure 3

MPGN type 3 has overlapping features of both MPGN type 1 in combination with membranous nephropathy, and has also been described as a pattern of HCV-associated glomerulonephritis.

The remaining patterns of glomerulonephritis are relatively uncommon and have been reported as small series and case reports, with varying degrees of strength in their association with HCV. Of these relatively uncommon forms of HCV-associated glomerulonephritis, membranous nephropathy is the most often quoted. In a study by Yamabe and colleagues (1995), 2 of 24 patients (8.3%) with membranous nephropathy had evidence of HCV infection. A separate Japanese study of 2 patients with membranous nephropathy demonstrated pathogenic linkage to HCV by detecting HCV core protein in the affected glomeruli using immunofluorescence (Okada et al., 1996). In contrast to HCV-associated MPGN, HCV-

associated membranous nephropathy does not appear associated with cryoglobulinaemia, rheumatoid factor or hypocomplementaemia (Uchiyama-Tanaka et al., 2004).

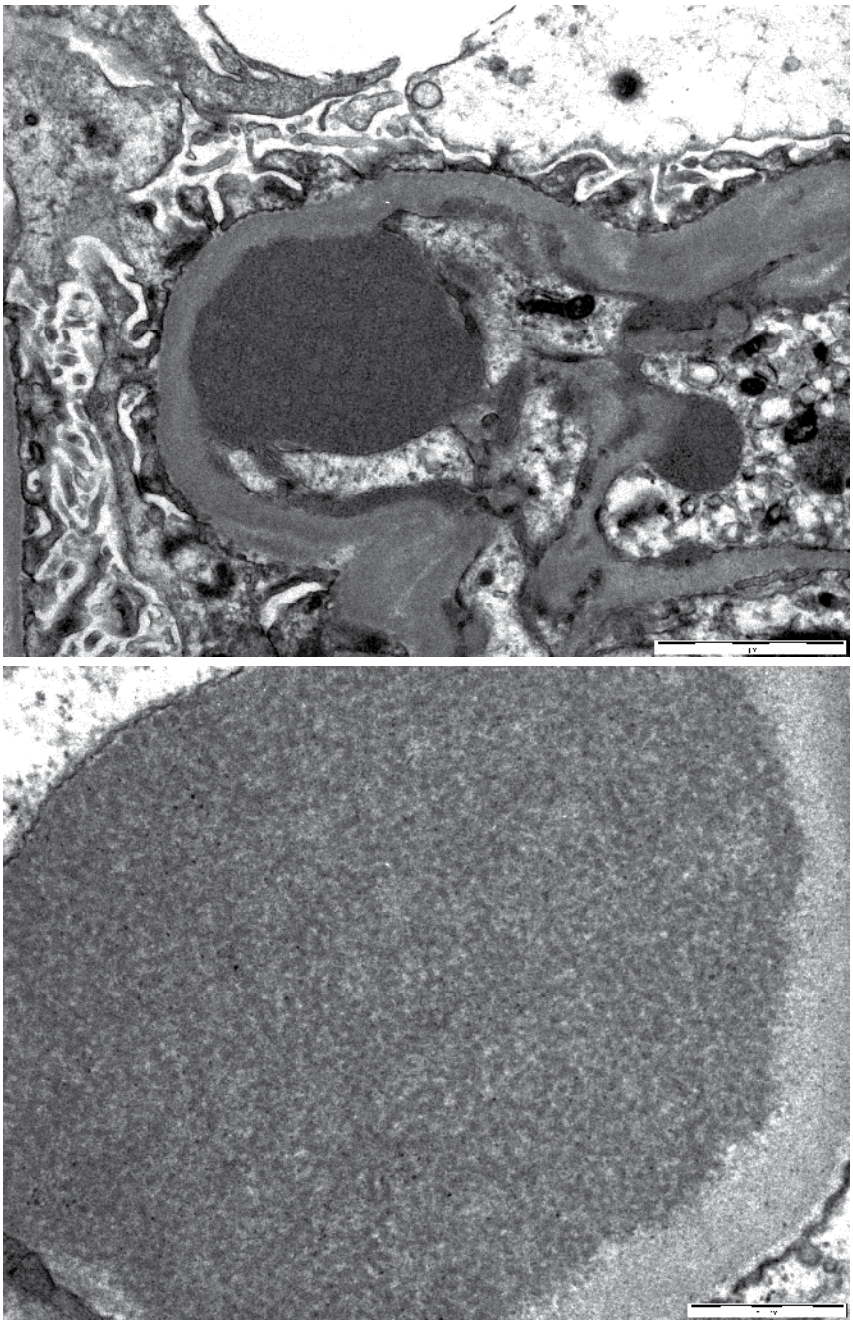

Fig. 3. (3a above and 3b below). Cryoglobulin deposits classically show organised substructure such as microtubular aggregates (EM; photos courtesy of Mr Paul Kirwan, EM unit Concord Repatriation General Hospital Sydney Australia).

A case series from the University of Alabama at Birmingham examined the kidney biopsies of 30 patients receiving liver transplants for HCV-induced cirrhosis at the time of liver engraftment (McGuire et al., 2006). Three types of disease were observed: membranoproliferative glomerulonephritis type 1, IgA nephropathy, and "mesangial glomerulonephritis". Cryoglobulins were not detected, even in rheumatoid factor-positive patients with urinary abnormalities.
Other rare, somewhat speculative, reported associations with HCV include focal segmental glomerulosclerosis (Stehman-Breen et al., 1999), fibrillary glomerulonephritis/immunotactoid glomerulopathy (Markowitz et al., 1998), pauci-immune glomerulonephritis (Usalan et al., 1998), and thrombotic microangiopathy (Herzenberg et al., 1998).
Lastly, it should be noted that the development of de novo immune complex glomerulonephritis in the transplanted kidney is one mechanism leading to the increased graft failure seen in HCV-infected renal transplant recipients (Scott et al., 2010).

3. Clinical manifestations

Glomerulonephritis can develop several years or even decades after initial infection with HCV. As described previously the principal renal manifestation of HCV infection is membranoproliferative glomerulonephritis (MPGN). This is usually accompanied by cryoglobulinaemia. Classically, HCV-associated MPGN is found in persons with long-standing infection and patients most often display mild subclinical liver disease. MPGN is rarely found in children. Clinically, patients may exhibit symptoms of cryoglobulinaemia, including palpable purpura, arthralgias, myalgias, neuropathy, and fatigue. The triad of purpura, peripheral neuropathy and arthralgia is evident in nearly 30% of cases (Monti et al., 1995). The majority of cryoglobulinaemic HCV-infected patients however have either no symptoms or nonspecific clinical manifestations. Cryoglobulins, or immunoglobulins (Igs) that precipitate at cold temperature, are detected in approximately 50–70% of patients. Cryoglobulinaemic vasculitis, predominantly involving the small vessels, is observed in less than 10% of patients (Lamprecht et al., 1999). The most frequently affected tissues/organs are skin, nerves, and kidney. Renal involvement has been reported in about one-third of cryoglobulinaemic patients (Meyers et al., 2003), but the predilection for renal involvement in certain patients is unclear. Renal signs of cryoglobulinaemia include nephrotic or non-nephrotic proteinuria and microscopic haematuria with mild to moderate renal insufficiency (Baid et al., 2000; Johnson et al., 1993; Markowitz et al., 1998). Glomerular disease may manifest acutely as oliguric acute renal failure in 5% of cases (Meyers et al., 2003). Around 80% of patients develop hypertension (Tarantino et al., 1995) which can be severe and difficult to control.
Usually, the diagnosis of HCV-associated MPGN is made by positive tests for serum HCV antibodies and HCV RNA. However patients with HCV-associated glomerulonephritis in whom HCV RNA were not detected in the blood have been reported (Yamabe et al., 2010).
Serum aminotransferase levels are increased in the majority of patients and often low serum concentrations of complement components (C1q, C4, and C3) are found (Meyers et al., 2003). Elevated levels of serum cryoglobulins can be divided by the Brouet classification into three types (Brouet et al., 1974). Type 2 and 3 cryoglobulins which are strongly associated with hepatitis C, have rheumatoid factor activity and bind to polyclonal immunoglobulins (Ferri et al., 2002). These two types are known as mixed cryoglobulinaemia.

4. Natural history

A large prospective cohort study conducted in Northern Norway on 1010 HCV-positive patients found elevated alanine aminotransferases in 27.4%, decompensated liver disease in 2.9%, hepatocellular carcinoma in 0.4% but only 2 patients (or 0.2%) with end-stage renal failure caused by membranoproliferative glomerulonephritis (Kristiansen et al., 2010). The median observation period from estimated acquisition of the disease to follow-up in these patients was 26 years.

The long-term outcome of HCV-associated nephropathies is nebulous. A retrospective cohort study of 474,369 adult veterans in the United States (Tsui et al., 2007) found that patients with HCV infection were more likely to develop end-stage renal disease (4.3 per 1000 person year) than HCV-seronegative patients (3.1 per 1000 person year). For patients aged 18 to 70 years with an estimated glomerular filtration rate of at least 30 mL/min per 1.73 m2, HCV seropositivity was associated with a nearly threefold higher risk of developing ESRD (adjusted hazard rate, 2.80; 95% confidence interval, 2.43-3.23).

Another cross-sectional study (Dalrymple et al., 2007) showed that HCV-positive veterans after adjustment for age, race, gender, diabetes and hypertension, had 40% higher odds for renal insufficiency (odds ratio 1.40; 95% confidence interval 1.11 to 1.76) as compared with HCV-negative veterans.

An early literature review of patients with essential mixed cryoglobulinaemia (Ponticelli et al., 1986) found that of 11 patients with nephrotic syndrome and renal dysfunction who received supportive treatment alone, 4 patients (36%) died or exhibited progressive renal failure, 2 patients (18%) had stable renal disease, and spontaneous improvement occurred in the other 5 patients (45%).

Finally Tarantino and colleagues (1995) reported the overall poor clinical outcome of 105 essential mixed cryoglobulinaemia patients with renal involvement collected throughout 25 years in three renal units in Milan. Patient survival was 49% at 10 years after renal biopsy. Forty-two patients died primarily from cardiovascular disease, liver disease or infection, whereas 15 patients developed chronic renal failure. Two patients had a complete remission of the disease while 15 had a remission only of renal manifestations. Thirty-one patients were alive at the end of the study with persistent renal and extrarenal manifestations. Thus only a minority of patients eventually developed renal failure because most patients died from cardiovascular disease, liver disease or infection.

5. Therapy

5.1 Renoprotective therapies

As hypertension, proteinuria, and progressive renal failure are the main clinical manifestations of HCV-associated chronic renal disease, it is essential that renoprotective therapies be instituted. Diuretics, renin–angiotensin system inhibitors (angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers), and lipid-lowering agents, have been proven to be beneficial in HCV patients with chronic renal disease (Chadban and Atkins, 2005; Ruggenenti et al., 2001; Ruggenenti et al., 2004).

5.2 Immunosuppressive therapy

High-dose methylprednisolone has been used to treat exacerbations of mixed cryoglobulinaemia for over 30 years (Tarantino et al., 1981; De Vecchi et al., 1983). In one of

the earlier studies (De Vecchi et al., 1983), 3 pulses of intravenous methylprednisolone (0.5-1 gm each) was given for 3 consecutive days during episodes of acute renal function deterioration in 16 patients with essential mixed cryoglobulinaemia. The intravenous administration was followed by oral prednisone 0.5 mg/kg per day with a slow taper until withdrawal of steroids after 4-6 months. Intravenous methylprednisolone pulse therapy did have a dramatic effect on renal function with cumulative mean plasma creatinine values decreasing from 3.3 ± 1.3 mg/dL to 2.2 ± 0.7 mg/dL ($p<0.001$). Proteinuria levels were not found to be significantly changed as a result of therapy. The basal cryocrit level decreased after pulse therapy, however again this was not found to be significant.

In one case report of rapidly progressive MPGN type 1 with HCV and nephritic syndrome, intravenous pulsed methylprednisolone appeared to be useful in establishing rapid remission but as antiviral therapy was used concurrently it is impossible to ascertain the effect of methylprednisolone alone (Ahmed et al., 2008).

Unlike intravenous pulse steroid therapy, oral steroids however have not been found to be effective in the acute setting. Ponticelli and colleagues (1986) reported 27 patients with mixed cryoglobulinaemia and acute renal disease that were treated with oral corticosteroids alone or in combination with other cytotoxic agents. 10 patients (37%) died or showed progressively worsening renal function, 4 patients continued to have stable renal disease, and the other 13 patients (48%) had improved renal function. This does not appear to differ markedly from their reporting of the natural outcomes of such patients with supportive treatments alone.

Indeed there are conflicting results on the use of oral steroids for HCV-associated MPGN. A Japanese study (Komatsuda et al., 1996) found that only 2 out of 6 patients with MPGN responded to steroids but paradoxically found that the serum titre of HCV RNA decreased in 5 out of 7 treated patients.

Other studies have subsequently confirmed that HCV RNA levels increase with steroid exposure (Fong et al., 1994; Lake, 2003; McHutchinson et al., 1993).

Cyclophosphamide has been used successfully in the treatment of HCV-infected patients with cryoglobulinaemia and progressive loss of kidney function due to MPGN. In one case report a patient with HCV-associated MPGN and progressive renal failure displayed disappearance of serum cryoglobulins and a marked improvement in creatinine clearance with the institution of cyclophosphamide (Quigg et al., 1995). However cyclophosphamide treatment similar to steroids produces a rise in HCV RNA levels.

It is generally agreed that immunosuppressive medications do increase HCV RNA levels but their selective use does not appear to worsen the underlying hepatic disease (D'Amico and Fornaserieri, 2003). A review by D'Amico (1998) reported no evidence of acute liver damage in more than 100 treatment courses (steroids, cyclophosphamide, plasma exchange) in Italian patients with HCV-associated cryoglobulinaemic glomerulonephritis.

The expert consensus currently is that in patients with HCV-associated renal disease, treatment of acute flares does require immunosuppressive therapy to preserve renal function however prolonged therapy does not confer any additional benefit (Campise and Tarantino, 1999).

5.3 Antiviral therapy

Antiviral therapy in the form of alpha interferon was first used in a small pilot study of 7 patients with cryoglobulinaemic vasculitis in 1987, before the discovery of the critical role of HCV in its pathogenesis (Bonomo et al., 1987).

After a link was established between HCV infection and the occurrence of cryoglobulinaemic MPGN, a number of studies have examined the efficacy of antiviral treatment to achieve both sustained virological response (clearance of HCV from the serum for at least 6 months after completing an antiviral course) and to improve renal injury. The use of antiviral therapy in HCV-positive patients with glomerulonephritis is targeted at eliminating the virus and reducing the generation of HCV-related antibodies and immune complexes.

In the early 1990s, standard recombinant alpha interferon (α-IFN) was used by itself. The first prospective randomised controlled trial by Misiani and colleagues (1994) reported that 15 out of 25 patients with HCV-associated type II cryoglobulinaemia receiving recombinant alpha-interferon had a complete clearance of hepatitis C viral RNA and that all these patients reported improvements in cutaneous vasculitis and renal function. There was no effect on proteinuria. Unfortunately after treatment with interferon alpha-2a was discontinued, viraemia and cryoglobulinaemia recurred in all 15 HCV RNA-negative patients.

Johnson and colleagues (1994) reported the results of a prospective uncontrolled trial of fourteen patients receiving interferon alpha for 6 to 12 months. There was a significant reduction in proteinuria but no improvement in renal function. Although a good clinical response correlated with disappearance of HCV RNA from the serum during treatment, relapse of viraemia and renal disease was common after the completion of therapy.

It was clear at this stage that alpha-interferon was useful but the optimal treatment strategy was yet to be defined.

An advance came with the discovery that ribavirin played a synergistic role with an interferon-based regimen to increase the possibility that an on-treatment responder would become a sustained responder. Ribavirin monotherapy itself was found to be disappointing (Pham et al., 1998) although one case report referenced a patient with refractory nephritic syndrome secondary to HCV-associated membranous nephropathy who had a complete remission following the initiation of ribavirin monotherapy (Hu and Jaber, 2005).

Small scale studies examined the combination of standard interferon plus ribavirin for HCV-associated cryoglobulinaemic glomerulonephritis. Rossi and colleagues (2003) treated 3 patients with HCV-associated cryoglobulinaemic glomerulonephritis with standard interferon and ribavirin for 12 months and showed that all had sustained virological response, with reductions in daily proteinuria and rheumatoid factor at the end of follow-up. Brucheld and colleagues (2003) treated 7 patients with HCV and renal insufficiency (2 patients with cryoglobulinaemic vasculitis, 4 patients with MPGN, 1 patient with focal segmental glomerulosclerosis) with a combination of interferon and ribavirin. 4 of the 7 patients had maintained virological and renal remission. The frequency of haematuria and amount of proteinuria decreased after the course of antiviral treatment.

The next important clinical breakthrough was the introduction of a polyethylene glycol side chain (pegylation) to the interferon to give it a much longer bioavailability, allowing for weekly injections rather than three injections per week. Pegylated interferon was shown to double the sustained viral response rate in hepatitis C treatment (Lindsay et al., 2001).

Saadoun and colleagues (2006) carried out a study on 72 consecutive patients with HCV-associated mixed cryoglobulinaemic vasculitis receiving recombinant interferon or pegylated interferon, both in combination with oral ribavirin. A complete clinical response of the cryoglobulinaemic vasculitis occurred in 45 patients, a sustained virologic response occurred in 42 patients, and cryoglobulins cleared in 33 patients. Compared with patients

treated with IFN alpha-2b plus ribavirin, those receiving PEG-IFN alpha-2b plus ribavirin had a higher sustained clinical (67.5% versus 56.3%), virologic (62.5% versus 53.1%), and immunologic (57.5% versus 31.3%) response, regardless of HCV genotype and viral load.

Fabrizi and colleagues (2007) then undertook a meta-analysis looking at clinical controlled trials of the 2 treatments (antiviral versus immunosuppressive) for HCV-associated glomerulonephritis. Six studies involving 145 patients with HCV-associated glomerulonephritis were identified (Alric et al., 2004; Beddhu et al., 2002; Johnson et al., 1994; Komatsuda et al., 1996; Mazzaro et al., 2000; Misiani et al., 1994). The primary endpoint was the frequency of patients with significant reduction in proteinuria (return of proteinuria to normal or decrease of >50%) at the conclusion of therapy. It was shown that standard interferon alpha therapy was more effective than immunosuppressive therapy in lowering proteinuria of patients with HCV-associated glomerulonephritis (OR 3.86, 95% CI 1.44-10.33; P=0.007). However renal dysfunction was not significantly improved with either therapy (Fabrizi et al., 2007).

This meta-analysis was methodologically flawed by the inclusion of studies where patients received immunosuppressive agents during antiviral treatment, making it difficult to ascertain the effect of each treatment alone.

A later meta-analysis (Feng et al., 2011) examined the results before and after stable regimens of antiviral therapy in subjects with HCV-associated glomerulonephritis and compared the results of those subjects who achieved sustained virological response (SVR) to those that did not. Improvement of proteinuria and serum creatinine levels after antiviral therapy were taken as the end points of interest. Eleven clinical trials involving 225 patients were included in the meta-analysis. At the end of antiviral therapy, the mean decrease in proteinuria was 2.71 g/24 h [95% confidence interval (CI) 1.38-4.04, $P < 0.0001$]. The pooled decrease in mean serum creatinine levels was 0.23 mg/dL (95% CI 0.02-0.44, $P = 0.03$). Comparison of nonsustained virological response (nonSVR) to SVR groups demonstrated a significant mean difference of proteinuria decrease in the SVR group of 1.04 g/24 h (95% CI 0.20-1.89, $P = 0.02$) but the serum creatinine decrease of 0.05 mg/dL was not significant (95% CI -0.33 to 0.43, $P = 0.80$).

A limitation of this meta-analysis is the small number of study subjects making it difficult to perform subgroup analysis on the basis of cryoglobulinaemia or baseline proteinuria. Another weakness is the lack of randomized controlled trials (RCTs) of interferon alpha-based therapy in HCV-associated glomerulonephritis. Indeed only 1 of the 11 studies in this analysis was an RCT (Misiani et al., 1994).

Thus antiviral therapy based on interferon alpha can significantly decrease proteinuria and hence should be undertaken in patients with HCV-associated glomerulonephritis.

It should be acknowledged that currently there are no long-term follow-up studies of antiviral therapy on HCV-associated glomerulonephritis patients. It is important to ascertain whether interferon alpha-based treatments can delay or halt the progression of chronic renal disease in the long term. This will require investment in large RCTs with longer durations of follow-up.

5.4 Rituximab

Treatment with interferon alpha in combination with ribavirin can suppress HCV RNA in 50-60% of patients with a subsequent decrease in cryoglobulins. Many patients however fail to respond to interferon therapy and half the responders relapse.

Rituximab is a monoclonal antibody against the CD20 antigen on the cell surface of B lymphocytes. Rituximab can thus reduce rheumatoid factor-producing B lymphocytes, resulting in a reduction in cryoglobulin production. In recent years the concept of anti-CD20 for mixed-type cryoglobulinaemia has emerged as an effective and safe treatment, inducing a rapid remission of disease activity (Sansonno et al., 2003; Zaja et al., 2003).

One study examined 20 HCV-positive patients with mixed-type cryoglobulinaemia (who were refractory to interferon therapy) that were treated with rituximab 375 mg/m² weekly for 4 weeks (Sansonno et al., 2003). Patients had a follow-up period of 12 months. Sixteen patients (80%) had a complete response defined as a 75% or greater reduction in cryoglobulins and resolution of at least 2 major clinical signs and symptoms. In these patients, rituximab treatment resulted in a reduction in both the IgM and IgG components of the cryoglobulin. Only 1 of the 20 patients had nephritis that did not respond to treatment.

Another study looked at the treatment of 15 patients with rituximab with a follow-up period of 9 to 31 months (Zaja et al., 2003). 12 patients were HCV-infected and 3 patients had mixed cryoglobulinaemia unrelated to HCV. All patients had early improvement in their cutaneous manifestations with rituximab however only 1 patient had complete resolution of the cryoglobulin at 6 months and only 3 lost their rheumatoid factor. It is worthwhile noting that in 7 of 8 patients, maintenance corticosteroids were successfully withdrawn by the second post-treatment month.

Quartuccio and colleagues (2006) carried out a study where 5 patients with HCV-associated mixed cryoglobulinaemia were treated with 4 weekly infusions of rituximab 375mg/m² without accompanying steroids. Renal function improved within 2 months in all 5 cases treated. There were no relevant short-term or delayed side effects reported. However 3 out of 5 patients showed a recurrence of disease at 5, 7 and 12 months. A repeated cycle of rituximab infusion induced rapid remission of disease activity in 2 of these patients. Only one patient achieved persistent remission after a single cycle and thus the results of the study suggest the need for repeated rituximab administrations for adequate control of nephritis. The optimal dosage and frequency of rituximab administrations in HCV-associated mixed cryoglobulinaemia remains unclear as all studies to date have been based on the rituximab prescribing regimen used in non-Hodgkin lymphoma (Coiffier et al., 1998).

6. Renal transplantation and HCV-related renal disease

It should be recognised that the most common cause of proteinuria and renal insufficiency after kidney transplantation in HCV-positive patients is not HCV-related damage but chronic allograft nephropathy (Cosio et al., 1996; Nampoory et al., 2001). Renal diseases that have been reported in HCV-infected patients after kidney transplantation include recurrent or *de novo* MPGN, membranous nephropathy, minimal change disease, thrombotic microangiopathy, acute transplant glomerulopathy, and chronic transplant glomerulopathy (Baid et al., 2000; Cruzado et al., 2001; Gallay et al., 1995; Gloor et al., 2007; Hammoud et al., 1996; Morales et al., 1997; Roth et al., 1995).

MPGN is the most commonly reported, with an incidence as high as 54% in HCV-positive renal transplant recipients (Cruzado et al., 2001; Hammoud et al., 1996; Roth et al., 1995). In these patients proteinuria or nephrotic syndrome is the commonest clinical presentation (Cruzado et al., 2001; Nampoory et al., 2001; Virgilio et al., 2001). Serum cryoglobulins are very often detected (Roth et al., 1995).

Early studies on patient and graft survival in HCV-positive renal transplant recipients have concentrated on recurrent liver disease as causes of morbidity and mortality rather than examining recurrent renal disease or graft loss (Batty et al., 2001; Meier-Kriesche, 2001; Pereira and Levy, 1997). Small single centre studies have shown that both graft and patient survival are lower for HCV-positive than HCV-negative patients (Batty et al., 2001; Pereira and Levy, 1997).

More recently two large population based studies have published long term results of patient and graft survival (Morales et al., 2010; Scott et al., 2010).

The outcomes of a large cohort of renal transplant patients was reviewed recently using the Australian and New Zealand Dialysis and Transplant registry (Scott et al., 2010). Survival outcomes, causes of mortality, and causes of graft failure were examined. 140 (1.8%) patients were HCV antibody positive. Patient survival among HCV antibody positive and HCV antibody negative groups was 77% versus 90% and 50% versus 79% at 5 and 10 years respectively. The adjusted hazard ratio for patient death was 2.38 (95% CI 1.69-3.37). Higher rates of death due to cardiovascular disease (adjusted hazard ratio 2.74), malignancy (adjusted hazard ratio 2.52) and hepatic failure (adjusted hazard ratio 22.1) were observed.

A large national study in Spain used data on 4304 renal transplant recipients, 587 of them with HCV antibody collected over a long period (1990-2002), to estimate graft and patient survival at 4 years (Morales et al., 2010). 4-year graft survival was found to be significantly better in HCV-negative versus HCV-positive patients (94.4% versus 89.5%, $P < 0.005$). Patient survival was 96.3% in the entire group with a demonstrable difference between HCV-negative and HCV-positive patients (96.6% vs 94.5%, $P < 0.05$). HCV-positive patients were characterised as having more episodes of acute rejection, a higher degree of proteinuria with impaired renal function and a greater need for renal graft biopsies. In particular *de novo* glomerulonephritis and transplant glomerulopathy rates in HCV-positive and HCV-negative renal graft biopsies was 9.3% versus 5.2% and 11.4% versus 5.0% respectively.

6.1 Hepatitis C treatment implications in renal transplantation

A meta-analysis of 12 trials of interferon alpha-based therapy in 102 kidney transplant patients showed that sustained virological response is extremely variable ranging from 0-50% with a variable and often extremely high drop-out rate (0% to 100%) (Fabrizi et al., 2006). HCV genotype is an important determinant of sustained virological response with genotype 1 being the most resistant (Lock et al., 1999). Any conferred benefit on the underlying disease is mitigated by a 15-60% increased risk of acute cellular or vascular rejection (Baid et al., 2003; Fabrizi et al., 2006; Weclawiack et al., 2008). Unfortunately graft rejection is often severe and resistant to steroid therapy (Fabrizi et al., 2006).

Cessation of standard interferon therapy leads to a surge in hepatitis C viral load (104,105). Avoidance of interferon in HCV-positive renal transplant patients has been recommended because of the potential to precipitate acute graft rejection. However combined therapy with ribavirin and pegylated interferon achieved sustained virological response in 5 out of 8 patients (62%) without unduly affecting renal function (Montalbano et al., 2007; Mukherjee and Ariyarantha, 2007; Schmitz et al., 2007). This suggests a therapeutic role in certain settings albeit with an appreciable risk of graft dysfunction.

Rituximab appeared to be safe in one study of 7 HCV RNA-positive kidney transplant patients with *de novo* cryoglobulinaemia-related MPGN. HCV infection remained stable

during and after rituximab therapy (Kamar et al., 2007). Larger long-term studies will be necessary to establish efficacy.

7. References

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12

Glomerular Pathology in Patients with HIV Infection

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1. Introduction

The course and prognosis of patients infected with the human immunodeficiency virus (HIV) is changing dramatically following the introduction of highly active antiretroviral therapy (HAART), with increased patient survival and decreased morbidity (Mocroft et al, 1998).

Current therapy offers patients increased survival by making it more susceptible to certain comorbidities (Fang et al, 2007). Cardiovascular and renal diseases are the prototype of diseases whose prevalence increases progressively with the prolonged survival and aging (Braithwaite et al, 2005). On the other hand, besides specifically associated nephropathy or HIV coinfection with hepatitis C virus (HCV) with prolonged survival of the HIV-infected population, the spectrum of kidney disease in patients with HIV also reflects the growing burden of comorbid diabetes and hypertension and development chronic renal disease (Wyatt et al, 2007; Mocroft et al 2007; Szczech, 2004).

The prevalence of chronic kidney disease in HIV patients may be between 5 to 15% depending on the series. However, it has been manifested as albuminuria or proteinuria may be present up to 30% in some cohorts of HIV-infected patients (Szczech et al, 2002). The prevalence of renal histological involvement has ranged from 1-15% depending on the different autopsy series (Shahinian et al, 2000). Patients with HIV infection may develop different types of glomerular diseases, vascular lesions and tubulointerstitial nephritis related in some cases with the virus itself or other co-infections (Williams et al, 1998).

In this chapter we will review at different types of glomerular diseases found in patients with HIV infection, highlighting its etipopatogenia, clinical presentation, diagnosis and therapeutic alternatives.

2. Glomerular diseases in patients with HIV infection

Association between HIV infection and renal disease was first described in 1984, shortly after isolation of the virus (Rao et al, 1984). The glomerular diseases are relatively common complication in patients infected with HIV, with a wide range of clinical presentations and with a poor prognosis in most cases. Although HIV-associated nephropathy (HIVAN) has

been considered the most frequent and most representative glomerular disease, after the generalized treatment of HIV-infected patients with HAART therapy has allowed the emergence of other glomerular diseases with a higher incidence than the general population, **see Table 1**.

- HIVAN
- HIV-Associated glomerulonephritis. Immune complex glomerulonephritis
  - Membranoproliferative glomerulonephritis
  - Membranous nephropathy
  - Mesangial glomerulonephritis (IgA)
  - Focal segmental glomerulosclerosis non-collapsing
  - Lupus-like glomerulonephritis
  - Postinfectious glomerulonephritis
- Other nephropathies
  - Thrombotic Microangiopathy
  - Malignant hypertension
  - Amyloidosis

Table 1. Glomerular diseases in patients with HIV infection

2.1 HIV-associated nephropathy

This type of glomerular disease is HIV-associated nephropathy best described (Rao et al, 1987). The disease affects mostly males, intravenous drugs users and black patients (D'Agati & Appel, 1997). U.S. is the third leading cause of end-stage renal disease (ESRD) in African Americans between 20 and 64 years of age and the most common cause of ESRD in HIV-1 seropositive patients (Eggers & Kimmel, 2004; Shahinian et al, 2000). Prevalence is variable, ranging from 4% in clinical studies and 10% in autopsy (Mazbar et al, 1989). Although these renal findings can be seen in patients with asymptomatic or primary HIV infection, most of patients have low CD4 cells count and advanced disease.

Among the specific mechanisms involved, in addition to genetic susceptibility (several polymorphisms that influence susceptibility to HIV infection), the virus can be seen in glomerular epithelial cells (Kimmel et al, 1993; Röling et al, 2006). Transgenic mice were crated with HIV-DNA construct inserted into the genome and developed a histological picture and clinical presentation very similar to that of humans (Bruggeman et al, 1997). The viral genome is detected in different renal structures (tubular cells, podocytes, glomerular parietal epithelial cells), even in cases without renal involvement, indicating that the presence in the renal tissue is not sufficient to cause nephropathy (Lu & Ross, 2005; Mikulak & Singhal, 2010). There are many mediators and the expression of nonstructural viral proteins, cytokines and growth factors can modulate and amplify renal injury. The mechanism of viral entry into renal epithelial cell is still unknown. CCR5 and CXCR4 are coreceptors to CD4 that mediate HIV-1 entry into lymphocytes. These receptors have not been demonstrated on renal epithelial cells in vivo (Eitner et al, 2000). Recently, have been described the DEC-205 receptor, which facilitates the entry of the virus in renal tubular cells (Hatsukari et al 2007).

HIVAN is a characterized by a constellation of pathologic findings involving glomerular, tubular and interstitial compartments. Glomerular pathologic findings include a collapsing

focal segmental glomerulonephritis, with a marked retraction of glomerular capillary loops, and occlusion of the lumen (D'Agati & Appel, 1998). Podocytes show intense hypertrophy and hyperplasia, which is associated with decrease expression of podocyte maturity markers and accumulations of protein in their cytoplasm. On the other hand, there is a characteristic intense tubulointerstitial involvement, which gives it a matter of some exclusivity over other nephrotic syndromes. These changes consist of dilatation of the renal tubules, which sometimes are proliferative microcyst, and large proteinaceous cylinders in light, cell infiltrates and areas of fibrosis (D'Agati & Appel, 1998; Klotman, 1999). Immunofluorescence usually shows mesangial deposits of IgM and C3 and electron microscopy, are frequently observed tubuloreticular inclusions in the cytoplasm of endothelial cells. These structures are synthesized by the stimulation of alpha-interferon, as in the cases of patients with lupus nephritis. The histological differential diagnoses of focal segmental glomerulosclerosis are the collapsing nephropathy heroin abuse, caused by bisphosphonates, interferon or parvovirus (Klotman, 1999).

Main manifestation of HIV-associated nephropathy is a nephrotic syndrome (Herman & Klotman, 1998). However, although the proteinuric can be massive in many cases (more than 8-10 g/24 h), most patients with HIVAN do not have significant peripheral edema. Moreover, patients with HIVAN are usually not hypertensive, a remarkable finding considering that more than 90% of black patients with renal insufficiency of other causes exhibit hypertension. The kidneys are usually normal in size or larger. Most patients also have advanced renal failure at the time of diagnosis.

Evolution without HAART is poor, with rapid development of renal failure requiring dialysis within the first year of diagnosis, and with a high mortality (Winston et al, 1998). The beneficial effects of HAART on HIVAN have been shown in individual clinical observations. There are reports of resolution of renal disease with the administration of HAART, with a recurrence of renal disease after stopping treatment (Scialla et al, 2007).

Although no controlled clinical trials have demonstrated the effectiveness of any therapeutic measure in the HIVAN, it is recognized that HAART prevents or reduces the risk of developing HIVAN and if this occurs, the patients may have a slower course and lower mortality than in untreated patients (Lucas et al, 2004). Another treatment option is recommended in these patients using drugs that blocker renin-angiotensin-aldosterone system (BRAAS). These drugs are a part like any other chronic proteinuric nephropathy (Wei et al, 2003). Some studies have shown a decrease in proteinuria and a trend towards stabilization of renal damage in patients with HIVAN treated with corticosteroids. However, this treatment was not without significant side effects (Smith et al, 1994), so it should be limited to cases in which treatment with HAART have not produced any improvement and if we rule out opportunistic infections. Finally, we can find some curious case of spontaneous improvement of the glomerular pathology (Morales et al, 2002). The indications for renal replacement therapy with dialysis or transplantation in patients with HIVAN are similar to those followed in other chronic renal disease in the general population.

2.2 Immune-mediated glomerulonephritis

Patients with HIV infection have a higher incidence of other glomerulonephritis whose pathogenesis is generally attributed to glomerular deposition of immune complexes (Balow, 2005; Nochy et al, 1993), see **Table 2.**

	City/Country	Number of patients	Gender (M/F)	HCV (+)%	Race	Types of Glomerular diseases	Ref
Williams DI et al	London	17	13 M, 4 F		47% blacks 53% caucasians	HIVAN 41%, MN 23%, HUS 12%, others 24 %	9
Rao TKS et al.	Nueva York	55	49 M, 6 F		100% blacks	HIVAN 90%, Mesangial GN 10%	11
Mazbar SA et al.	San Francisco	27	26 M, 1 F		63% blacks 37% caucasians	HIVAN 27%, MPGN 27%, Interstitial nephritis 9%, immune complex GN with IgG-IgM 9%, 28 % others	15
NocheD et al.	Paris	60	51 M, 9 F		48% blacks 52% caucasians	43 % HIVAN, Immune complex GN 37%, lupus like nephritis 16%, HUS 11.5%	33
Casanova S et al.	Italy	26	21 M, 5 F		100% caucasians	Immune complex GN 65.5%, MPGN 15.5%, lupus like nephritis 11.5%, minimal change 7.5%	34
Connolly JO et al.	London	34	25 M, 9 F		55.8% blacks 41.1%caucasians	50 % HIVAN, 14.5 % MN, 6% MPGN, 12 % HUS, 3% Immune complex GN, 14.5 % others	53
Shahinian V et al.	Texas	389	362 M, 27 F		54 % blacks 35% caucasians	26% unknown, 7 % HIVAN, 7% immune complex GN, 17 % ATN, 25% crystal induced nephropathy	14
Szczech La et al.	EEUU	89	73 M, 16 F		88% blacks 12% others	47% HIVAN, 53 % non-HIVAN	6
Cheng JT et al.	EEUU	14	8 M, 6 F	100	93% blacks 7% caucasians	79% MPGN, 21% MN	36
Stokes MB et al.	EEUU	12	11 M, 1 F	100	58% blacks 42% caucasians	41% MPGN, 41 % mesangial GN, 8% MN, 8 % HIVAN	37
Gutiérrez et al.	Madrid, Spain	27	23 M, 4 F	77.8	11% blacks 89% caucasians	29.6% MPGN, 25.9% Non-collapsing FSGS, 22.2% mesangial GN, 14.8% HIVAN.	45

Table 2. Different studies with renal disease in HIV patients

In Europe, especially in Caucasians, glomerular immune complex glomerular diseases are more common than HIVAN and may take several forms. The immune-mediated glomerulonephritis would be the counterpart among the white population to what is represented by the HIVAN in the black (Casanova et al, 1995).

The pathogenesis of these types of glomerulonephritis remains largely unknown; there is a local formation or glomerular deposition of circulating immune complexes that may contain HIV antigens and polyclonal antibodies. We can not rule out the involvement of an immune response against associated viral infections. The clinical features are very striking (gross hematuria, edema, acute renal failure, and hypertension), although cases of more subtle presentation and are diagnosed incidentally, see **Table 3.** Hypergammaglobulinemia is common, reflecting a polyclonal B cell activation. However, the role of some viral co-infections very frequent among HIV patients, such hepatitis C (HCV) and hepatitis B virus (HBV), appears to be decisive, particularly in MPGN and NM.

Very limited information is presently available regarding treatment and clinical outcomes in patients with immune-mediated GN in patients with HIV infection. Until now we have not information about the therapeutic interventions used in patients without HIV infection (steroids, immunosuppressants, calcineurin inhibitors) can change the course of immune-complex glomerulonephritis.

	HIVAN	**MPGN**	**IgAN**	**FSGS-NC**	**MN**	**PIGN**	**Lupus-like**	**Amyloidosis**	**MAT**
Nephrotic syndrome	4	3	0	3	4	1	4	4	1
Macroscopic hematuria	0	2	4	0	0	3	2	0	2
Acute renal failure	1	1	2	1	1	3	1	1	4
Hypertension/ Malignant hypertension	1	1	3	1	1	1	1	0	3
Hypo-complementemia	0	3	0	0	0	4	1	0	0
Cryglobulins (+)	0	3	0	0	0	1	1	0	0
Coinfection HCV/HBV	0	4	1	1	2	0	2	1	0
Treatment	HAART BSRAA S	BSRAA **VHC (+) (INF+RBV) (S+PF+RTX)**	BSRAA	BSRAA	BSRAA **Several clinical (S+IMS)**	BSRAA **Several clinical (S)**	HAART S	HAART Secondary aetiology	Plasma P

HIVAN: HIV-associated nephropathy; MPGN: membranoproliferative GN; IgAN: IgA nephropathy; FSGS-NC: Not collapsing forms of FSG; MN: membranous nephropathy; PIGN: Postinfectious GN; MAT: Thrombotic microangiopathy; HAART: Highly active antiretroviral therapy; BSRAA: Blockers of the system renin-angiotensin-aldosterone; S: steroids; INF: interferon; RBV: ribavirin; RTX: rituximab; P:Plasmapheresis. Degrees: (0-4): (Never-Always)

Table 3. Most common presentations and laboratory markers in glomerular diseases in patients with HIV infection

2.2.1 Membranoproliferative glomerulonephritis

The most common clinical presentation is nephrotic syndrome with macroscopic hematuria or microhematuria and normal renal function or mild renal function impairment. Up to 90% of cases there are co-infected with HCV (Morales et al, 1997; Cheng et al, 1999). Cryoglobulins are detected in most cases with elevated rheumatoid factor and decrease of complement, especially C4. The clinical and serological profile is very similar to the membranoproliferative GN associated with HCV patients without HIV infection. For all these reasons it is considered that this is a pathogenetically GN induced by HCV, without the concomitant presence of HIV play a prominent role (Stokes et al, 1997).

In some cases the extrarenal manifestations of cryoglobulinemia include clinical presentation, with vasculitis cutaneous, and even gastrointestinal manifestations alveolar hemorrhage. In these cases we can find an acute renal failure with hematuria and proteinuria, and characteristic histological finding of membranoproliferative GN with deposits of cryoglobulins in glomerular capillary lumens. Cryoglobulins are usually of a mixed IgG-IgM.

The safety and efficacy of HCV treatment with interferon and ribavirin in patients coinfected with HIV has been evaluated in numerous series in the literature (Kadan & Talal, 2007). There are series of cases in which an effective antiviral treatment correlated with improvement in renal manifestations (Kamar et al, 2006). However, we find cases in which there is no negativity of cryoglobulins despite the negativity of HCV-RNA, and can even clinical manifestations (Morales et al, 2007). There are cases in which clinical aggressiveness may be amenable to immunosuppressive treatment. High-dose steroids and plasmapheresis may improve the clinical course, although has increased HCV replication and risk of worsening liver disease. Rituximab is an anti-CD20 monoclonal antibody that has produced a prolonged improvement of renal manifestations in cases that can not be eradicated HCV (Kamar et al, 2006).

In cases of membranoproliferative GN is not associated with HCV; the experience is limited and can recommend the use of drugs that block the RAAS, since its effect antiproteinuric, renoprotective and antihypertensive.

2.2.2 IgA nephropathy

Series have described several cases of IgA nephropathy in patients infected with HIV. The actual incidence is unknown, but in a study of 116 autopsies in patients with HIV infection were detected IgA glomerular deposits by 7.7% (Beaufils et al, 1995).

Patients infected with HIV develop IgA antibodies against specific HIV antigens and this seems to be the pathogenic basis of this nephropathy. On the other hand, can produce idiotype IgA antibodies against other antibodies IgG and IgM directed against viral antigens. Renal lesions may result from HIV antigen-specific immune complexes that are derived from the circulation and from in situ complex formation (Kimmel et al, 1992).

Clinical presentation is similar to that of idiopathic IgA nephropathy: microhematuria with occasional episode of gross hematuria after infection mainly located in the upper respiratory tract and different proteinuria degrees. The long-term prognosis depends mainly on the amount of the proteinuria. In addition to these developments chronic, slowly progressive, characteristic of IgA nephropathy, patients may also develop acute complications similar to those of the IgA idiopathic acute renal failure that is associated with macroscopic hematuria

is a widely known complication (Gutierrez et al, 2007) and development of hypertension malignant (MHT) (Chen et al, 2005). In episodes of gross hematuria may develop acute tubular necrosis, tubular abnormalities consist in a high proportion of tubules that are filled by red blood cells (RBC) casts and signs of tubular necrosis that are more evident in tubules that are occupied by RBC casts, see **Figure 1**.

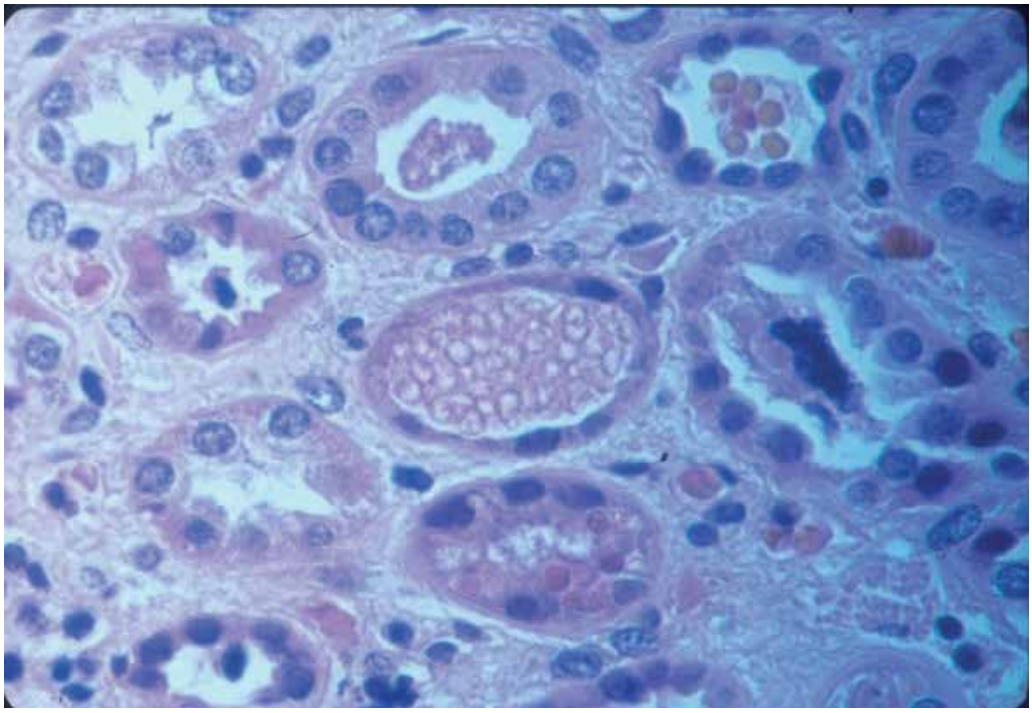

Fig. 1. Tubules filled by red blood cells, showing diminished number of lining epithelial cells and nuclear pyknosis.

Regarding the MHT, is observed in a significant percentage (5% -7%) of cases of idiopathic IgA, but some series indicate that its incidence may be even higher in HIV-associated IgA nephropathy. In our experience, most of the cases with HIV-infection and IgA nephropathy presented malignant hypertension (Gutierrez et al, 2007). The prognosis is poor and many cases progress to irreversible end-stage renal failure, see **Figure 2**. However, in some patients the effective control of blood pressure drug type inhibitors of angiotensin converting enzyme (ACE) inhibitors and / or antagonists of the angiotensin receptor (ARB) manages to partially reverse renal failure.

In some patients with HIV infection, as also in Idiopathic IgA renal manifestations may be associated with systemic manifestations of the type of vasculitis cutaneous, arthritis and various digestive disorders, all components of the Schönlein-Henoch syndrome.

At the present there is a little information about the treatment and outcomes of patients with HIV infection. Therefore, according to the recommendations in idiopathic IgA, it is recommended to treat all patients who develop significant proteinuria (>0.5-1 g / day) or hypertension, with ACE inhibitors or ARB, since the antiproteinuric and renoprotective effect of these drugs, also tested with IgA nephropathy (Praga et al, 2003).

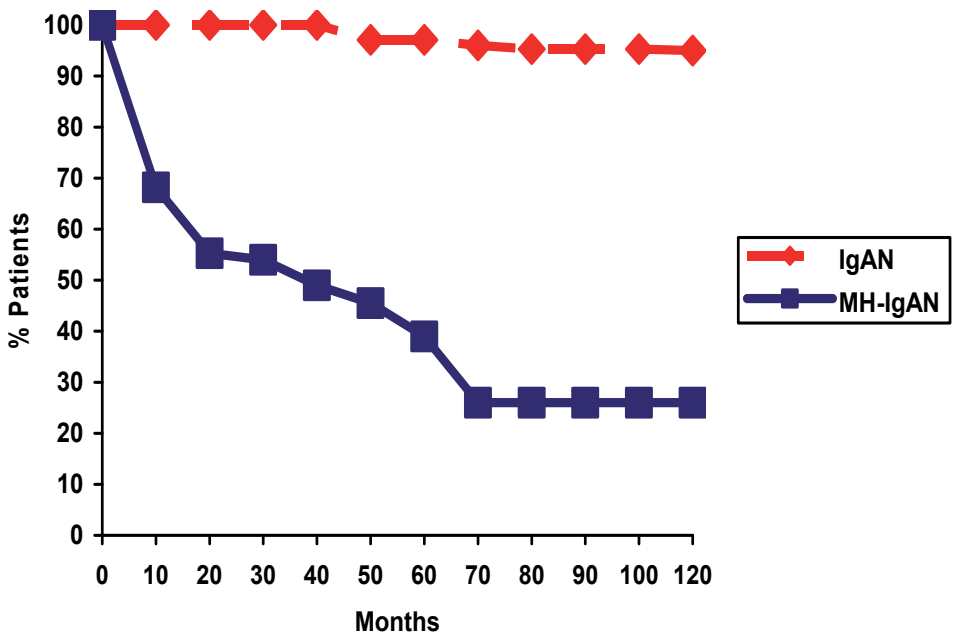

Fig. 2. Renal survival of patients with mesangial IgA nephropathy and MHT and mesangial nephropathy patients without MHT.

2.2.3 Focal segmental glomerulosclerosis non-collapsing

Among the different histological forms of focal glomerulosclerosis, have also been reported not collapsing glomerulosclerosis in patients with HIV (Haas et al, 2005). The pathogenesis and the exact relationship of these GN may have HIV or other pathogens have not been investigated.

Very limited information is available about the real incidence, clinical and developmental characteristics and therapeutic possibilities of this entity. In our experience, this type of glomerular disease is the second most common presentation in our HIV-infected patients (Gutierrez et al, 2007). The most common clinical presentation was proteinuria, and nephrotic syndrome was observed in 72% of cases. All patients were treated with HAART and BSRAA; at the end of treatment no patient need dialysis and 60% had renal insufficiency (Gutierrez et al, 2007).

In idiopathic forms, not associated with HIV, proteinuria and nephrotic syndrome are common manifestations. Some cases progress rapidly to ESRD. Corticosteroids administered for a long time for months and calcineurin inhibitors drugs are treatment options supported by experience. However, given the absence of data on HIV-infected patients and the susceptibility of these patients to infections, careful observational studies are needed before recommending these options. The optimization of antihypertensive and antiproteinuric drugs (ACEI/ARB) can exert a favorable effect, as in idiopathic less aggressive forms.

2.2.4 Membranous nephropathy

Membranous nephropathy (MN) is a typical example of glomerular disease mediated by deposition of immune complexes. MN is a recognized complication of renal malignancies

and infections. Among these, HBV and HCV have been associated with the development of MN and syphilis, and various parasitic diseases.
In this context, the frequent presence of HBV and HCV co-infection and the increasing incidence of syphilis and other infectious processes suggests that there may be a predisposition of patients with HIV infection to the development of MN, although not known actual incidence and its outcome.
The characteristic clinical presentation is nephrotic syndrome, although minorities of cases show no nephrotic range proteinuria. In forms idiopathic, there are two therapeutic strategies that have proven effective in controlled prospective studies, immunosuppression with steroids plus cyclophosphamide or chlorambucil, and calcineurin inhibitors drugs (cyclosporine, tacrolimus) (Praga, 2008). However, consider that a high percentage (up to 50% in some series) have spontaneous remission within the first years of the disease (Polanco et al, 2010) for this reason may be preferable initially attitude conservative in patients with HIV-infection. All chronic proteinuric nephropathies may be beneficial to use renin-angiotensin-aldosterone system blockers (ACEI/ARB). If we can identify an infectious agent as pathogenic factor, the treatment could potentially resolve the kidney problem.

2.2.5 Postinfectious glomerulonephritis

Although the descriptions are limited (Enriquez et al, 2004), patients with HIV infection could be theoretically exposed to the development of postinfectious GN due to its higher rate of infection. The clinical picture is generally abrupt, with hypertension difficult to control, often gross hematuria and mild and transient impairment of renal function frequently. There are typical red blood cell casts present in urinary sediment and hypocomplementemia. Renal biopsy shows a diffuse endocapillary GN with proliferation of mesangial and endothelial cells, Glomerular and interstitial infiltration of monocytes and lymphocytes is present. Glomerular accumulation of neutrophils is common and is termed "exudative". Ultrastructural studies demonstrate the subepithelial "humps" which are typical although not pathognomonic of postinfectious GN.
The history of an infection, usually 1-2 weeks, followed by a short asymptomatic period and the typical abrupt onset of nephritic syndrome for the diagnosis in many cases, along with the study of the sediment and the detection of hypocomplementemia. The majority of cases have benign course with conservative treatment of the infectious process and complications (hypertension, edema). The descriptions of acute postinfectious GN in patients with endocarditis or localized infections are characteristic, although they have published very few cases among HIV-infected patients.

2.2.6 Lupus-like glomerulonephritis

Lupus-like glomerulonephritis, defined by the presence of a "full house" of glomerular immunoglobulin and complement deposits on immunofluorescence in the absence of serologic evidence of systemic lupus erythematosus (SLE). There is presently little information known about the etiology, its treatment, or its long-term outcome.
Its main histological features are cell proliferation and mesangial matrix, the presence of hyaline thrombi and massive deposit of immune complexes in capillary walls, which reach mimic the "wire loops" typical of lupus nephritis and are the origin of name ("lupus-like") that has been proposed for these cases. In immunofluorescence, as occurs in lupus nephritis, deposit detects all types of immunoglobulins (IgG, IgA, IgM) and various fractions of

complement (Haas et al, 2005; Weiner et al, 2003). However, the term may be misleading, because the similarities end with lupus in the histological aspects of renal biopsy. There are no systemic symptoms similar to lupus flares and serology (ANA and anti-DNA, hypocomplementemia) is negative.

Most reported cases present with nephrotic syndrome and progressive deterioration of renal function. No information about the natural history of this process and whether or not modify therapeutic interventions. Regarding the pathogenesis, there are only speculations. It is proposed that, in addition to frequent infections with HBV, HCV and other infectious agents, HIV itself through several of its proteins (p24, gp41, gp120) can induce a systemic response of immunoglobulin, causing a large amount of circulating immune complexes that would be trapped nonspecifically in the glomerulus, alternatively, the HIV proteins could be initially deposited in the glomerulus (planted antigens) and subsequently be detected by the antibody in situ formation of immune complexes (Weiner et al, 2003).

Although we can be exceptionally patient with HIV infection and the coexistence of lupus with renal involvement, and it would be important to make a diagnosis difference between the two entities. In patients with both HIV infection and a diagnosis of SLE, three patterns of disease occurrence have been described: HIV following SLE diagnosis, SLE following HIV infection, and simultaneous diagnosis of HIV and SLE. Since the appearance of an autoimmune disorder in patients with a pre-existing immunodeficiency would not be expected, at least from a mechanistic standpoint, we will focus our review on patients with existing HIV who developed signs and symptoms consistent with a new diagnosis of lupus. SLE and HIV infection are two diseases whose clinical and serologic presentations may occasionally mimic one another, but with pathogenic mechanisms that theoretically are mutually exclusive. The seemingly paradoxical coexistence of these two immune disorders offers intriguing insights into the complex cellular and humoral immune networks that govern autoimmune phenomena and self tolerance.

Finally, the clinical management of the HIV positive lupus patient represents a therapeutic challenge for the physician due to the delicate equilibrium that needs to be achieved between SLE remission and HIV control (Gindea et al, 2010).

2.3 Other nephropathies in patients with HIV infection

2.3.1 Thrombotic microangiopathy

Thrombotic microangiopathy (TMA) is a known complication of HIV infection (Connolly et al, 1995; Alpers, 2003). Published reports of the incidence of TMA evaluated during the pre-HAART era vary considerably, depending on the type of study performed, diagnostic criteria used to evaluate patients, and stage of HIV disease (Connolly et al, 1995). It is likely that this under-diagnosed entity, as shown in autopsy studies (Gadallah et al, 1996) and patients with progressive deterioration of renal function, the presence of MAT in renal biopsy exceeded the incidence of HIVAN (Peraldi et al, 1999).

Endothelial cell injury appears to be the primary event causing platelet activation and deposition in the microvasculature. On these microthrombi mechanical destruction would occur, anemia, schistocytes, elevated LDH and Coombs test negative. Direct cytopathic roles of HIV as well as other factors such as malignancy, drugs, and infectious agents have been implicated in the pathogenesis of HIV-TMA. It is known that various HIV proteins can directly damage endothelial cells, inducing apoptosis therein (Alpers, 2003).

In experimental animals infected with HIV often develops MAT, so it is suspected that HIV itself plays a key role in endothelial damage (Eitner et al, 1999). It is also the possibility that anticardiolipin antibodies/antiphospholipid frequently detected in HIV patients (Boue et al, 1990) have pathogenic importance. In the primary antiphospholipid syndrome or lupus erythematosus associated with MAT may be triggered. Although not able to demonstrate that HIV-infected patients with anticardiolipin antibodies are at higher risk for MAT, it is an intriguing association. In idiopathic MAT has been achieved in many cases to elucidate the molecular basis of the disorder, especially those with family history: gaps ADAMS-13 and other enzymes that degrade the von Willebrand factor in type forms PTT and alterations of the complement system in the SHU. There is no evidence that these disorders occur in the MAT of patients infected with HIV. Although the majority of patients present in a more advanced stage of HIV disease, TMA can be the initial presenting symptom of HIV infection.
Clinical features are those of idiopathic TMA (Eitner et al, 1999), and the diagnosis should be suspected in any patient with new onset thrombocytopenia and microangiopathic haemolytic anaemia. Most patients are male and young and progressive deterioration of renal function is associated with the typical hematological findings MAT: schistocytes anemia with peripheral blood, thrombocytopenia, elevated LDH. Although most cases present a clear and rapidly progressive deterioration of renal function, similar to pictures of hemolytic uremic syndrome (HUS), others can have predominant neurologic manifestations, as in thrombotic thrombocytopenic purpura (TTP).
Kidney biopsy shows changes similar to idiopathic MAT: platelet-fibrin thrombi in preglomerular arterioles and the glomerular capillaries, arterioles with endothelial edema imaging in "onion skin" and mesangiolysis with widening of subendothelial space. When there are extrarenal manifestations (neurological, cardiac) vascular lesions are similar in the vessels of the affected organs.
Therapy with plasma exchange or infusion appears to be efficacious. A rapid diagnosis and institution of plasmapheresis is crucial for a favorable outcome. The long term prognosis of HIV-TMA is unfavorable and may depend on the stage of HIV infection. The recent data after the use of highly active retroviral treatment, however, are unavailable and current prognosis is therefore uncertain.

2.3.2 Malignant hypertension

Malignant hypertension is defined by the presence of unacceptably high blood pressure with grade III hypertensive retinopathy (hemorrhages and exudates) or IV (papilledema more vascular lesions). As we noted earlier, is a casual presentation of idiopathic IgA nephropathy, but its impact on the rest of idiopathic GN is rare. By contrast, preliminary studies indicate incidence of MHT special not only in IgA nephropathy patients with HIV, in which may be the most frequent presentation of this entity, but in other glomerular diseases associated with HIV (Morales et al, 2008). On the other hand, entities such as membranoproliferative GN, membranous and focal glomerulosclerosis, in which the MHT is a rare complication, there are a significant number of cases when associated with HIV infection. In our experience, malignant nephrosclerosis (arteriolar fibrinoid necrosis with intimal thickening and luminal narrowing; see **Figure 3**) was detected in six cases (three of them with IgAN, one with C-FSG, one with NC-FSG and one with MGN).

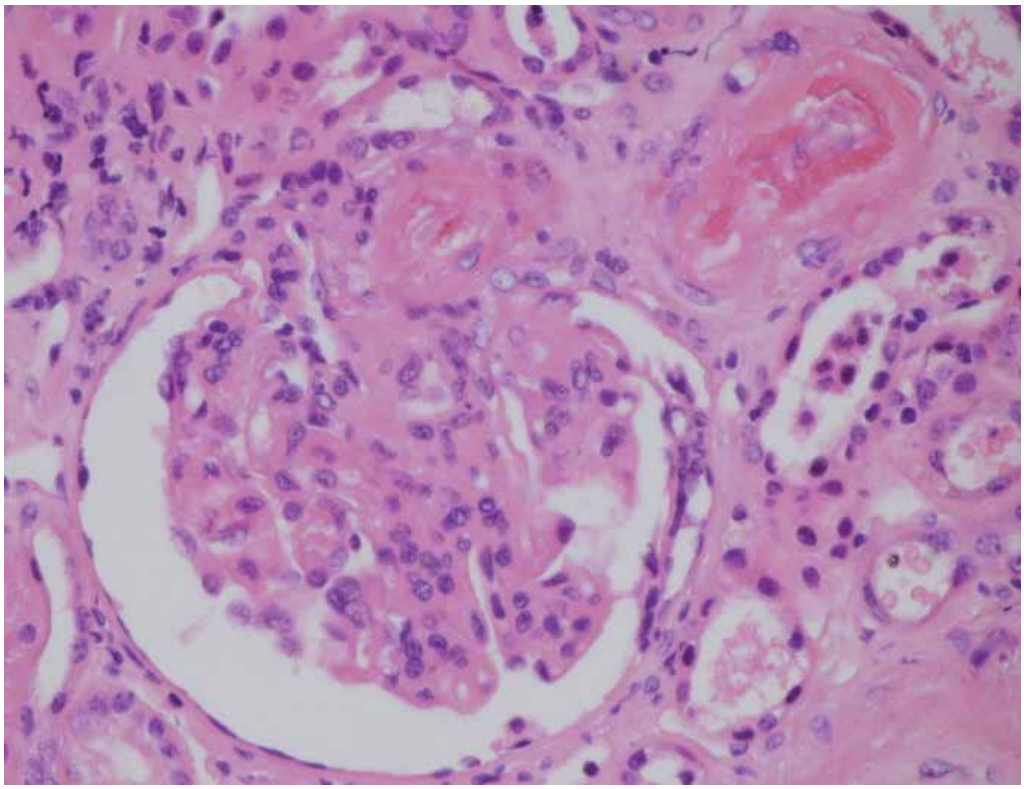

Fig. 3. Renal biopsy of patient 1 (Table 1), showing a glomerulus with mesangial proliferation (the immunofluorescence showed predominant deposits of IgA) and vascular lesions of malignant nephrosclerosis (fibrinoid necrosis) in the afferent arteriole. (hematoxylin;eosin; x400).

The typical symptoms of MHT (markedly increased BP, blurred vision due to retinal haemorrhages and severe headache). To interpret this catastrophic prognosis in HIV patients with GN and MHT, we speculate that the development of MHT has an irreversible detrimental influence on glomerular diseases already having a rather poor prognosis by themselves, see **Figure 4**.

On the other hand, the development of chronic renal failure could likely exacerbate the appearance of infectious and cardiovascular complications in these HIV-infected patients because both patients with chronic renal failure and HIV infection are particularly prone to the appearance of such complications (Grinspoon & Carr, 2005; Kamin & Grinspoon, 2005; Gupta et al, 2005). Other possible pathogenic pathways to explain this particular propensity of HIV patients with glomerular diseases to MHT could rely on the already known higher incidence of thrombotic microangiopathy (TMA) among HIV patients (Alpers, 2003). Both TMA and MHT could be interpreted as clinical manifestations of a systemic endothelial injury, due to a direct toxic effect of HIV, other infectious agents (particularly HCV and HBV) or other factors (such as drug addictions) linked to the environment of HIV infection. On the other hand, the presence of antiphospholipid antibodies is also more common among HIV patients, and these autoantibodies could play a role in endothelial vascular damage (Alpers, 2003; Galrao et al, 2007).

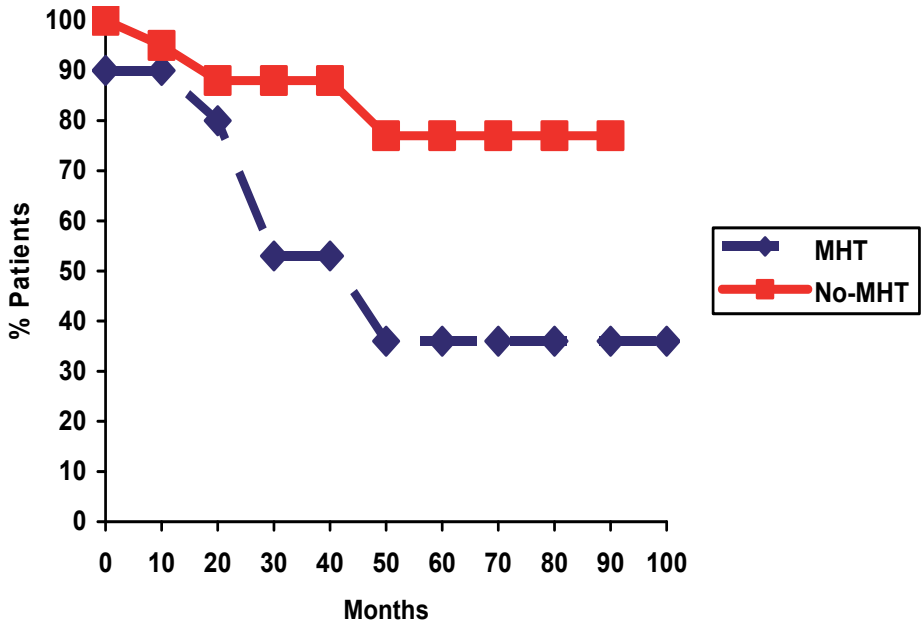

Fig. 4. Probability of renal survival (absence of chronic dialysis) in HIV patients with or without malignant hypertension.

In addition, considering the dismal prognosis of our patients in spite of HAART treatment and a satisfactory control of blood pressure, early detection and treatment of hypertension in HIV patients with glomerular diseases are mandatory.

2.3.3 Amyloidosis

In autopsy series of patients infected with HIV, as well as reviews of renal biopsies, amyloidosis is a relatively common finding (Lanjewar et al, 1999; Joseph et al, 2000). This is a secondary amyloidosis, type AA. Many studies have been suggested that frequent chronic infections may be responsible for this complication. However, it is possible that HIV infection itself plays a pathogenic role. Described a high level of SAA, acute phase reactant which is the precursor of AA amyloid in patients infected with HIV (Husebekk et al, 1986) and experimental models have shown that a significant proportion of animals infected develop amyloidosis (King et al, 1983).

As is common in amyloidosis, massive proteinuria and nephrotic syndrome were the most common renal manifestations in the cases described. There are no studies about specific therapeutic options in these patients, apart from trying to eradicate the underlying infectious process.

2.3.4 Hypertensive and diabetic nephropathy

Metabolic complications of HAART (dyslipidemia, changes in body fat, insulin resistance, diabetes mellitus) and the aging of the infected population suggest that kidney damage secondary to diabetes and hypertension may have increasing importance in patients infected HIV (Masia-Canuto et al, 2006).

In some series of renal biopsies of patients with HIV infection has been reported the presence of diabetic nephropathy in 6% of cases, hypertensive nephropathy in 4% (Szczech et al, 2004).The recommended treatment is similar to that used in the uninfected population and should include strict control of blood pressure and the early use of BSRAA to try to reduce proteinuria.

3. Tubular and interstitial renal disease

Patients infected with HIV can present a wide variety of tubular and interstitial renal disease secondary to drugs, infections and/or tumors; see Table 4 (GESIDA, 2010; Blok & Weening, 1999).

- Acute tubular necrosis
- Interstitial nephritis associated with drugs
  - Renal failure and Fanconi syndrome (certain reverse transcriptase inhibitors)
- Allergic interstitial nephritis
- Cristal-induced nephropathy (certain protease inhibitors)
- Interstitial nephritis associated with infections (cytomegalovirus, tuberculosis, hongos, Salmonella, Legionella, etc)
- Kaposi sarcoma and Lymphoma infiltrative
- Rhabdomyolysis

Table 4. Tubular and interstitial renal disease

The recommendations for the treatment of these nephropathies consist of drug withdrawal or correction of the precipitating cause, corrections, electrolyte, and in interstitial nephritis immunoallergic the administration of a short course of corticosteroids.

4. Conclusion

In conclusion, due to the wide range of kidney damage in people with HIV infection, it is difficult to predict the renal histology according to clinical criteria, so renal biopsy is mandatory for histologic diagnosis. In addition, the course and prognosis of these patients has changed radically since the introduction of antiretroviral therapy, with higher survival, so early diagnosis is essential and the establishment of an alternative therapy to prevent progression of renal disease.

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Part 3

Vasculitis and Autoimmune Glomerulopathies

13

Henoch-Schönlein Purpura Nephritis in Childhood

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1. Introduction

Henoch-Schönlein purpura is one of the most common causes of systemic vasculitis. Henoch–Schönlein purpura typically affects children between the age of 3 and 10 years. The aetiology is unknown. Diagnosis includes palpable purpura (essential) in the presence of diffuse abdominal pain, acute arthritis/arthralgia, renal involvement characterized by haematuria and/or proteinuria (Ozen et al., 2006) and skin biopsy showing predominant IgA deposition in the walls of cutaneous vessels.
In the majority of cases it is a self-limiting disease. Therefore, up to 40% of children with Henoch–Schönlein purpura require hospitalization for management of acute disease manifestations which may include nephritis, hypertension, severe pain, gastrointestinal bleeding or arthritis. Purpura occurs in all cases, joint pains and arthritis in 80% of cases, and abdominal pain in 62% of cases.
The purpura typically appears on the legs and buttocks (Fig.1), but may also be seen on the arms, face and trunk. The abdominal pain is colicky, and may be accompanied by nausea, vomiting, constipation or diarrhea. There may be blood or mucus in the stools. Sometime finding includes a gastrointestinal haemorrhage, occurring in 33% of cases, due to intussusceptions (Saulsbury, 1999). The joints involved tend to be the ankles, knees, and elbows but arthritis in the hands and feet is possible; the arthritis is non-erosive and hence causes no permanent deformity. Problems in other organs, such as the central nervous system (brain and spinal cord) and lungs may occur, but much less commonly than the skin, bowel and kidneys (Saulsbury, 2001)

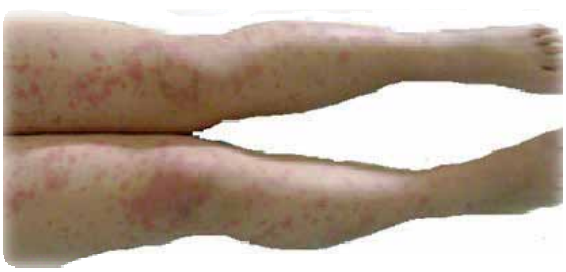

Fig. 1. Henoch-Schönlein purpura in a 8 years old female child

Paediatric patients may develop glomerulonephritis within 4 to 6 weeks of the initial purpura presentation (Saulsbury, 2007). Renal involvement in Henoch-Schönlein purpura is transitory in most cases. Therefore, the long-term prognosis in Henoch-Schönlein purpura depends on the severity of renal involvement and can be poor when complicated by severe nephritis and chronic renal failure.

2. Pathogenesis

Henoch-Schönlein purpura nephritis is a systemic immune-complex mediated disease according to the clinical or histological pattern of recurrences of Henoch-Schönlein purpura nephritis in some patients after transplantation (Soler et al., 2005). The histological hallmark of Henoch-Schönlein purpura is severe inflammation of small vessels, particularly post-capillary venules, with neutrophils, resulting in fibrinoid necrosis of vessel walls and extravasation of erythrocytes (Saulsbury, 1999). The clinical features are a consequence of general vasculitis due to IgA1 deposition in vessels and the renal measangium (Saulsbury, 1999). Therefore, the pathogenetic mechanisms are still not fully understood.

2.1 IgA immune complexes

Similarly to IgA nephropathy, deposits of IgA-binding M proteins of group A streptococci were found on Henoch-Schönlein purpura kidneys. All Henoch-Schönlein purpura patients have IgA1-circulating immune complexes of small molecular mass. Therefore, only those with nephritis have large-molecular-mass IgA1-IgG-containing circulating immune complexes (Levinsky & Barratt, 1979). Large-molecular mass IgA-IgG complexes in the circulation are the major factor responsible for the formation of the nephritogenic immune complexes in patients with Henoch-Schönlein purpura nephritis.

Some children with Henoch-Schönlein purpura nephritis subsequently have an episode or recurrent episodes of macroscopic hematuria, associated with upper respiratory tract infection without the other clinical features of Henoch-Schönlein purpura nephritis (Waldo, 1988). Thus, these children's clinical phenotype changes to that of IgA nephropathy. As the renal histologic and immunofluorescence microscopy findings in Henoch-Schönlein purpura nephritis are indistinguishable from those seen in patients with IgA nephropathy (Evans et al., 1973), it has long been ever speculated that Henoch-Schönlein purpura nephritis and IgA nephropathy have common pathogenetic mechanisms representing different ends of a continuous spectrum of disease (Waldo, 1988).

Henoch-Schönlein purpura nephritis is similar to IgA nephropathy since IgA1, but not IgA2, is found in the circulating immune complexes and in mesangial immune deposits (Novak et al., 2007). IgA1-containing immune complexes are excreted in elevated amounts in the urine in patients with IgA nephropathy and Henoch-Schönlein purpura nephritis and may provide a specific marker for disease activity and/or severity in these patients (Suzuki et al., 2008). IgA-binding M proteins may encounter circulatory IgA forming a complex with IgA-Fc that could deposit in renal tissues (Schmitt et al., 2010).

Reduced galactosylation of IgA1 O-glycans has been reported in patients with Henoch-Schönlein purpura nephritis (Allen et al., 1998). Glycosylation defects are due to complex changes in expression of specific glycosyltransferases with reduced expression of β1,3-galactosyltransferase and elevated expression of GalNAc-specific α2,6-sialyltransferase in patients with both IgA nephropathy and Henoch-Schönlein purpura nephritis, but not in patients with Henoch-Schönlein purpura without nephritis or healthy controls (Suzuki,

Moldoveanu et al., 2008). Due to their size, Galactose-deficient IgA1 containing immune complexes are less efficiently taken up by the asialoglycoprotein receptor in the liver and catabolised and their amounts increase in the circulation (Moura et al., 2004). Galactose-deficient IgA1 leads to the formation of the circulating immune complexes. These complexes may then deposit in the renal mesangium and incite, likely due to the binding to mesangial cells, to cellular activation (Lau et al., 2010). Consequently, mesangial cells start to proliferate and overproduce extracellular matrix components, cytokines and chemokines (Davin & Weening, 2003) leading to glomerular injury contributing to the pathogenesis of Henoch-Schönlein purpura nephritis.

Glomerular depositions of other components, including kappa and lambda light chains, are also variably demonstrated in Henoch-Schönlein purpura nephritis. In patients with IgA nephropathy, lambda light chains were found predominantly over kappa light chains (Lai et al., 1996).

2.2 Complement

Complement activation appears to play an important role in the pathogenesis of IgA nephropathy and Henoch-Schönlein purpura nephritis, as glomerular complement activation may initiate the inflammatory cascade and enhance glomerular injury (Wyatt et al., 1987). Therefore, hypocomplementemia has been reported in some patients with Henoch-Schönlein purpura nephritis, and it is usually transient and not related to the severity of the diseases (Motoyama & Litaka, 2005).

3. Incidence

The estimated annual incidence of Henoch-Schönlein purpura in children is 10–20 per 100,000 children (Rostoker, 2001). The annual incidence of Henoch-Schönlein purpura in Asian children [4.9 per 100,000] and African children [6.2 per 100,000] was significantly lower than Caucasian children [17.8 per 100,000].

In childhood Henoch-Schönlein purpura, the male:female ratio ranges from 1.2−1.6 (Yang et al., 2005).

Renal involvement occurs less frequently in children than adulthood (Yang et al., 2005; Pillebout et al., 2002). The incidence of nephritis in patients with Henoch-Schönlein purpura has been reported to be 15-62% with an estimated annual incidence of 20.4 per 100,000 children (Gardner-Medwin et al., 2002; Shenoy, Bradbury, et al. 2007; Bogdanovic, 2009).

The overall incidence of Henoch-Schönlein purpura nephritis and the severity of Henoch-Schönlein purpura nephritis in patients between 1987 and 1997 were similar to those in children between 1998 and 2008 and the number of patients with severe Henoch-Schönlein purpura nephritis has not decreased (Kawasaki et al., 2010). The overall incidence of Henoch-Schönlein purpura nephritis is rather stable over time.

It could be estimated that 1–2% of all Henoch-Schönlein purpura nephritis patients will ultimately develop chronic kidney disease (Stewart et al., 1988; Narchi, 2005). A variable percentage of children (0−19%) with Henoch-Schönlein purpura nephritis may progress to renal failure or end stage renal disease (Ronkainen et al., 2002; Coppo et al., 1997; Goldstein et al., 1992; Kawasaki et al., 2003; Pillebout et al., 2002). In children with Henoch-Schönlein purpura nephritis followed up at tertiary centres the risk for progression to chronic kidney disease or end-stage renal disease is predicted to be 5–18% at 5 years, 10–20% at 10 years

and 20–32% at 20 years from the diagnosis (Goldstein et al., 1992; Coppo et al., 1997; Kaku et al., 1998; Bogdanovic, 2009).

4. Clinical patterns

The average duration of Henoch-Schönlein purpura symptoms is 4 weeks. The majority of patients experience resolution of symptoms within 2 to 3 months. Approximately 30% of patients have one or more recurrences after the resolution of initial symptoms (Saulsbury, 1999; Trapani et al., 2005). Therefore, purpura lasting longer than 1 month or relapsing disease are associated with the development of nephritis(Rigante et al., 2005; Shin, Park, et al., 2006). Patients showing abdominal pain as the initial symptom had a higher probability of developing nephrotic syndrome. Persistent rash was a poor prognostic factor for Henoch-Schönlein purpura nephritis (Hung et al., 2009).

Renal signs are manifested in the majority of Henoch-Schönlein purpura patients from 3 days to 17 months after onset of the disease (Kaku et al., 1998), occurring more frequently within the first 3 months (Sano et al., 2002). In few cases the renal disease may develop even years after the initial presentation (Mollica et al., 1992). While abnormalities on urinalysis may continue for a long time, only 1% of all Henoch-Schönlein purpura patients develop chronic kidney disease (Saulsbury, 2001).

Nephritis is the one feature of Henoch-Schönlein purpura that may have chronic consequences. The long-term prognosis is largely dependent on the severity of nephritis (Narchi, 2005; Mir et al., 2007). Renal manifestations of the disease ranged from mild, benign involvement, intermittent haematuria and proteinuria, to rapidly progressive or crescentic nephritis. Of the 40% of patients who develop kidney involvement, almost all have evidence (visible or on urinalysis) of blood in the urine. More than half also have proteinuria, which in one eighth is severe enough to cause nephrotic syndrome (Saulsbury, 2001). From a retrospective study, nephritis occurred in 46% of the Henoch-Schönlein purpura patients, consisting of isolated haematuria in 14%, isolated proteinuria in 9%, both haematuria and proteinuria in 56%, nephrotic-range proteinuria in 20% and nephrotic-nephritic syndrome in 1% (Jauhola et al., 2010).

Renal involvement is in most cases mild and self-limited in children. Henoch-Schönlein purpura nephritis in children had a lower risk of progression to renal insufficiency than adults. Gross hematuria and lower extremity edema were less frequent in the children than adults (Hung et al., 2009). Morbidity is low in patients with Henoch-Schönlein purpura who have hematuria and mild proteinuria at onset, while it is higher among those with more severe renal disease, as in a nephritic, nephrotic or a nephritic/nephrotic signs (Coppo et al., 2006; Ronkainen et al., 2002; Narchi, 2005; Goldstein et al., 1992). The main clinical signs of rapidly progressive Henoch-Schönlein purpura nephritis at presentation were edema, hypertension, gross hematuria, and oliguria (Oner, 1995). End-stage renal disease was associated with nephritic and/or nephrotic syndrome at presentation in nearly all children with Henoch-Schönlein purpura nephritis (Soylemezoglu et al., 2009). The highly variable clinical course of Henoch-Schönlein purpura nephritis has been related to the marked variability in histopathologic presentation at renal biopsy, with glomeruli ranging from histologically normal to diffuse proliferative and crescentic lesions (Assadi, 2009).

4.1 Classification

Five categories of Henoch-Schönlein purpura nephritis were identified according to renal manifestations at disease onset (Falkner et al., 2004): (A) micro/macroscopic hematuria or

persistent mild proteinuria (< 1 g/L or urine albumin/creatinine ratio < 200 mg/mmol); (B) persistent mild proteinuria (< 1 g/L or urine albumin/creatinine ratio < 200 mg/mmol) and micro- or macroscopic hematuria; (C) nephritic syndrome (moderate proteinuria and urine albumin/creatinine ratio ≥ 200–400 mg/mmol), decreased glomerular filtration rate, hematuria and/or hypertension, or nephrotic syndrome (urinary albumin excretion > 40 mg/hour/m^2 body surface area or urine albumin/creatinine ratio ≥ 400 mg/mmol, serum albumin < 25 g/L; (D) acute progressive glomerular nephritis; (E) chronic glomerular nephritis. Classes A and B were considered as mild renal disease, and classes C to E as severe disease (Meadow et al., 1972).

Another clinical evaluation categorized the patients according to four stages. Stage (A) is considered normal: the patient was normal on physical examination, with normal urine and renal function; stage (B) had minor urinary abnormalities: the patient was normal on physical examination, with microscopic hematuria or proteinuria of less than 20 mg/m^2/h; stage (C) had persistent nephropathy: the patient had proteinuria of 20 mg/m^2/h or greater or hypertension and a 24-h creatinine clearance of 60 ml/ min/1.73 m^2 or greater; stage (D) had renal insufficiency: the patient had a 24-h creatinine clearance of less than 60 ml/min/1.73 m^2, including dialysis/transplant or death (Kawasaki et al., 2010).

4.2 Serum IgA

Although serum IgA levels are higher in children with Henoch-Schönlein purpura / Henoch-Schönlein purpura nephritis than in controls, this serum abnormality does not constitute a sensitive diagnostic marker of Henoch-Schönlein purpura with or without nephritis. In particular, over 40% children with Henoch-Schönlein purpura had elevated serum IgA levels at presentation. Therefore, the difference in serum IgA levels between patients with and without nephritis was not statistically significant.

4.3 Renal biopsy

Renal involvement can be severe but may resolve completely. Therefore, some children will develop long-term sequelae. The renal biopsy is helpful in determining the need for treatment with immunosuppression in the acute phase (McCarthy & Tizard, 2010).

The criteria for renal biopsy were defined as follows: (1) the patients had proteinuria of 20 mg/m^2/hour or greater and haematuria or (2) the patients had proteinuria of less than 20 mg/m^2/hour and recurrent macrohematuria (Kawasaki et al., 2010). According to another recent review a renal biopsy has been recommended in the following situations: (1) acute renal impairment/nephritic syndrome at presentation; (2) nephrotic syndrome with normal renal function persisting at 4 weeks; (3) nephrotic range proteinuria (urine protein/creatinine ratio, >250 mg/mmol) at 4–6 weeks (if not improving spontaneously); (4) persistent proteinuria-urine protein/creatinine ratio >100 mg/mmol for more than 3 months. Consider biopsy particularly if the diagnosis is not clear (McCarthy & Tizard, 2010).

Renal involvement in Henoch-Schönlein purpura is quantified by means of a kidney biopsy (Fig. 2), which may demonstrate positive mesangial staining and positive anti-IgA antisera on immunofluorescence, with glomerular changes graded chiefly according to the Henoch-Schönlein purpura nephritis classification described in the International Study of Kidney Disease in Children (Rai et al., 1999; Sheno, Bradbury, et al. 2007; Ronkainen et al., 2006). The grading of renal histology has been considered as an important marker of outcome (Farine et al., 1986). The classification provided by the Study of Kidney Disease in Children included grade I: minimal alterations; grade II: mesangial proliferation; grade III: focal or diffuse

proliferation or sclerosis with <50% crescents; grade IV: focal or diffuse mesangial proliferation or sclerosis with 50–75% crescents; grade V: focal or diffuse mesangial proliferation or sclerosis with >75% crescents; grade VI: membranoproliferative-like lesions (Counahan et al., 1977).

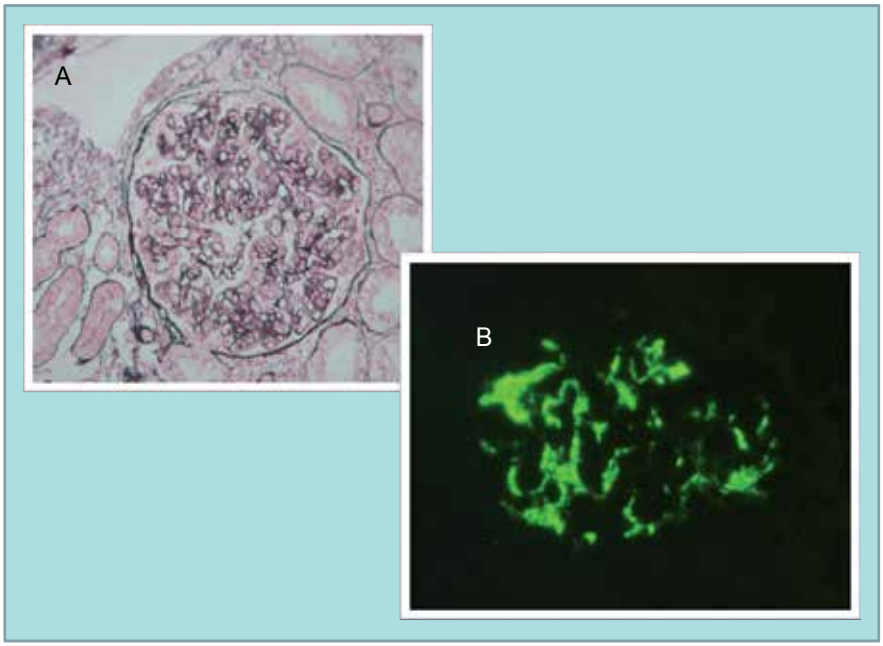

Fig. 2. Methenamine-silver stain of a glomerular of a patient with Henoch-Schönlein purpura nephritis showing diffuse mesangial and focal endocapillary proliferation (panel A). The immunofluorescence studies showed intense IgA positivity (3+) (panel B); complement C3 staining was also mildly positive (1+).

5. Risk factors

5.1 Renal involvement in Henoch-Schönlein purpura

Some authors attempted to identify prognostic factors for a child with Henoch-Schönlein purpura to develop nephritis by using univariate and multivariate analysis models. The independent risk factors for Henoch-Schönlein purpura nephritis were persistent purpura, severe abdominal symptoms and age above 4 or 7 or 10 years. Other independent risk factors were relapse or decreased serum factor XIII activity (Kaku et al., 1998; Sano et al., 2002; Rigante et al., 2005; Shin, Park, et al., 2006; Ronkainen et al., 2006). Persistent purpura, severe abdominal symptoms and an older age were confirmed as the most significant risk factors for later nephropathy (Bogdanovic, 2009). A prospective study showed that age over 8 years at onset (OR 2.7), abdominal pain (OR 2.1) and a recurrence of Henoch-Schönlein purpura disease (OR 3.1) were independent risk factors for developing nephritis(Jauhola et al., 2010).

5.2 Long-term renal impairment

Severe renal involvement at onset of Henoch-Schönlein purpura is in general predictive of a poor renal outcome. The independent predictors of a poor renal outcome were severe initial

presentations with renal failure, nephritic, nephrotic syndrome or mixed syndrome and the percentage of glomeruli with crescents (Mir et al., 2007). Some few children with mild renal symptoms at onset have a poor long-term outcome. Thus, long-term follow-up is mandatory also for these patients (Algoet & Proesmans, 2003; Goldstein et al., 1992).

6. Follow-up

A clinical pathway was recommended if there is evidence of haematuria, proteinuria, renal impairment or hypertension in patients with Henoch-Schönlein purpura (Tizard & Hamilton-Ayres, 2008). Prospectively and systematically collected data suggested that weekly urine dipstick tests should be continued for 2 months from the onset of Henoch-Schönlein purpura. Beyond that point frequent routine follow-up is neither cost-effective nor necessary in patients with no urine abnormalities during follow-up. However, the length of follow-up time should be increased at least up to 6 months individually in the case of Henoch-Schönlein purpura recurrence and in those developing nephritis (Jauhola et al., 2010).

7. Prevention of Henoch-Schönlein purpura nephritis

Intervention to shorten the duration of Henoch-Schönlein purpura and prevent relapses may be helpful in preventing the development of nephritis. However, no therapy has yet been shown to decrease the duration of Henoch-Schönlein purpura, prevent recurrences, or prevent the development of nephritis (Saulsbury, 2009).

Studies reported on patients with Henoch-Schönlein purpura lacking clinical signs of nephropathy at admission were treated with prednisone at doses ranging from 1.0 to 2.5 mg/kg/day over a period of 7–21 days. Early prednisone treatment did not succeed in reducing the risk of further renal complication from Henoch-Schönlein purpura. Although prednisone is effective in alleviating the abdominal pain and joint pain associated with Henoch-Schönlein purpura, it did not short the duration of the disease, prevented the recurrences, or prevented the development of nephritis (Ronkainen et al., 2006). The relatively small subgroup of Henoch-Schönlein purpura patients who may benefit from corticosteroids included those who present with renal involvement and probably those with severe abdominal symptoms requiring medical attention (Mollica et al., 1992; Saulsbury, 1999; Narchi, 2005).

In general, the prophylactic treatment with prednisone at 2 mg/kg/day in Henoch-Schönlein purpura must be considered of value if (1) there is a quicker resolution of abdominal pain, considering the cost and potential damage from prolonged treatment; (2) the treatment performed during the acute phase reduce the rates of abdominal surgery for exploration or actual intestinal injury; (3) the avoidance of several late-onset medical conditions, including hypertension, preeclampsia, and persistent nail-fold capillary changes, suggesting a chronic vasculitis (Gibson et al., 2008). Such measure at this time has not been demonstrated. However, 2– 4 weeks of prednisone administration at doses ranging from 1.0 to 2.5 mg/kg per day over a period of 7–21 days or intravenous methylprednisolone prophylaxis (5 mg/kg four to six times per day for 3–5 days) failed to prevent renal involvement in Henoch-Schönlein purpura after 0.5–1 years (Zaffanello et al., 2009).

Dapsone, an antileprotic drug, has been used at 1–2 mg/kg/day daily in a few patients with prolonged in Henoch-Schönlein purpura, improving the time of purpuric rush, but it has not been studied in a rigorous fashion in children with nephritis (Iqbal & Evans, 2005).

The clinical course of patients in a report suggested that colchicines, an ancient anti-inflammatory drug, may be effective treatment at dosages of 1.2 mg/day in children with prolonged Henoch-Schönlein purpura possibly preventing the development of nephritis (Saulsbury, 2009). Therefore, at the current time insufficient data are available to support a recommendation for prophylaxis.

8. Conventional treatment

Conventional therapies were defined as drugs or procedures that act as immune modulators.

8.1 Treatment of mild form of Henoch-Schönlein purpura nephritis

Patients with mild renal symptoms have showed lower proportion of poor outcome than those with severe renal symptoms. In particular, patients with isolated hematuria showed a good prognosis, but 18% of patients with mild proteinuria at onset showed a poor outcome (Edström Halling et al., 2010). For this reason patients would be followed until full clinical resolution of renal symptoms. Since the level of proteinuria at onset does not seem to be a reliable predictor of outcome, the persistence of mild proteinuria in the long-term follow-up (one year) may require renal biopsy. Finally, the treatment of mild clinical form must be weighed according to International Study of Kidney Disease in Children grading score (see 8.2. and 8.3. sections).

8.2 Treatment of moderately severe Henoch-Schönlein purpura nephritis

Patients affected by moderately-severe proteinurias were treated with prednisone, intravenous gamma globulins and tonsillectomy, while pulse methylprednisolon and cyclophosphamide were introduced according to the degree of severity of renal histology.
Three Henoch-Schönlein purpura nephritis patients treated by means of intravenous (2 g/kg/month) and intramuscular gamma globulins (0.35 ml/kg every 15 days) showed improved degrees of proteinuria and acuity index at renal biopsy (Rostoker et al., 1994; Rostoker et al., 1995). The administration of the angiotensin converting enzyme inhibitor (enalapril 10 mg/day) combined with fish oil led to a significant reduction in protein excretion rate after a few weeks of treatment in case series (Dixit et al., 2004). Additionally, tonsillectomy proved to be effective in five patients affected by Henoch-Schönlein purpura nephritis (Sanai & Kudoh, 1996) as well as in 16 children with Henoch-Schönlein purpura nephritis in combination with intravenous pulse methylprednisolon at a dose of 1 gram/1.73 m^2 of body surface area (three to four cycles), prednisone (2 mg/kg/day) and cyclophosphamide (2 mg/kg/day) (Inoue et al., 2007).
Data obtained from the literature are insufficient to support the use of specific treatments, such as intravenous gamma globulins and angiotensin converting enzyme inhibitors, in moderate-severe Henoch-Schönlein purpura nephritis, based on case series (Zaffanello et al., 2009).

8.3 Treatment of rapidly progressive or crescentic Henoch-Schönlein purpura nephritis

Predictors of treatment with immunosuppression drugs were higher albuminurias and urine immunoglobulin G, and lower glomerular filtration rate at onset and a higher International Study of Kidney Disease in Children grading score in the biopsy. Unfortunately, no significant difference in outcome was found between the treated and

untreated patients with crescents. Neither was there any significant difference in glomerular filtration rate between treated and untreated patients (Edström Halling et al., 2010).
Several studies have reported that patients with severe Henoch-Schönlein purpura nephritis may benefit from an intravenous pulse of methylprednisolone (Kawasaki et al., 2003; Niaudet & Habib, 1998; Edström Halling et al., 2010), cyclosporine A (Ronkainen et al., 2003), cyclophosphamide (Kawasaki et al., 2004; Tarshish et al., 2004; Shenoy et al., 2007; Kawasaki et al., 2004; Edström Halling et al., 2010), urokinase pulse therapy (Kawasaki et al., 2003; Zaffanello et al., 2007), azathioprine (Zaffanello et al., 2007; Foster et al., 2000), and plasma exchange therapy (Scharer et al., 1999). All severe cases almost received angiotensin converting enzyme inhibitors and/or angiotensin II receptor blockers combined with immunosuppressant drugs (Edström Halling et al., 2010).
Some protocols of treatment for Henoch-Schönlein purpura nephritis included the prescription of a single specific immunosuppressive treatment; other included two or multiple immunosuppressive drugs.

8.3.1 Steroids

Steroids included prednisolone or methylprednisolone at 30 mg/kg/day for 3 consecutive days or dexamethasone at 5 mg/kg/day (Kawasaki et al., 2003; Niaudet & Habib, 1998). Fifty-six patients with renal lesions graded as IIIb or higher were treated with intravenous/oral steroids, along with dipyridamole and anticoagulant warfarin. The acuity index decreased at second biopsy, whereas the chronicity index did not differ significantly (Kawasaki et al., 2003).

8.3.2 Cyclosporine A

Single treatment included cyclosporine A performed for 6 months - 2 years (Ronkainen et al., 2003).
Cyclosporine A, at the initial dose of 4–8 mg/kg/day, with blood level kept at 150–200 µg/L, and at the maintenance dose of 1–5 mg/kg/day, with blood level kept at 80–100 µg/L, proved to be effective in some case series with biopsy-proven steroid-resistant Henoch-Schönlein purpura nephritis. In particular, the treatment was effective in reducing the nephrotic proteinuria range after an average of 2 months in seven patients. Stable remission after a mean follow-up of 6 years was achieved in four subjects (Ronkainen et al., 2003). Treatment with cyclosporine A was effective in one patient with renal crescents following the failure of pulse steroid, oral prednisone and an 8 months course of azathioprine at 2 mg/kg/day (Shin et al., 2006). In seven patients, treatment with pulse or oral prednisone and cyclosporine A with or without angiotensin converting enzyme inhibitor cilazapril displayed a marked efficacy in reversing nephrotic-range proteinuria and reducing histological grading post-treatment (Shin et al., 2005). In another study involving a group of 82 children with varying degrees of renal manifestation and histology (Mir et al., 2007), the majority received steroid treatment while only a few were treated with angiotensin converting enzyme inhibitor or cyclophosphamide in combination with steroids. Despite treatment, the long-term prognosis worsened markedly in those children manifesting severe clinical presentation.

8.3.3 Cyclophosphamide

Single treatments included cyclophosphamide performed for 8-12 weeks (Tarshish et al., 2004; Zaffanello et al., 2007).
A case series of patients with biopsy-proven Henoch-Schönlein purpura nephritis displayed varying degrees of response to cyclophosphamide at 2-2.5 mg/kg/day. Several patients in

whom the initial renal biopsy had revealed ≥80% crescentic glomeruli received cyclophosphamide in combination with pulse of methylprednisolone and/or long-term oral prednisone. The combined treatment was effective in aiding clinical recovery, maintaining normal renal function and reducing grade histology. Unfortunately, the lack of control patients and the small number of patients treated or short follow-up period hamper the drawing of firm conclusions (Zaffanello et al., 2007; Zaffanello et al., 2009).
Long-term outcome of patients treated with daily cyclophosphamide expressed as end-stage renal disease did not differ with controls (Tarshish et al., 2004), indicating failure at level II of evidence. In a retrospective investigation, 21 children with crescents in 40% of glomeruli who were treated with an association of azathioprine at 1-2 mg/kg/day and steroids displayed an effective clinical outcome. Unfortunately, the considerable variability of the histological patterns complicated any interpretation of the results obtained (Bergstein et al., 1998; Foster et al., 2000). Moreover, nine patients with severe histologically graded lesions were prescribed aggressive therapy with azathioprine and steroids, leading to marked clinical improvement. Regrettably, histological outcome was not reported, thus hindering the drawing of sustainable conclusions (Singh et al., 2002). Lastly, ten children treated with steroids and azathioprine were compared to ten patients receiving steroids alone. The initial biopsy revealed histological lesions comparable to those observed at follow-up, although mesangial IgA depositions were reduced in the majority of patients (Shin et al., 2005). However, the small number of patients studied led to difficulties in interpreting results.

8.3.4 Mycophenolate mofetil

Mycophenolate mofetil at 900-1,200 mg/m^2/day was tested in patients with vasculitis and connective tissue disease involving the kidney, one of whom was affected by Henoch-Schönlein purpura nephritis. This drug was administered subsequent to the failure of treatment with steroids and azathioprine (Filler et al., 2003). However, two patients featuring a prolonged course of nephritis were first prescribed steroids and azathioprine with the subsequent addition of mycophenolate mofetil (Algoet & Proesmans, 2003). Once again, due to the exceedingly low number of patients treated, no firm conclusions could be drawn.

8.3.5 Single or combined treatment

Other protocols were performed with double immunosuppressant therapy, including steroid– cyclophosphamide (Oner et al., 1995; Iijima et al., 1998; Flynn et al., 2001; Tanaka et al., 2003; Kawasaki et al., 2004; Mir et al., 2007), steroid combined with azathioprine for 8-15 months (Bergstein et al., 1998; Foster et al., 2000; Singh et al., 2002; Shin et al., 2005; Zaffanello et al., 2007), and steroid– cyclosporine A or steroid – (angiotensin converting enzyme inhibitors) – cyclosporine A (Shin et al., 2005; Shin et al., 2006; Shin et al., 2007).
Triple immunosuppressant therapy was carried out using steroid– cyclophosphamide – azathioprine (Shenoy et al., 2007) and steroid– azathioprine –mycophenolate mofetil (Algoet et al., 2003; Zaffanello et al, 2007).

8.3.6 Evidence based treatment

The majority of reports provide scarce support to the various treatment options identified in cases of severe childhood Henoch-Schönlein purpura nephritis (Zaffanello et al., 2009). As the Henoch-Schönlein purpura nephritis and IgA nephropathy have identical pathogenesis and renal lesions, for which current evidence supports the use of immunosuppressive drugs

in patients with severe disease (Samuels et al., 2003; Cheng et al., 2009), the treatment protocols with proven significant benefit in IgA nephropathy should be used in children having Henoch-Schönlein purpura nephritis of comparable severity (Bogdanovic, 2009). A recent, single-centre, retrospective review looking at treatment of severe Henoch-Schönlein purpura nephropathy and IgA nephropathy demonstrated that therapy with differing combinations of steroids, cyclophosphamide, angiotensin converting enzyme inhibitors and angiotensin receptor blockers produced a good outcome in 54% of children with severe (>stage III) histological changes on initial renal biopsy (Edström Halling et al., 2009). For this reason, many physicians prescribe similar treatments in Henoch-Schönlein purpura patients with nephritis, despite the lack of disease-specific data, although IgA nephritis lesions however, tend to have a less severe inflammatory component and clinical trials on IgA nephropathy often include adult patients, while Henoch-Schönlein purpura nephropathy is primarily a disease that develops in children (Zaffanello et al., 2010).
Moreover, several patients with moderately severe Henoch-Schönlein purpura nephritis (histological grade I–III and serum albumin >2.5 g/dl) were treated with angiotensin-converting enzyme inhibitors and/or angiotensin receptor blockers. Patients with Henoch-Schönlein purpura nephritis exceeding grade III or serum albumin ≤ 2.5 g/dl received combination therapy comprising prednisolone at 2 mg/kg/day, given in three divided doses (maximum dose, 80 mg/day) for the first 4 weeks, followed by prednisolone at 2 mg/kg given as a single dose every other morning for 8 weeks, immunosuppressant azathioprine at 2 mg/kg/day as a single dose (maximum dose 100 mg) or mizoribine at 5 mg/kg/day as a single dose (maximum dose 300 mg) maintained for 6 months, and warfarin at 1 mg/day given as a single dose each morning. The warfarin dose was then adjusted to give a thrombo-test result of 20–50%, and dipyridamole started at 3 mg/kg/day given in three divided doses, increased to 6 mg/kg/day (maximum dose, 300 mg/day) maintained for 8 weeks if the patient experienced no adverse effects, such as headache. The resolution of proteinuria, without renal dysfunction, was 50% at 5.2 months, 80% at 8.5 months, and 90% at 11.1 months (Ninchoji et al., 2011).

8.4 Plasma exchange

Patients featuring rapid progression of Henoch-Schönlein purpura nephritis, despite the treatment with immunosuppressive medications (steroids, azatioprine and cyclophosphamide), were treated with plasma exchange. Among these children, 36% developed end-stage renal disease between 1 and 7 years after the initiation of treatment (Gianviti et al., 1996). The other children showed a reduction of glomerular filtration rate and crescents > 50% of glomeruli and continued plasma exchange treatment (Hattori et al., 1999). A case series of eight children with a rapidly progressive course of disease treated with plasma exchange, the beneficial effects produced were only transient, and, despite repeated plasma exchange, the subjects progressed to end-stage renal disease after 1–4 years (Scharer et al., 1999). In case series study, children with extensive crescent formation underwent plasma exchange followed by multiple drug therapy, including steroids, pulse urokinase and cyclophosphamide. The patients manifested a decrease in acuity index and percentage of glomeruli with crescents, whereas the chronicity index remained unchanged (Kawasaki et al., 2004). Plasma exchange was recently performed in patients with a histology grading of at least III at renal biopsy. At follow-up, only one child treated later in the course of the disease underwent a kidney transplant. At the end of the study, the investigators reported that the early performing of plasma exchange may delay the rate of progression of cellular crescents to the fibrotic stage and end-stage renal disease (Shenoy et al., 2007).

Plasma exchange in patients with Henoch-Schönlein purpura nephritis, either alone or in combination with immunosuppressant, cannot currently be recommended due to the paucity of data available (Zaffanello & Fanos, 2009).

8.5 Adjuvant treatment

Adjuvant therapies were defined as any secondary treatment used in addition to the primary or conventional treatments that increased the likelihood of cure (eg, intravenous immunoglobulins, anticoagulants, enzymes and vitamins). Results on moderate urinary abnormalities and severe Henoch-Schönlein purpura nephritis are reported separately because the renal implications, clinical monitoring, and conventional treatment modalities differ in terms of outcome measures (Zaffanello et al., 2009).

8.5.1 Moderate urinary abnormalities

Adjuvant treatment in children with isolated proteinuria with or without nephrotic syndrome has been reviewed (Zaffanello et al., 2009).

Two studies (Rostoker et al., 1995; de Almeida et al., 2007) reported the usefulness of 3 months treatment with intravenous immunoglobulins (2 g/kg/month) followed by intramuscular immunoglobulins (0.35 mL/kg) twice a month for 8 months in treating nephritis with significant proteinuria. Both the severity of proteinuria and the acuity index significantly improved in the majority of these patients with renal histology of stage III and normal renal function. In patients with severe gastrointestinal involvement who did not respond to methylprednisolone, were administered intravenous immunoglobulins at 2 g/kg in a single dose (Rostoker et al., 1998; Aries et al., 2005). Finally, the efficacy of intravenous immunoglobulins (2 g/kg, infused over 10–12 hours) in inhibiting the progression of the disease has been tested although only in unstructured case series.

Intravenous administration of Factor XIII concentrate from 30 to 50 U/kg for 3 days was associated with significant improvements in the severity of proteinuria and hematuria compared with non-treated group (Erdoğan et al., 2003).

In children aged 3 to 15 years with Henoch-Schönlein purpura nephritis, tocopherol 300 mg/day was to be administered for 6-17 weeks (Kaku et al., 1998). Oxidative damage and worsening of the clinical course were observed despite significant increases in mean plasma vitamin E concentration. In 5 children with biopsy-proven Henoch-Schönlein purpura nephritis, treatment with an angiotensin-converting enzyme inhibitor (enalapril) for hypertension and adjuvant treatment with fish oil 1 g twice a day as an antioxidant was associated with significantly decreased severity of proteinuria, decreased blood pressure, and stable serum creatinine concentration and glomerular filtration rate. The limitation of this study was the small sample size and the confounding antiproteinuric effect of the angiotensin-converting enzyme inhibitor (Dixit et al., 2004; Chiurchiu et al., 2005).

A systematic review reported no clear benefit of the use of dipyridamole (3-6 mg/kg/day for 8 weeks) and heparin (adjusted to maintain activated partial thromboplastin time between 60 and 80 seconds for 4 weeks) in treating Henoch-Schönlein purpura nephritis, with limitations being the small number of studies, small sample size, and poor methodology of the studies (Chartapisak et al., 2009).

8.5.2 Severe Henoch-Schönlein purpura nephritis

Adjuvant treatments of severe Henoch-Schönlein purpura nephritis with crescent formations in children have been studies from a systematic review (Zaffanello et al., 2009).

The patients with severe Henoch-Schönlein purpura described in these articles had nephrotic syndrome, Henoch-Schönlein purpura nephritis, rapidly progressive glomerulonephritis, and/or kidney failure. Histology of kidney biopsies found a significant proportion with crescent formations (International Study of Kidney Disease in Children grade IV–V). These patients were at high risk for kidney failure despite aggressive treatment with cocktails that included adjuvant anticoagulants or antiplatelet agents such as heparin (Iijima et al., 1998), warfarin (Kaku et al., 1998; Iijima et al., 1998; Kawasaki et al., 2004) dipyridamole (Oner et al., 1995; Kaku et al., 1998; Iijima et al., 1998; Mir et al., 2007) and acetylsalicylic acid (Mir et al., 2007). Treatments associated conventional with adjuvant therapy including three days intravenous pulse of methylprednisolone and/or long-term oral prednisolone administration for 6 months (Oner et al., 1995; Kawasaki et al, 2003; Kawasaki et al., 2004; Mir et al., 2007). Intravenous steroids were used alone (Kawasaki et al., 2003) or in combination with (or to replace) cyclophosphamide(Oner et al., 1995), or comparable steroid pulse therapy was used with cyclophosphamide (Kawasaki et al., 2004; Mir et al., 2007) and Cyclosporine A (Mir et al., 2007). Oral steroid therapy was used alone or in combination with cyclophosphamide (Iijima et al., 1998; Tanaka et al., 2003; Shekelle et al., 1999).

In a case series of 12 children aged 6 to 14 years with Henoch-Schönlein purpura, quadruple therapy with cyclophosphamide for 2 months, intravenous pulse methylprednisolone for 3 days, oral prednisone for 3 months, and adjuvant oral dipyridamole for 6 months, glomerular filtration rate normalized in 11 patients and 7 patients had complete remission (Oner et al., 1995). In a case series of 14 children followed up for a mean (standard deviation) of 7.5 (0.9) years, combination treatment consisted of prednisone for 12 weeks, cyclophosphamide for 8 weeks, and adjuvant intravenous heparin for 4 weeks followed by warfarin 1 mg/day for 4 weeks, and dipyridamole for 8 weeks . The histologic abnormalities of the kidney significantly improved in the meantime (Iijima et al., 1998).

In 2 clinical trials, cyclosporine A was used for the treatment of severe Henoch-Schönlein purpura nephritis in children, either alone (Ronkainen J, 2003) or in combination with steroids and adjuvant dipyridamole (Mir et al., 2007). In particular, a retrospective, nonrandomized study in 82 children, prednisone or pulse methylprednisolone and cyclophosphamide and cyclosporine were given; asetil salicylic acid or dipyridamole were given as adjuvant therapy, although their dosing were not reported as well. In 35% of nephrotic patients and 62% of nephritic patients showed complete remission after 6 months and long-term course (Mir et al., 2007). In a case series of 13 patients with renal histology grade IIIb or IVb, prednisone was administered for 26 weeks and cyclophosphamide was administered for 8 weeks (Tanaka et al., 2003). Adjuvant therapy was dipyridamole 5 mg/kg/day (maximum, 300 mg/day). At study end, acuity index was significantly decreased, chronologic index was unchanged, and renal histology grade was improved significantly (Tanaka et al., 2003).

In a controlled study, 26 in 37 children with severe Henoch-Schönlein purpura nephritis, triple therapy with oral cyclophosphamide for 12 weeks, pulse methylprednisolone for 3 days, and intravenous pulse urokinase at 5000 U/kg/day (maximum, 180,000 U) for 7 consecutive days was effective. Thus, after 6 months, severity of proteinuria and mesangial IgA deposition were significantly reduced (Kawasaki et al., 2003; Kawasaki et al., 2004).

Observations were reported in retrospective case series, and the literature lacks reports of substantial clinical trials. Because the literature search did not find any well-structured studies reporting benefits of the use of antiplatelet agents or heparin in children with

Henoch-Schönlein purpura nephritis, the use of such therapy is not recommended at this time since lack of evidence from well structured clinical trials (Zaffanello et al., 2009).

9. Prognosis

9.1 Prognostic factors and clinical findings

Age at onset has not been shown to be a predictor of poor outcome (Ronkainen et al., 2002; Coppo et al., 1997; Counahan et al., 1977; Edström Halling et al., 2010). In the long-term, the morbidities of Henoch-Schönlein purpura are predominantly attributed to the intensity of renal involvement at presentation. Anymore, the majority of patients that first manifest nephropathy during childhood will not reach end stage renal disease before adulthood (Wyatt & Hogg, 2001).

Female had a markedly greater risk of a poor long-term outcome (Edström Halling et al., 2010).

Patients with isolated hematuria showed a good prognosis (Narchi, 2005), with some exceptions (Goldstein et al, 1992). Most children with Henoch-Schönlein purpura nephritis who present only with hematuria and/or low-grade proteinuria at onset have good probabilities of recovery (Mir et al., 2007). Only patients showing normal urinalysis for 6 months may be discharged from follow-up. In view of the possibility of late deterioration in those with mild renal involvement, long-term albeit annual follow-up was recommended (Narchi, 2005). It has been calculated that 18% of patients with mild proteinuria at onset progress towards poor outcome (Ronkainen et al., 2002; Butani & Morgenstern, 2007; Edström Halling et al., 2010).

Of children who at onset displayed mild symptoms, 72% achieved a complete recovery, compared to 47% of those with severe symptoms. Of children who had mild symptoms at onset, 15% had a poor outcome compared to 41% of those with severe symptoms (Edström Halling et al., 2010).

Henoch-Schönlein purpura nephritis patients with nephritic/nephritic syndrome and massive proteinuria (Niaudet & Habib, 1998) are considered to have severe disease (Kawasaki et al., 2003; Niaudet & Habib, 1998). Therefore, no significant difference in outcome was found between patients with nephrotic versus non-nephrotic proteinuria at onset. Of patients with nephrotic or nephritic-nephrotic features, 68% had a good outcome and specifically 59% of them achieved complete recovery. Level of urinary albumins/creatinine ratio at 1 year above or below 144 mg/mmol discriminated between poor and good outcome with a sensitivity of 95% and specificity of 40%, positive predictive value 82%, negative predictive value 73% (Edström Halling et al., 2010).

Patients with a poor outcome had lower Glomerular Filtration Rate than patients with a good outcome. An initial renal insufficiency was a predictor of poor renal outcome in Henoch-Schönlein purpura nephritis (Edström Halling et al., 2010).

There was no difference in outcome between patients who were normotensive and those who were hypertensive at the first investigation (Edström Halling et al., 2010).

9.2 Prognostic factors and biopsy findings

International Study of Kidney Disease in Children grading score and proteinuria at the 1-year follow-up were the best discriminators of a good and poor outcome (Edström Halling et al., 2010).

Kawasaki (Kawasaki et al., 2003) classified Henoch-Schönlein purpura nephritis patients with International Study of Kidney Disease in Children grade IIIb or higher as having severe disease. Shenoy (Shenoy et al., 2007) concluded that most children with Henoch-Schönlein purpura nephritis grade IIIb or higher on initial biopsy had persistent renal abnormalities at long-term follow-up. The risks for long-term renal impairment are highest in children who present and/or with more than 50% of glomeruli (grade IV or above) occupied by large crescents or sclerosing lesions (Bogdanovic, 2009) because they showed higher probabilities for development of progressive renal disease, renal failure, or end-stage renal disease after long-term follow-up (Iijima et al., 1998; Kawasaki et al., 2004; Niaudet & Habib, 1998).

Patients with segmental glomerulosclerosis had a poorer outcome than those without segmental glomerulosclerosis. The comparison of patients with crescents to those without or those with global glomerulosclerosis to those without revealed no difference in outcome. Patients with poor outcome had a higher degree of mesangial matrix expansion, mesangial proliferation, interstitial inflammation and interstitial fibrosis than did those with a good outcome. Crescents were not a factor of poor prognosis, but the majority of the patients with crescents were treated, which may have improved the course of the disease (Edström Halling et al., 2010).

10. Conclusion

At this time, randomized controlled trials are needed to demonstrate whether the present management with conventional and adjuvant treatments improve truly renal survival of patients with Henoch-Schönlein purpura nephritis. Anymore, the choice of the treatment must depend on both the histological and clinical severity of the Henoch-Schönlein purpura nephritis. In particular, recommendations include a combination therapy for clinically and histologically severe Henoch-Schönlein purpura nephritis, which is unnecessary for moderate Henoch-Schönlein purpura nephritis.

11. References

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14

Lupus Glomerulonephritis

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1. Introduction

Systemic lupus erythematosus (SLE) is a multi-systemic autoimmune disease of unknown etiology. The onset of SLE is believed to be triggered by ill-defined environmental factors in genetically susceptible individuals (Mok, 2003a). Although the exact pathogenetic mechanisms have yet to be elucidated, recent works have revealed a myriad of immunological abnormalities in patients with SLE. These include aberrant apoptosis and defective clearance of apoptotic materials such as nuclear autoantigens and nucleosomes, and immune complexes by macrophages and the complement system (Katsiari, 2010), increased maturation of myeloid dendritic cells which drive the development of the proinflammatory Th17 cells (Fransen, 2010), and defective functions of the regulatory T cells (Tregs) leading to hyperactivity of the helper T cells and autoreactive B cells causing production of autoantibodies (Tucci, 2010).

Of the numerous clinical manifestations of SLE, renal disease is one of the commonest and most serious. Lupus renal disease appears to be more prevalent in certain ethnic groups such as the African and Hispanic Americans, as well as the Asians (Mok, 2005a). Renal involvement in SLE adversely affects its ultimate prognosis as reflected by the rates of patient survival and renal survival (survival without the need for renal replacement therapy), and is a major determinant for morbidity and impairment of quality of life (Mok, 1999).

The glomerulus is the commonest site of kidney involvement by lupus. However, the renal interstitium and tubules, as well as the vessels may also be affected (Cross, 2005). The presentation of renal disease in SLE is variable, ranging from no symptoms, trace proteinuria or urinary sediments to frank nephrotic syndrome, chronic renal insufficiency, and nephritic syndrome with rapid progression leading to acute renal failure. Early recognition of renal disease and close monitoring of renal parameters for progress after treatment is an essential part of the management. Conventional serological markers and clinical renal parameters for active lupus nephritis are not sensitive or specific enough, and novel biomarkers for early detection of renal disease and prediction of renal prognosis are under ongoing evaluation. It is believed that a combination of conventional parameters with one or more serological or urine biomarkers may yield better sensitivity and specificity for predicting renal activity or flare of nephritis in patients with SLE. This may help to abate the need for more invasive investigations such as renal biopsy in the assessment of renal activity and allow early institution of therapy (Mok, 2010a).

Therapy of lupus nephritis should target at symptomatic control, preservation of renal function, reduction of renal flares, prevention of treatment-related complications, and ultimately reduction in mortality (Mok, 2003b). The treatment schedule of lupus nephritis is now divided into an induction phase and a maintenance phase. Induction treatment aims at controlling inflammation and minimizing glomerular injury, whereas maintenance therapy is to reduce the risk of renal flares and renal function decline in the long-run. A combination of glucocorticoids with a non-glucocorticoid immunosuppressive agent has been shown to be more effective than glucocorticoid monotherapy in reducing the risk of progression into end stage renal failure in lupus nephritis (Austin, 1986). Of the many non-glucocorticoid immunomodulating agents, mycophenolate mofetil (MMF) has emerged to be the first-line treatment of lupus nephritis around the world because studies have shown that it is associated with fewer adverse effects than cyclophosphamide, particularly on the ovarian functions (Ginzler, 2005). Recent evidence also reveals that maintenance therapy with MMF is more effective than azathioprine in reducing the composite endpoint of renal flare and deterioration of renal function (Wolfsy, 2010).

In this chapter, the prevalence, presentation and significance of renal involvement in patients with SLE is discussed. An update on the current therapies of lupus nephritis is also presented based on the results of recent randomized controlled trials. Finally, promising biomarkers for the detection and monitoring of lupus nephritis is briefly reviewed.

2. Prevalence of renal disease in SLE

Lupus renal disease appears to be more prevalent in certain ethnic groups such as the African and Hispanic Americans, as well as the Asians (Mok, 2005a; Dooley, 1997). In a comparative study of the clinical manifestations of SLE in three ethnic groups, it was reported that renal disease, as defined by the American College of Rheumatology (ACR) criteria, namely persistent daily proteinuria of more than 500mg, presence of cellular casts or biopsy evidence of lupus nephritis, occurred in 45% of African American, 42% of Chinese and 30% of Caucasian patients, respectively (Mok, 2005a). Another multi-ethnic US cohort of SLE patients reported that renal disease occurred in 51% of Africans and 43% of Hispanics but only in 14% of Caucasians (Bastian, 2002). In a prospective study of 216 Chinese patients with new onset SLE, 31% patients had active renal disease at the time of initial presentation (Mok, 2004). Of 148 patients without overt renal disease at SLE onset, 33% developed active renal disease after a median of 14 months. The overall cumulative incidence of renal disease as defined according to the ACR renal criteria in this cohort of patients was 60% at 5 years post-SLE diagnosis (Mok, 2004). The actual incidence of renal disease might have been underestimated as the renal definition does not include subtle renal involvement such as proteinuria of less than 500mg/day or microscopic hematuria, or both. These studies illustrate that lupus renal involvement is more common in the Africans, Hispanics and Chinese than the Caucasians.

3. Clinical presentation of lupus renal disease

The presentation of renal disease in SLE is variable, ranging from no symptoms (detected by routine renal biopsy or "silent" lupus nephritis), trace proteinuria or active urinary

sediments (microscopic hematuria, pyuria or cellular casts), to more serious proteinuria (nephrotic syndrome), and acute nephritic syndrome with rapid progression to acute renal failure. Occasionally, patients may present with chronic renal failure, isolated renal insufficiency and hypertension as the initial manifestation.
The wide range of presentations of lupus nephritis does not necessarily correlate with the histological findings from renal biopsy. "Silent" lupus nephritis has long been recognized in the literature. A retrospective study of 21 SLE patients with low level of proteinuria (<1gm/day) who underwent renal biopsy showed that proliferative lupus nephritis was present in 57% patients (Christopher-Stine, 2007). This emphasizes the frequent discordance of the histological severity with clinical presentation, and the need for renal biopsy, especially for new onset renal disease as evidenced by abnormal urinalysis and/or renal function impairment.

4. Renal biopsy

Renal biopsy is the gold standard of confirming the diagnosis of lupus glomerulonephritis. The finding of positive staining for immunoglobulin G, A and M, together with C1q, C3 and C4, constitutes the "full house" staining pattern for lupus nephritis. In addition to establishment of the diagnosis of lupus renal disease and confirming renal flares, renal biopsy also provides information on the histological classes of lupus nephritis, and the degree of inflammation and damage in the kidneys so as to guide therapeutic decision. Renal biopsy should be considered in SLE patients with new onset of proteinuria of more than 1g/day with and without active urinary sediments, especially in the presence of active lupus serology or impaired renal function. Some experts recommend renal biopsy at a lower threshold of proteinuria (eg. ≥500mg/day).
Patients with lupus nephritis that is refractory to treatment should be evaluated for other possible causes for the persistence of proteinuria or deterioration in renal function such as the nephrotoxic side effects of medications (eg. the calcineurin inhibitors and non-steroidal anti-inflammatory drugs), renal vein thrombosis, infections, overdiuresis and poorly controlled hypertension. Treatment compliance should be checked. A repeat renal biopsy should be considered in patients with persistently active serological markers because it provides information on the following: (1) histological transformation of the classes of lupus nephritis; (2) the degree of residual activity in the kidneys; and (3) the extent of chronic irreversible changes and its progression since the initiation of immunosuppressive treatment. These data may help to guide further treatment decisions.

5. Histological classification of lupus glomerulonephritis

The histological classification of lupus nephritis has undergone several modifications. The first WHO classification was formulated in 1974 and was last revised in 1995. According to this system, lupus glomerulonephritis was classified according to the extent and pattern of immune deposits and inflammation, which were detected by immunohistochemistry on light microscopy. There were 5 histological subtypes of lupus nephritis (class I to V) in the 1974 WHO classification (McCluskey, 1975). The differentiation of class III and class IV disease was based on the percentage of glomeruli affected by proliferative lesions (>50% was classified as Class IV). No qualitative differences between class III and class IV lesions

were described. Tubulointerstitial and vascular lesions were not included in the classification.

The WHO classification was revised in 1982 (Churg, 1982). Class I disease was subdivided into 2 subclasses based on the presence and absence of immune deposits on immunofluorescence or electron microscopy. Class III was denoted focal segemental glomerulonephritis and Class IV was referred to diffuse proliferative glomerulonephritis. There were no description on the percentage of involvement of glomeruli for the differentiation between class III and class IV disease. Class III and IV disease was subdivided into active, chronic, or mixed types of glomerular injury. Class V was denoted membranous glomerulonephritis, which was subdivided into 4 subclasses: pure membranous nephropathy without or with mesangial hypercellularity (Va and Vb, respectively), membranous nephropathy with segmental endocapillary proliferation and/or necrosis (Vc) and membranous nephropathy with diffuse endocapillary proliferation and/or necrosis (Vd). Class VI was introduced to denote advanced sclerosing glomerulonephritis.

The WHO system was further revised in 1995 (Churg, 1995), with the emphasis of segmental glomerular capillary wall necrosis to be the defining feature of class III lesions, regardless of the percentage of glomeruli affected. For membranous lupus nephropathy, as the long-term prognosis is dependent on the proliferative than membranous component, the 1995 WHO classification removed Vc and Vd to be included into class III and class IV lupus nephritis, respectively. Class V retained only the subclasses Va and Vb, under the category "diffuse membranous glomerulonephritis".

The histological classification system was modified once again in 2003 by the International Society of Nephrology and the Renal Pathology Society (Weening, 2004) (Table 1). One of the reasons was the demonstration of the poor outcome of diffuse segmental necrotizing glomerulonephritis involving over 50% of glomeruli, (a "severe" form of class III disease), as compared to class IV lupus nephritis. Class III disease referred to focal lupus nephritis, which was defined as involvement of less than 50% of glomeruli by segmental endocapillary proliferative lesions, with or without capillary wall necrosis and crescents, and subendothelial deposits. Class IV disease was denoted diffuse lupus nephritis which involved more than 50% of the glomeruli. This class is subdivided into diffuse segmental lupus nephritis (class IVS) when >50% of the involved glomeruli showed segmental lesions, and diffuse global lupus nephritis (class IVG) when >50% of the glomeruli having global lesions. The proportion of glomeruli with active and chronic lesions, fibrinoid necrosis or crescents, tubulointerstitial and vascular pathology should be separated reported.

Class V, or membranous lupus nephritis, was defined as global or segmental continuous granular subepithelial immune deposits, often in the presence of concomitant mesangial immune deposits and hypercellularity. The distinction between pure membranous nephropathy and membranous nephropathy superimposed on mesangial changes was eliminated. When a diffusely distributed membranous lesion is associated with an active lesion of class III or IV, both diagnoses are reported ('V+III' or 'V+IV'). Finally, minimal change nephropathy (class I) was renamed minimal mesangial lupus nephritis, which was characterized by normal light microscopy of the glomeruli with accumulation of mesangial immune complexes identified by immunofluorescence and/or electron microscopy. A complete lack of renal abnormalities by light microscopy, immunofluorescence, and electron microscopy no longer qualified Class I lupus nephritis.

Class I	Minimal mesangial lupus nephritis Normal glomeruli by light microscopy, but mesangial immune deposits by immunofluorescence
Class II	Mesangial proliferative lupus nephritis Purely mesangial hypercellularity of any degree or mesangial matrix expansion by light microsocpy, with mesangial immune deposits. A few isolated subepithelial or subendothelial deposits may be visible by immunofluorescence or electron microscopy, but not by light microscopy
Class III	Focal lupus nephritis Active or inactive focal, segmental or global endo- or extracapillary glomerulonephritis involving <50% of all glomeruli, typically with focal subendothelial immune deposits, with or without mesangial alterations
III (A)	Active lesions: focal proliferative lupus nephritis
III (A/C)	Active and chronic lesions: focal proliferative and sclerosing lupus nephritis
III (C)	Chronic inactive lesions with glomerular scars: focal sclerosing lupus nephritis
Class IV	Diffuse lupus nephritis Active or inactive diffuse, segmental or global endo- or extracapillary glomerulonephritis involving ≥50% of all glomeruli, typically with diffuse subendothelial immune deposits, with or without mesangial alterations. This class is divided into diffuse segmental (IV-S) lupus nephritis when ≥50% of the involved glomeruli have segmental lesions, and diffuse global (IV-G) lupus nephritis when ≥50% of the involved glomeruli have global lesions. Segmental is defined as a glomerular lesion that involves less than half of the glomerular tuft. This class includes cases with diffuse wire loop deposits but with little or no glomerular proliferation.
IV-S (A)	Active lesions: diffuse segmental proliferative lupus nephritis
IV-G (A)	Active lesions: diffuse global proliferative lupus nephritis
IV-S (A/C)	Active and chronic lesions: diffuse segmental proliferative and sclerosing lupus nephritis
IV-G (A/C)	Active and chronic lesions: diffuse global proliferative and sclerosing lupus nephritis
IV-S (C)	Chronic inactive lesions with scars: diffuse segmental sclerosing lupus nephritis
IV-G (C)	Chronic inactive lesions with scars: diffuse global sclerosing lupus nephritis
Class V	Membranous lupus nephritis Global or segmental subepithelial immune deposits or their morphologic sequelae by light microscopy and by immunofluorescence or electron microscopy, with or without mesangial alterations. Class V lupus nephritis may occur in combination with class III or IV in which case both will be diagnosed Class V lupus nephritis may show advanced sclerosis
Class VI	Advanced sclerotic lupus nephritis ≥90% of glomeruli globally sclerosed without residual activity

Table 1. ISN/RPS 2003 classification of lupus nephritis

6. Prognosis of lupus renal disease

Renal involvement of SLE carries significant morbidity and mortality. The renal survival (survival without dialysis) rates of lupus nephritis in the 1990's range from 83-92% in 5 years and 74-84% in 10 years (Mok, 1999; Donadio, 1995; Bono, 1999; Neumann, 1995). The risks of end stage renal failure were particularly high in patients with diffuse proliferative glomerulonephritis, with figures ranging from 11-33% in 5 years (Mok, 1999; Dooley, 1997, Donadio, 1995; Neumann, 1995; Bakir, 1994; Nossent, 2000; Korbet, 2000). The prognosis of lupus nephritis depends on a large number of demographic, racial, genetic, histopathological, immunological and time-dependent factors (Mok, 2005b). Renal disease that fails to remit with conventional immunosuppressive therapies is a major risk factor for subsequent deterioration of renal function and poor outcome (Mok, 1999; Korbet, 2000; Mok, 2006b). Other unfavorable prognostic factors for lupus neprhitis include younger age, male sex, histological cellular crescents, fibrinoid necrosis, subendothelial deposits, glomerular scarring, tubular atrophy and interstitial fibrosis, impaired renal function at presentation, persistent hypertension, hypocomplementemia, low hematocrit, as well as delay in treatment due to problems of access to health care and poor compliance (Mok, 2005b).

A recent hospital registry study of 5686 patients with SLE showed that there was a loss in life expectancy of 20 years in female and 27 years in male patients, respectively (Mok, 2011). Among 514 lupus deaths, direct complications of renal disease accounted for 9% of all cases (Mok, 2011). This reiterates that the prognosis of renal disease in SLE has yet to be improved by novel therapies in the future.

7. Current treatment of lupus glomerulonephritis

The immunosuppressive therapy of lupus nephritis is divided into an induction phase which targets at reducing inflammation and glomerular injury and a maintenance phase that aims to reduce the long-term risk of renal flares and renal function decline. Adjunctive therapies such as vigorous control of blood pressure to less than 120/80mmHg may retard the deterioration of renal function. The early use of renal protection agents such as the angiotensin converting enzyme inhibitors (ACEIs) and the angiotensin II receptor antagonists is mandatory. Hyperlipidemia should also be aggressively controlled to offer protection against accelerated vascular disease, especially in the membranous type of lupus nephritis. Calcium and vitamin D should be adequately supplemented to reduce the risk of aggravation of disease activity related to vitamin D deficiency, and to protect against loss in bone mineral density. Low-dose aspirin should be considered in patients with histological evidence of antiphospholipid syndrome nephropathy, although there is still no published evidence that this will protect against renal function decline. Anticoagulation may be considered in patients with persistent nephrotic range of proteinuria and the presence of the antiphospholipid antibodies.

8. Induction therapy for lupus nephritis

Milder form of lupus nephritis (ISN/RPS Class I, II) is usually manageable with corticosteroids (Mok, 2010b). Azathioprine (AZA) can be added as a corticosteroid sparing agent and for the treatment of concomitant extra-renal manifestations. Mild class V disease can be treated with ACEIs. Proliferative lupus nephritis (class III and IV or mixed III/V and IV/V) and more serious class V (nephrotic range of proteinuria or deteriorating renal

function) disease requires more aggressive induction regimens consisting of corticosteroids and a non-corticosteroid immunosuppressive agent.

The standard therapy for severe proliferative lupus nephritis has been a combination of high-dose glucocorticoid and cyclophosphamide (CYC). From the series of randomized controlled trial conducted by the National Institute of Health (NIH), it was demonstrated that prednisone combined with intravenous (IV) pulse CYC offered better long-term protection against renal function decline than prednisone alone (Austin, 1986; Gourley, 1996; Illei, 2001). However, the use of CYC is associated with a number of untoward side effects, which include infection, ovarian and bladder toxicities, leukopenia, increased risk of cervical intraepithelial neoplasia and malignancy. Some of these toxicities are dose dependent, with a higher risk related to a higher cumulative dose (Mok, 1998). IV pulse CYC has gained popularity over continuous daily oral CYC because it is associated with less toxicity on the bladder and the gonads. Whether oral CYC is more efficacious than IV pulse CYC in lupus nephritis remains controversial because of the lack of large controlled trials (Austin, 1986; Mok, 2001). A recent analysis of a large cohort of patients with diffuse proliferative lupus nephritis showed a trend of better efficacy of oral CYC than IV pulse CYC in preserving renal function after a mean follow-up of 8.8 years (Mok, 2006b). In a multivariate model, the cumulative dose of CYC delivered instead of the route of CYC was an independent factor for a complete renal response. This suggests that the higher potency of the oral CYC regimen is probably related to the higher cumulative dose delivered instead of the route of administration per se. However, ovarian toxicity leading to premature menopause was more frequent in users of oral CYC.

Although the optimal route of CYC and duration of therapy in lupus nephritis remains to be defined, recent evidence supports the use of a shorter course and lower dose of CYC to minimize toxicities (Mok, 2001; Mok, 2002; Houssiau, 2010a). Houssiau et al. (2010a) compared the efficacy and toxicity of two less intensive intravenous pulse CYC regimens for the initial treatment of lupus nephritis. Eighty-four patients (predominantly Caucasians) were randomized to receive either 8 intravenous pulses of CYC (0.5g/m² to a maximum of 1.5gm) or 6 biweekly low dose pulses of CYC (500mg each). In both regimens, CYC was later substituted with AZA for long-term maintenance. Patients who participated in the study had milder renal disease compared to other lupus nephritis trials, as reflected by a lower proportion of patients having class IV disease, nephrotic syndrome and renal function impairment. After 10 years, rates of mortality, sustained doubling of serum creatinine and end stage renal disease did not differ between the two groups (36). The incidence of cardiovascular events and was also similar. Cancers, however, were numerically more common in patients who had received the low-dose regimen. Thus, for less serious lupus nephritis, a low-dose CYC regimen, followed by AZA is a viable strategy if there are no alternatives to CYC for initial treatment.

Nevertheless, CYC remains the treatment of choice for high-risk patients with proliferative lupus nephritis such as those with impaired or rapidly deteriorating renal function, histological cellular crescents or a combination of high activity and chronicity scores (Tang, 2009). The course of CYC should be limited to less than 6 months, with subsequent replacement by another immunosuppressive agent, to reduce the incidence of toxicities (Mok, 2002).

9. Recent controlled trials for induction therapy of severe lupus nephritis

Six randomized controlled trials comparing the efficacy and adverse effects of different treatment protocols for the induction therapy of severe lupus nephritis have recently been

presented (Appel, 2009; Grootscholten, 2006, Bao, 2008; Chen, 2011; Mok, 2008; Furie, 2009). These are briefly summarized in Table 2.

In the largest lupus nephritis controlled trial to-date, called the Aspreva Lupus Management Study (ALMS), 370 patients with histologically ISN/RPS class III, IV or V lupus nephritis were randomized to receive either monthly IV pulse CYC (0.5-1.0g/m^2) or MMF (target 3g/day) on top of high-dose prednisone (60mg/day initially and then tapered) (Appel, 2009). Two-third of the participants had class IV disease. Asians and Hispanics comprised 33% and 35% of the participants, respectively. Three hundred and six (83%) patients completed the 24-week protocol. Clinical response, defined by a decrease in urine protein/creatinine ratio (P/Cr) to <3 in patients with baseline nephrotic range P/Cr ≥3, or by ≥50% in patients with subnephrotic baseline P/Cr (<3), and stabilization (±25%) or improvement in serum creatinine at 24 wk as adjudicated by a blinded clinical endpoints committee, was not significantly different between the CYC (53%) and MMF (56%) group. Subgroup analyses revealed that MMF was associated with a significantly higher response rate than CYC (60% vs 39%; p=0.03) in the non-Caucaisan non-Asians, which were mainly Hispanics. The rates of adverse events and serious adverse events were not significantly different between the two groups. Specifically, nausea, vomiting and alopecia were numerically more frequent in the CYC group, whereas diarrhea was more commonly reported in the MMF group. The induction phase of the ALMS study did not allow comparison of long-term side effects such as sustained amenorrhea and malignancies. There were 9 and 5 deaths in the MMF and CYC group, respectively. Of the 9 deaths in the MMF group, 7 were Asians (mainly Chinese), suggesting that Asian patients tolerated high-dose prednisone and MMF (3g/day) less well.

A controlled trial comparing the efficacy of CYC and azathioprine (AZA) in lupus nephritis was reported by Grootscholten et al. (2006). In this study, 87 patients with proliferative lupus nephritis (class III and IV) were randomized to receive either oral prednisone combined with intravenous pulse CYC (750mg/m^2 monthly for 6 months and then quarterly for another 7 doses) or intravenous pulse methylprednisolone (1 gram daily for 3 days for 9 pulses) together with AZA (2mg/kg/day). At the end of the third year, both groups of patients received AZA for long-term maintenance (2mg/kg/day). The dosage of AZA was reduced to 1mg/kg/day after 4 years of treatment. This cohort of patients consisted mainly of Caucasian patients (76%) who had serious renal disease as evidenced by a high proportion of patients having hypertension (57%), nephrotic syndrome (53%) and impaired creatinine clearance (56%) at presentation. In the first 2 years, no significant difference in the rates of complete and partial renal remission could be demonstrated between the two regimens. After a median follow-up of more than 5 years, significantly more patients in the AZA arm relapsed and there was a trend of higher incidence of doubling of serum creatinine in the AZA-treated patients. Interestingly, the incidence of herpes zoster infection was lower in the CYC than AZA arm during the first two years of treatment.

Although this was a randomized controlled trial, the number of patients assigned to the two treatment arms was unequal (50 patients in the CYC arm vs 37 patients in the AZA group). The corticosteroid regimens of the two treatment arms were also different, which confounded a proper interpretation of whether CYC was more effective than AZA by its own. However, taking the observation that relapse of nephritis and renal function decline was more common in AZA-treated patients despite the use of a more intensive corticosteroid regimen, it was not unreasonable to conclude for the superiority of CYC over AZA in the treatment of severe lupus nephritis.

Author, year	N	Study duration	Histological classes of lupus nephritis	Steroid regimen	Comparators	Primary end points	Adverse events
Houssiau, 2010a	84	10 yrs	WHO III, IV, Vc,Vd	Prednisolone (0.5mg/kg/d) for 4wks, then taper to 5-7.5mg/d for at least 30mths	IV CYC (0.5g/m^2 to a max of 1.5g) monthly for 8 doses vs 6 biweekly low dose pulses of 500mg, followed by AZA in both	Rates of mortality, sustained doubling of serum creatinine and end stage renal disease similar between the two groups	Cardiovascular events similar; but cancers were numerically more common in the low dose CYC group
Appel, 2009	370	24 wks	ISN/RPS III,IV,V	Prednisolone 60mg/day then taper	IV CYC (0.5-1.0g/m^2) monthly for 6 doses vs MMF (3g/d)	Clinical response similar at 6 months; MMF higher reponse rate than CYC in non-Caucasians non-Asians	Nausea, vomiting and alopecia more common in CYC group; diarrhea more common with MMF; numerically more deaths in MMF group
Grootscholten, 2006	87	5.7 yrs	WHO III, IV, Vc, Vd	Prednisone 1mg/kg/day, tapered to 10mg/d after 6 mths vs IV MP for 9 doses + prednisone 20mg/d and taper	IV CYC (750mg/m^2) monthly for 6 then 3-monthly for another 7 doses followed by AZA vs AZA (2mg/kg/d) following pulse MP	Complete and partial response rate similar at 2 years; at 5 years, significantly more relapses in AZA group with a higher incidence of doubling of serum creatinine	More herpes zoster in the AZA group than CYC; major infection rate similar; more ovarian toxicities in the CYC-treated patients
Bao, 2008	40	9 mths	Mixed IV+V	Pulse MP (0.5g/day x 3d) + prednisolone (0.6-0.8mg/kg/day) then taper	IV CYC (0.5-1g/m^2/ monthly for 9 months) vs MMF (1g/d) + Tac (4mg/d)	Complete response rate significantly higher in MMF + Tac than CYC group at 6 and 9 mths	Gastrointestinal upset, leucopenia, alopecia, menstrual irregularities and upper respiratory tract infection more common in CYC group
Chen, 2011	81	6 mths	ISN/RPS III,IV,V	Prednisolone (1mg/kg/d) then taper	IV CYC (0.5-1g/m^2/ monthly for 6 months) vs Tac (0.05mg/kg/d) titrating to a level of 5-10ng/ml	Clinical response at 6 months similar between the two groups	Infection rate similar; more leucopenia and gastrointestinal upset with CYC
Mok, 2008	130	6 mths	ISN/RPS III,IV,V	Prednisolone (0.6mg/kg/d) then taper	MMF (2-3g/d) vs Tac (0.1-0.06mg/kg/d)	Clinical response similar at 6 months	Herpes zoster more common with MMF; alopecia, tremor and reversible increase in serum creatinine more common with Tac
Furie, 2009	144	52 wks	ISN/RPS III,IV	High-dose prednisone	MMF (2-3g/d) in both; rituximab x 2 courses (1g x2 each course) vs placebo	Clinical efficacy similar at 52 wks	Infection rate and major infection rate similar between the two groups

Yrs = years; mths = months; CYC = cyclophosphamide; MMF = mycophenolate mofetil; AZA = azathioprine; Tac = tacrolimus

Table 2. Recent randomized controlled trials of induction therapy for lupus nephritis

Bao et al. (2008) studied 40 patients with mixed proliferative and membranous lupus nephritis (ISN/RPS IV+V) by randomizing them to receive either IV pulse CYC (0.5-1g.m^2 monthly) (N=20) or low-dose combination of MMF (500mg BD) and tacrolimus (Tac) (2mg BD) (N=20), on top of high-dose prednisolone (0.6-0.8mg/kg/day) after 3 daily pulses of methylprednisolone (0.5g). The mean creatinine clearance at recruitment was 97.6ml/min and 85% patients had normal serum creatinine level. At 6 months, the rate of complete response, defined as daily proteinuria <0.4g/day with normal urinary sediments and stabilization of serum creatinine (<15% increase), was significantly higher in the MMF / Tac group (50%) than the CYC group (5%). The corresponding rates at 9 months of treatment were 65% and 15%, respectively. Leukopenia, gastrointestinal upset, upper respiratory tract infection, alopecia and irregular menses were more common in the CYC than MMF/Tac group of patients.

A randomized controlled trial comparing the short-term efficacy of IV pulse CYC with tacrolimus (Tac) in lupus nephritis were recently presented (Chen, 2011). In this study, 81 patients with class III, IV or V lupus nephritis were randomized to receive IV pulse CYC (0.5-1g.m^2 monthly) (N=39) or Tac (0.05mg/kg/day titrating to a level of >5ng/mL) (N=42) in combination with high-dose prednisolone (1mg/kg/day). The study population consisted of moderate to high-risk patients as shown by a high proportion of class IV disease (77%) and impaired renal function (11%) at presentation. At 6 months, the rate of complete remission, which was defined as proteinuria <0.3g/day, stabilization of serum creatinine and normalization of urinary sediments, was not significantly different between the CYC and Tac group of patients (38% vs 52%, p=0.2). Regarding adverse events, gastrointestinal upset and leucopenia were significantly more frequent in the CYC group but the rate of infection was similar between the CYC- and Tac-treated patients. Transient increase in serum creatinine was reported in 8% of patients receiving Tac.

Our group has conducted a controlled trial comparing the efficacy of MMF (2g/day, titrating to 3g/day if response suboptimal at 3 months) with Tac (0.1mg/kg/day in first 2 months with tapering to 0.06mg/kg/day) in combination of high-dose prednisolone (0.6mg/kg/day for 6 weeks and taper) for lupus nephritis (Mok, 2008). Up to March 2011, 130 patients with ISN/RPS class III, IV or V lupus nephritis were recruited. Our preliminary analysis showed that the clinical complete and partial response rates were not significantly different between the two treatment arms at month 6. The rate of infection, in particular herpes zoster reactivation, was higher in MMF than Tac-treated patients, whereas alopecia, tremor and reversible increase in serum creatinine was more frequent in the Tac group of patients. Dose-related neurological and metabolic adverse effects of Tac, and the possibility of early renal relapse upon completion of the induction phase and substitution of Tac have to be carefully monitored.

The LUNAR study is a phase III randomized, double-blind, placebo-controlled multicenter study to evaluate the efficacy and safety of rituximab in patients with active proliferative lupus nephritis (Furie, 2009). Patients with ISN/RPS Class III or IV lupus nephritis and urine protein to creatinine (UP/Cr) ratio >1 were randomized to receive rituximab (1000mg) or placebo infusion on days 1, 15, 168 (week 24) and 182 (week 26), on top of corticosteroid and MMF (>2g/day). Seventy-two patients were recruited in each treatment arm. Two-third of the patients had class IV nephritis and the mean UP/Cr at entry was 4.0±2.8. At week 52, no statistically significant differences in the primary and secondary endpoints were observed between the rituximab and placebo groups of patients, although there were numerically more responders in the rituximab group (57% vs 46% in the placebo group). Africans and Hispanics treated with rituximab tended to have better response compared to

placebo than the Whites. Rituximab had a greater effect than placebo on anti-dsDNA and complement levels at week 52. Serious adverse events and infection rates were similar between the two groups but two deaths occurred in the rituximab-treated patients.
Taken the evidence from these recent studies together, it appears that MMF should be used as the first line treatment in combination with corticosteroids for severe lupus nephritis because of its stronger evidence (largest sample size) compared to other agents and lower incidence of toxicities compared to conventional CYC. Although Tac has similar efficacy with either CYC or MMF, it has been tried in a smaller population of patients and disadvantages such as transient and long-term nephrotoxicity, as well as higher relapse rate upon substitution with another immunosuppressive agent are of concern. However, Tac is a definite option when patients are contraindicated for or intolerant to MMF. Moreover, Tac is indicated as salvage therapy for refractory lupus nephritis. Tac is preferred to cyclosporin A for the lower incidence of cosmetic side effects. The initial results of the B cell depleting agents such as rituximab are disappointing. Although evidence does not support an additional benefit of rituximab on top of MMF treatment for lupus nephritis, rituximab is an option to be considered in recalcitrant lupus nephritis, as evidenced by a number of uncontrolled case series (Jonsdottir, 2010; Melander, 2009; Vigna-Perez, 2006).

10. Maintenance therapy for lupus nephritis

There are no randomized controlled trials with the main objective of delineating whether maintenance therapy of lupus nephritis is effective or not. However, some indirect evidence suggests that maintenance therapy is probably necessary in severe lupus nephritis. In a long-term follow-up of 145 patients who participated in the NIH lupus nephritis studies, renal flares occurred in 45% of the patients when immunosuppression was completely stopped (Illei, 2002). A recent retrospective review of 32 patients with predominantly diffuse proliferative lupus nephritis described a relapse of lupus activity in 53% of patients after immunosuppression was discontinued (Moroni, 2006). In our experience with 212 patients with diffuse proliferative lupus nephritis (Mok, 2006b), despite maintenance treatment was given to 73% of patients, more than one-third of patients still had renal flares which might be serious. The use of maintenance therapy for more than 3 years was independently associated with an increased likelihood of having the composite outcome of doubling of serum creatinine, end stage renal failure or death (hazard ratio 4.62 [1.35-15.8]; p=0.02).
In a 2006 retrospective review of 32 patients with proliferative lupus nephritis in whom immunosuppressive therapy was stopped for a median of 203 months, clinical remission persisted in 47% of patients (Moroni, 2006). Patients who experienced sustained remission had received a longer total median duration of immunosuppressive treatment since renal biopsy than those who did not experience remission (median 57 months vs 30 months; $p<0.01$). This finding, coupled with the observation that maintenance treatment for less than 3 years after successful cyclophosphamide induction was a predictor of poor renal outcome in proliferative lupus nephritis (Mok, 2006b), suggests that maintenance immunosuppressive therapy should be continued for at least 3 years after a complete clinical response is achieved.
Four recent randomized controlled trials compare the efficacy of different immunosuppressive agents in maintaining remission in lupus nephritis (summarized in Table 3). Contreras et al. (2004) randomized 59 patients with lupus nephritis (mainly African and Hispanic Americans; 78% had class IV disease) to receive one of the three treatment arms after induction with 4-7 pulses of intravenous CYC: (1) MMF (0.5-3g/day); (2) quarterly pulse CYC; (3) AZA (1-

3mg/kg/day). Long-term observation showed that either MMF or AZA was superior to CYC in the prevention of the composite outcome of renal failure and death. MMF was more efficacious than pulse CYC in the prevention of renal flares. Moreover, maintenance treatment with CYC was associated with more side effects such as nausea, vomiting and infection. Although the sample size is small, this study shows that maintenance treatment of lupus nephritis with either AZA or MMF is safe and effective. However, whether MMF is more cost-effective than AZA is not clear because significant difference in all outcomes is not apparent between MMF- and AZA-treated patients. Moroni et al. (2006) studied 69 patients (mainly Caucasians) with lupus nephritis and compared the efficacy of cyclosporin A (CSA) with AZA for maintenance therapy. After initial induction treatment with pulse methylprednisolone, prednisone and oral CYC (91.5±23.8 mg/day for a median of 3 months), patients were randomized to receive either cyclosporin A (Neoral; 4.0 to 2.5-3.0mg/kg/day) (N=36) or AZA (2mg/kg/day) (N=33) for maintenance. At 4 years of follow-up, flare occurred in 24% of AZA-treated and 19% of CSA-treated patients, respectively (no significant difference). Minor infections and leucopenia were more commonly reported with AZA treatment whilst arthralgia and gastrointestinal symptoms were more common in CSA-treated patients.

Author, year	N	Follow-up duration	Histological classes of lupus nephritis	Induction regimen	Comparators	Primary end points	Adverse events
Contreras, 2004	59	Beyond 5 yrs	WHO III, IV, Vb	IV CYC (0.5-1g/m²) for 4-7 pulses	IV CYC (0.5-1g/m²) every 3 months vs MMF (0.5-3g/d) vs AZA (1-3mg/kg/d)	Renal flare and renal function deterioration was significantly more common with CYC than MMF; MMF no better than AZA in the above outcomes	Nausea, vomiting, major infection rate and sustained amenorrhea more common with CYC than the other 2 groups
Moroni, 2006	69	4 yrs	Class IV nephritis	Pulse MP + high dose prednisone + oral CYC for 3 mths	CSA (4mg/kg/d) and taper to 2.5-3mg/kg/d vs AZA 2mg/kg/d	7 flares in CSA (19%) vs 8 flares in AZA (24%) group; reduction in proteinuria, blood pressure and creatinine clearance similar in both groups	Gum hypertrophy, hypertrichosis, hypertension, arthralgia, gastrointestinal symptoms more common with CSA; Infections and leucopenia more common with AZA
Houssiau, 2011	105	53 mths	WHO class III, IV, Vc, Vd	Pulse MP + high dose prednisone + IV CYC (500mg) x 6 doses	AZA (2mg/kg/d) vs MMF (2g/d)	Frequency of renal and extra-renal flares, doubling of serum creatinine similar in both groups	Infection rate similar; but drug-related cytopenias more common with AZA; withdrawal due to pregnancy wish more common with MMF
Wofsy, 2010	227	2.1 yrs	ISN/RPS III,IV,V	High dose prednisone + either IV CYC (6 pulses) or MMF (3g/d) x 6 mths	AZA (2mg/kg/d) vs MMF (2g/d)	Treatment failure, defined as the composite outcome of renal flares, doubling of serum creatinine or end stage renal failure, death or need for rescue therapy significantly less common in MMF than AZA group	No information yet

Yrs = years; mths = months; CYC = cyclophosphamide; MMF = mycophenolate mofetil; AZA = azathioprine; CSA = cyclosporin A

Table 3. Recent randomized controlled trials of maintenance therapy for lupus nephritis

In the MAINTAIN study conducted by Houssiau et al. (2010b), 105 patients with class III, IV, Vc and Vd lupus nephritis were randomized to receive either MMF (2g/day) (N=53) or AZA (2mg/kg/day) (N=52) after an initial induction regimen that consisted of IV pulse methylprednisolone, high-dose prednisone and IV pulse CYC (500mg 2-weekly for 6 doses). Participants were mainly Caucasians and 10% of patients had impaired renal function at study entry. After a mean follow-up of 53 (15-65) months, 24 (23%) patients withdrew from the study mainly because of pregnancy wish (in the MMF group) and adverse effects. Frequency of renal and extra-renal flares, doubling of serum creatinine and incidence of infections occurred at similar frequency in the two arms. However, drug-related cytopenias were more common with AZA.

Results of the maintenance phase of the ALMS study was released in the 9th International Lupus Congress at Vancouver in 2010 (Wofsy 2010). Two hundred and twenty-seven patients who had completed the induction phase of the ALMS (IV pulse CYC or MMF 3g/day) were randomized to receive either MMF (2g/day) (N=116) or AZA (2mg/kg/day) (N=111) for maintenance treatment. The mean daily doses received by the patients were 1.87±0.43g and 120±48mg, respectively, for MMF and AZA. After a mean follow-up of 2.1 years, the rate of treatment failure, defined as renal flare, doubling of serum creatinine or end stage renal disease, need for rescue therapy or death, was significantly less common in MMF than AZA-treated patients. The results were similar in patients induced by CYC or MMF at recruitment.

Taken these studies together, it appears that MMF is the preferred agent for long-term maintenance therapy for lupus nephritis. However, the cost-effectiveness of this approach has to be evaluated in future analysis. AZA and CSA are alternative options for patients who are intolerant to MMF or plan for pregnancy. The long-term use of the calcineurin inhibitors such as Tac and CSA is not encouraged because of the increased risk of nephrotoxicity, hyperlipidemia and atherosclerosis.

11. Membranous lupus nephropathy

Membranous lupus nephropathy (MLN), defined as global or segmental continuous granular subepithelial immune deposits, often in the presence of mesangial immune deposits and mesangial hypercellularity, comprises only one-fifth of all cases of histologically confirmed lupus nephritis (Mok, 2009). Reported rates of patient survival and end-stage renal disease in MLN vary considerably, because of substantial heterogeneity among the published studies. The risk of progression of MLN to renal failure is generally reduced in the absence of proliferative lesions, but patients are nevertheless at risk of thromboembolic complications.

The optimal therapy for MLN remains elusive because of the paucity of clinical trials. Mixed membranous and proliferative lupus nephritis should be treated in the same way as pure proliferative lupus nephritis. If MLN is not accompanied by proliferative lesions but is associated with clinically relevant proteinuria, renal insufficiency or failure to respond to supportive therapies, immunosuppressive treatment is indicated. In addition, cardiovascular protection and blockade of the renin-angiotensin system should be instituted early in all patients.

Austin et al. (2009) randomized 42 patients (71% Blacks or Hispanics) with MLN to receive one of the following regimens: (1) alternate day prednisone (1mg/kg/day for 8 weeks and taper to 0.25mg/kg/day throughout); (2) similar prednisone regimen plus IV pulse CYC (0.5-1.0g/m^2

every two months); or (3) similar prednisone regimen plus CSA (5mg/kg/day). At 12 months, the cumulative probability of complete (<0.3g/day proteinuria) or partial (<2.0g/day proteinuria or improvement by 50% from baseline) remission was highest with CSA (83%), followed by IV pulse CYC (60%) and prednisone alone (27%). The response rates of either CSA or CYC were significantly better than prednisone alone. However, relapse of nephrotic syndrome was significantly more common after discontinuation of treatment with CSA than IV pulse CYC. Adverse effects during the 12-month period included insulin-requiring diabetes (one with prednisone and two with CsA), pneumonia (one with prednisone and two with CsA), and localized herpes zoster (two with IVCY).

A recent pooled analysis of 65 patients with pure membranous lupus nephritis recruited for two randomized controlled trials and completed 24 weeks of treatment (Ginzler, 2005; Appel, 2009) showed that there were no differences in the measured end points, response rate, mortality and withdrawal rate between MMF and IV pulse CYC (Radhakrishnan, 2010). There was also no difference in the change in proteinuria or partial response rate between MMF and CYC in those patients presenting with nephritic syndrome.

Therefore, similar to the proliferative types of lupus nephritis, more serious MLN should be treated with a combination of glucocorticoids and non-glucocorticoid immunosuppressive agent. A number of uncontrolled series have reported efficacy of various regimens for MLN such as AZA, tacrolimus and MMF in combination with glucocorticoids (Mok, 2009). Taken these together, possible options for MLN include MMF, IV pulse CYC, CSA, AZA and tacrolimus. Many specialists will start with MMF or AZA for their lower incidence of adverse effects, reserving other agents for salvage therapy when the clinical response is not optimal. Controlled trials comparing existing immunosuppressive agents and experimental modalities such as rituximab, infliximab and sirolimus should be undertaken in the future (Jonsdottir, 2011).

12. Refractory lupus nephritis

There is no international consensus on the definitions of remission and treatment refractoriness in lupus nephritis. In the absence of reliable and readily available biomarkers for ongoing activity / inflammation in the kidneys and histological / immunological data from routine post-therapy renal biopsy, true remission of lupus nephritis is difficult to define. Despite the discrepancies in the clinical criteria used, up to 20% of patients with lupus nephritis are reported to be resistant to initial immunosuppressive therapy (Mok, 2006a). They are more likely to be patients with multiple unfavorable prognostic factors such as the African ethnicity, delayed institution of CYC, poor treatment compliance, impaired serum creatinine, severe nephrotic syndrome, arterial hypertension at presentation, and the presence of active crescents and a higher degree of chronicity in renal histology (Mok, 2005b).

Using the similar renal response criteria as suggested by the NIH investigators (Boumpas, 1998), we reported that 14% of a cohort of 212 patients with diffuse proliferative lupus nephritis did not respond to either continuous oral or intermittent pulse CYC therapy at the end of the induction courses (Mok, 2006b). The failure to respond to immunosuppressive treatment in the first year is associated with increased risk of renal function decline and the development of end stage renal disease (Mok, 1999).

Controlled trials in refractory lupus nephritis are unavailable. Open-labeled studies have reported success of newer immunosuppressive drugs, immunomodulatory therapies and

the biological agents such as MMF, calcineurin inhibitors (CSA and tacrolimus), leflunomide, intravenous immunoglobulin, immunoadsorption and rituximab in the treatment of CYC-refractory lupus nephritis. More aggressive CYC regimens such as daily oral CYC and the immunoablative CYC protocol have been used in lupus nephritis, but at the expense of more toxicities (Petri, 2010). Novel biological agents that are undergoing clinical trials in renal and non-renal lupus include epratuzumab, ocrelizumab, belimumab, abatacept and atacicept (summarized in Table 4) (Mok, 2010c).

B cell depletion
Fludarabine, rituximab, epratuzumab, ocrelizumab, belimumab, atacicept
B cell tolerization
Abetimus sodium
Blockade of the co-stimulatory pathways
Abatacept (CTLA4-Ig)
Neutralization of cytokines
IL-10, TNF, IL-6, type I interferons
Anti-complement
anti-C5b (eculizumab)

Table 4. Biological therapies for renal and non-renal lupus

13. Biomarkers for lupus nephritis

Current laboratory markers for lupus nephritis such as proteinuria, urine protein-to-creatinine ratio, creatinine clearance, anti-dsDNA and complement levels are unsatisfactory. They lack sensitivity and specificity for differentiating renal activity and damage in lupus nephritis. Significant kidney damage can occur before renal function is impaired and first detection by laboratory parameters. Persistent proteinuria may not necessarily indicate ongoing inflammation in the kidneys; and may be contributed by pre-existing chronic lesions or recent damage in the kidneys during the course of the disease. Flares of nephritis can occur without any observable and recent increase in the degree of proteinuria. Renal biopsy is the gold standard for providing information on the histological classes of lupus nephritis and the relative degree of activity and chronicity in the glomeruli. However, it is invasive and serial biopsies are impractical in the monitoring of lupus nephritis. Thus, novel biomarkers that are able to discriminate lupus renal activity and its severity, predict renal flares, monitor treatment response and disease progress, and stratify prognosis are necessary.

A biomarker refers to a biologic, biochemical or molecular event that can be assayed qualitatively and quantitatively by laboratory techniques. An ideal biomarker for lupus nephritis should possess the following properties: (1) Good correlation with renal activity as reflected by the degree of proteinuria and urine sediments; (2) Sensitive to change so that it can be used for serial monitoring of disease activity in the kidneys and defining treatment response and clinical remission; (3) Ability to predict renal activity / flares before an obvious change in conventional clinical parameters occurs so that early treatment / preventive strategies can be considered; (4) Specific to nephritis among patients with SLE; and (5) Specific to SLE for aiding early diagnosis of lupus nephritis. In addition, a useful biomarker should be easy to assay, simple to interpret and readily available in most laboratories with a reasonable cost.

Hitherto, quite a number of serum and urine biomarkers have been studied in lupus nephritis (summarized in Table 5). Many of these markers have only been tested in cross-sectional studies with small sample size, and none has been rigorously validated in large-scale longitudinal cohorts of patients with different ethnic background. It is unlikely at this juncture that a candidate biomarker stand-alone can replace conventional clinical parameters to monitor disease progress and detect early renal flares. Urine biomarkers appear to be more encouraging than serum biomarkers possibly because they are the direct products or consequences of kidney inflammation or injury. Future directions in SLE biomarker research should focus on a combination of novel markers with conventional clinical parameters to enhance the sensitivity and specificity for the prediction of renal flares and prognosis in lupus nephritis (Mok, 2010a).

Urinary monocyte chemoattractant protein-1 (uMCP-1)
Plasma and urine neutrophil gelatinase–associated lipocalin (NGAL)
Urinary tumor necrosis factor (TNF)-like inducer of apoptosis (uTWEAK)
Urine proteomics
Hepcidin
Anti-C1q antibodies
Anti-nucleosome antibodies
Anti-α-actinin antibodies
MAGE-B2 antibodies
Anti-CRP antibody
Serum and urine IL-12
Peripheral blood leukocyte chemokine transcriptional levels
Serum apoCIII
Serum ICAM-1
Anti-endothelial cell antibody
Urine osteoprotegerin (OPG)
FOXP3 mRNA expression in urinary sediments
Urine endothelin-1
Urine CXCR3+CD4+T cells
Urine VCAM-1, P-selectin, TNFR-1 and CXCL16
Urine TGFβ-1
TGFβ and MCP-1 mRNA expression in urine sediments
Chemokine and growth factor mRNA level in urinary sediments
Serum nitrate and nitrite level
Anti-ribosomal P antibody
Urine glycoprotein panel

Table 5. Novel biomarkers for lupus nephritis

14. Conclusions

Renal involvement is a major determinant of the prognosis of SLE. Lupus renal disease is more frequent in certain ethnic groups such as the Africans, Hispanics and Asians. Of the various histological types of lupus nephritis, diffuse proliferative lupus nephritis carries the worst prognosis. Treatment of lupus nephritis should target at disease remission, prevention of relapse and complications, and long-term preservation of renal function. The main stay of

treatment of lupus nephritis is immunosuppression using a combination of high-dose glucocorticoid and a non-glucocorticoid immunosuppressive agent. Mycophenolate mofetil combined with prednisone has emerged to be the standard regimen. Intravenous pulse or daily oral cyclophosphamide is reserved for more serious or refractory cases of lupus nephritis. The evidence for calcineurin inhibitors in lupus nephritis is less strong and these agents are reserved for patients intolerant or recalcitrant to standard therapies. B cell modulation is emerging as novel therapeutic modalities for lupus nephritis. While further evidence from controlled trials is eagerly awaited, the current use of B cell modulating agents is confined to recalcitrant lupus renal disease. Conventional markers for activity of lupus nephritis are neither sensitive nor specific. Novel biomarkers are being studied for earlier detection of renal flares and better prognostic stratification so that intervention can be instituted early to minimize damage to renal function.

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Anti-Glomerular Basement Membrane Disease

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1. Introduction

Anti–glomerular basement membrane (anti-GBM) disease is a rare autoimmune disorder characterized by rapidly progressive glomerulonephritis (RPGN) with diffuse crescentic formation on renal biopsy, and it is a well-characterized cause of glomerulonephritis.
In 1919, an autopsy of an 18-year-old male patient, who had developed hemoptysis and acute renal failure after experiencing flu-like symptoms, revealed massive alveolar hemorrhage, glomerulonephritis with fibrinous exudates in Bowman's capsule and necrotizing vasculitis in the spleen and gut (Goodpasture, 1919). Stanton and Tange reported 9 cases with alveolar hemorrhage and RPGN as Goodpasture's syndrome (Stanton & Tange, 1958). Anti-GBM disease was defined as the presence of serum autoantibodies to the noncollagenous domain of the alpha 3 chain of type IV collagen or a linear binding of IgG to glomerular capillary walls as detected by direct immunofluorescence in patients with RPGN. Anti-GBM disease was divided into two types: anti-GBM disease without alveolar hemorrhage was regarded as renal-limited anti-GBM disease, and that with alveolar hemorrhage was defined as Goodpasture's syndrome.
This review focuses on anti-GBM disease by comparing international differences in prevalence, clinical features, treatments and outcomes in order to improve the prognosis of anti-GBM disease.

2. Prevalence

Anti-GBM disease is relatively rare, with an estimated annual incidence of about 0.5-1.0/million population (Table 1). It has been estimated to cause 0.2-2.4% of biopsy-proven glomerulonephritis cases in Europe, but less than 0.2% in Asia. It causes about 10% of RPGN (or necrotizing and/or crescentic glomerulonephritis) in Europe, more than 10% of RPGN in the United States, and less than 10% in Asia. In Japan, to improve the prognosis of patients with RPGN, a nation-wide survey of patients with RPGN in 365 hospitals between 1989 and 2000 was conducted, and clinical characteristics including initial symptoms, laboratory findings and histological findings were investigated along with treatment methods and outcomes (Hirayama et al., 2008). Among patients with RPGN, 6.6% had anti-GBM disease. In comparison with foreign countries, this Japanese rate of anti-GBM disease in RPGN was lower.

Authors	Year	Nation	Incidence (/million/yr)	Frequency (%)		
				GN	2nd GN	RPGN
Rychlík et al.	2004	Czech	0.17	0.31 *	1.2 *	
Heaf et al.	1999	Denmark	0.6			12.8 +
Andrassy et al.	1991	Germany	0.55			7.9
Daly et al.	1996	Ireland		2.4 *		
Schena et al.	1997	Italy	0.1	0.20 *	1.5 *	
Grcevska et al.	1995	Macedonia				3.6 +
Naumovic et al.	2009	Serbia	0.02	0.18 *	0.74 *	
Rivera et al.	2002	Spain				14.6 +
Saxena et al.	1991	Sweden				13.4 +
Williams et al.	1988	United Kingdom	1.12(0.2 – 4.0)			
Angangco et al.	1994	United Kingdom		0.81 *		11.2 +
Parfrey et al.	1985	Canada				11.5 +
Wilson and Dixon	1973	United States		7.0 *		
Jennette	1993	United States				14.6 +
Briganti et al.	2001	Australia	0.99	0.8 *		
NZGS	1989	New Zealand		5.9 *		
Date et al.	1987	India		0.04 *		
Sumethkul et al.	1999	Thai		0.10 *		3.3 +
Tang et al.	2003	China		0.15 *		8.7 +
Li, FK. et al.	2004	China(HongKong)	0.6			
Li, LS. et al.	2004	China		0.21 *	0.86 *	
Hirayama et al.	2008	Japan				6.6

The incidence of patients with anti-GBM disease is expressed as the number per 1 million population per year. The frequencies of patients with anti–GBM disease in glomerulonephritis, secondary glomerulonephritis or rapidly progressive glomerulonephritis are expressed as percentages. *Biopsy-proven glomerulonephritis. Blanks are unavailable data. Abbreviations: yr, years; GN, glomerulonephritis; 2nd GN, secondary glomerulonephritis; RPGN, rapidly progressive glomerulonephritis (including +necrotizing and/or crescentic glomerulonephritis); NZGS, The New Zealand Glomerulonephritis Study.

Table 1. Prevalence of anti–GBM disease in various countries.

All age groups are affected, but the peak incidence of anti-GBM disease is in the third decade in young men, with a second peak in the sixth and seventh decades affecting men and women equally (Figure 1). Alveolar hemorrhage is more common in younger men, while isolated renal disease is more frequent in the elderly, with a near-equal gender distribution. In that survey (Hirayama et al., 2008), the mean age at onset of renal-limited anti-GBM disease was 52.6±17.0 years. There was only one peak incidence of anti-GBM disease, and this peak occurred in the fifth and sixth decades. The gender distribution was nearly equal in renal-limited anti-GBM disease (male: female = 1: 0.94).

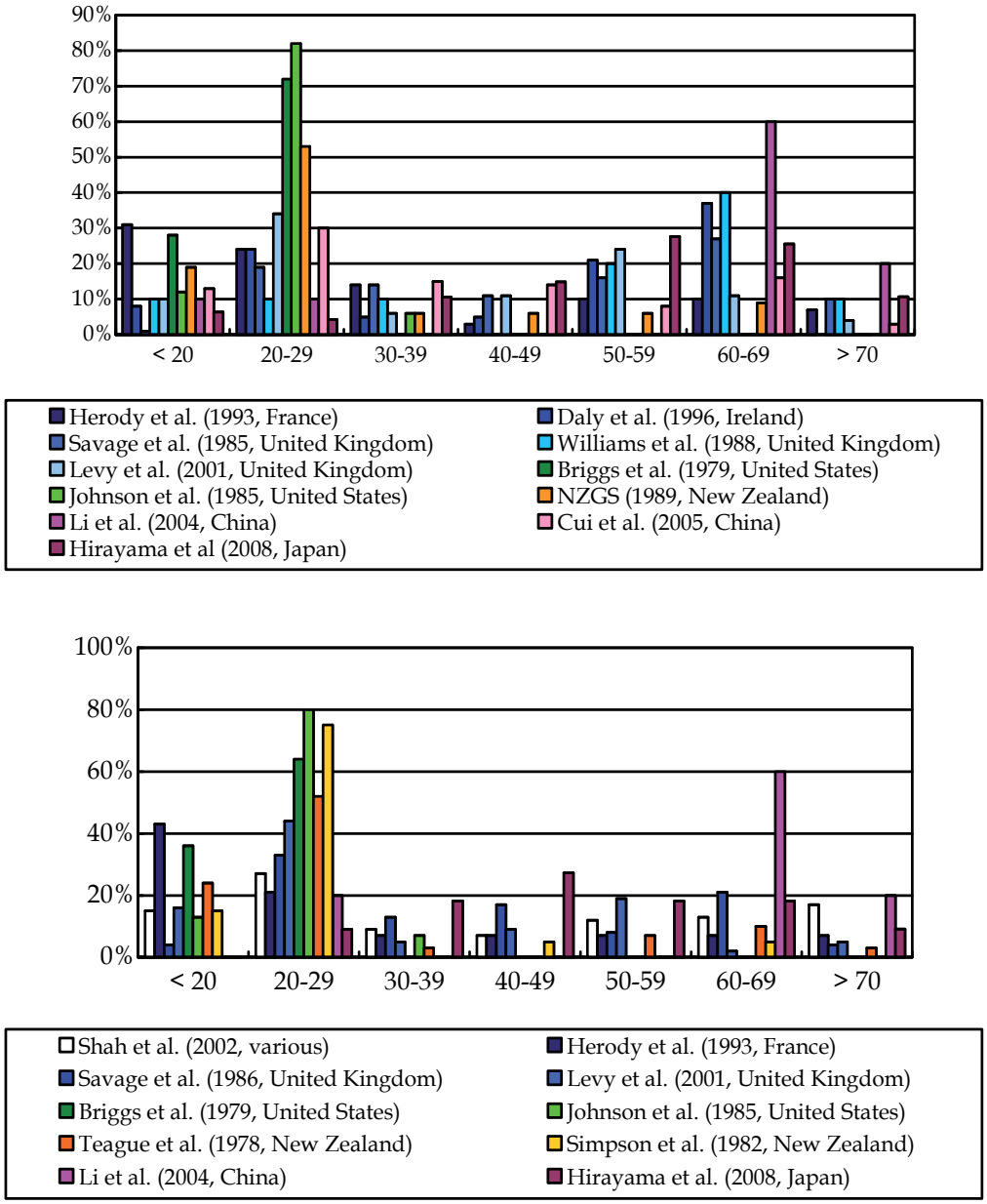

Fig. 1. Investigations of age distribution in anti-GBM disease (upper) and Goodpasture's syndrome (lower).

The histograms show the number of patients with anti-GBM disease classified by patient age at the onset of the disease. Abbreviations: GN, glomerulonephritis; Biopsy, biopsy-proven glomerulonephritis; RPGN, rapidly progressive glomerulonephritis; NZGS, The New Zealand Glomerulonephritis Study; N.A., not available.

Alveolar hemorrhage is observed in 35-62% of patients with anti-GBM disease in Europe, the United States, and China, and it is more common in younger patients and in men, whereas renal-limited anti-GBM disease is more common in older patients and in women. In Japan, alveolar hemorrhage of patients with anti-GBM disease was less frequent (23.4%) and the age at onset of Goodpasture's syndrome was lower (49.4±14.4 years), but it was more common in females (male: female = 1: 1.75).
This disease appears to be more common in Caucasians and very rare in those of African origin (Pusey, 2003; Ooi et al., 2008). There is apparently a higher incidence of onset of Goodpasture's disease in the spring and summer, as well as localized clustering of the disease, perhaps suggesting an infectious relationship (Pusey, 2003). Anecdotal associations with urinary tract infections and lithotripsy, which may subclinically affect the glomerular basement membrane, have also been reported (Pusey, 2003; Ooi et al., 2008).

3. Pathogenesis

In 1934, Masugi reported nephrotoxic glomerulonephritis induced by anti-kidney serum in an experimental model (Masugi, 1934), after which a linear binding of IgG to glomerular capillary walls was detected by direct immunofluorescence (Ortega & Mellors, 1956). In 1964, a linear immunostaining of IgG was observed in 2 patients with Goodpasture's syndrome (Scheer & Grossman, 1964), and in another study the kidney serum of patients with Goodpasture's syndrome and of patients with crescentic glomerulonephritis without alveolar hemorrhage contained antibodies that reacted with the GBM of humans and animals (Lerner et al., 1967). Those authors also demonstrated that those anti-GBM antibodies caused glomerulonephritis when injected into animals.

3.1 Structure of GBM

GBM, which exists between endothelial cells and podocytes, consists of type IV collagen, heparansulphate proteoglycan, laminine and fibronectin. Type IV collagen, which consists of 3 of 6 alpha-chains (α1 to α6) encoded by three pairs of genes on chromosomes 2, 13 and X, and its molecules were trimeric (α1α1α2, α3α4α5 and α5α5α6). This basement membrane, found in kidney, lung, cochlea and eye, comprises the surface on which epithelial cells rest. In kidneys, α3α4α5 molecules were found in the GBM, particularly the epithelial side; α1α1α2 molecules were in the mesangium, the endothelial side of the GBM, tubular basement membranes and Bowman's capsule; and α5α5α6 molecules were in tubular basement membranes and Bowman's capsule. Each alpha-chain was made by one long collagenous domain and two terminal noncollagenous globular domains: the C-terminal noncollagenous (NC1) domain and the N-terminal domain (the 7S domain). Mature GBM is a lattice-like structure comprised in part by heterotrimers of α3, 4 and 5 chains, which form a triple helix with short NC1 and 7S domains (Sado et al., 1998). The NC1 domain of the α3 chain of a tissue-specific type IV collagen [α3(IV)NC1] monomer is structured into the collagen IV network through the association of α3, α4 and α5 chains to form a triple helical protomer and through the oligomerization of these protomers via end-to-end associations and intertwining of triple helixes (Hudson et al., 2003).

3.2 Anti-GBM antibodies

The target of the anti-GBM antibodies was identified as α3(IV)NC1 (Saus et al., 1988). The two conformational epitopes of anti-GBM antibodies have been defined as E_A and E_B

(Kalluri et al., 1996; Borza et al., 2000). The E_A epitope is 17-31 amino acids on the N-terminal side, and the E_B epitope is 127-141 amino acids on the C-terminal side (Netzer et al., 1999). Alterations of the amino acid sequence translated by the COL4A3 gene, which encodes α3(IV)NC1, are not major factors, because no mutation of the COL4A3 gene was found (Persson et al., 2004). It was suggested that E_A and E_B, as cryptic B-cell epitopes, were enclosed in the quaternary structure of the hexamers created by sulfilimine crosslinks between the trimers of adjacent NC1 chains (Vanacore et al., 2008, 2009). Recently, in patients with Goodpasture's disease, elevated autoantibody titers to α3(IV)NC1 and α5(IV)NC1 monomers at diagnosis were associated with the eventual loss of renal function (Pedchenko et al., 2010). In that study, these anti-GBM antibodies bound to specific epitopes that encompassed region E_A in the α5(IV)NC1 monomer and regions E_A and E_B in the α3(IV)NC1 monomer, but did not bind to the native crosslinked α345(IV)NC1 hexamer. Thus, it is a dissociation of the NC1 hexamers that expose the pathogenic epitopes on the α3 and α5 chains, precipitating the production of anti-GBM antibodies (Pedchenko et al., 2010). It was suggested that the autoantibody itself may subsequently alter antigen conformation and expose further epitopes, causing an epitope-spreading phenomenon (Salant, 2010).

3.3 Crescent formations

The anti-GBM antibody bound to GBM ligates Fc receptors, leading to the activation of monocytes, neutrophils, eosinophils, basophils and macrophages. These release chemokines that attract a further influx of neutrophils into glomeruli, causing severe tissue injury, including the disruption of the GBM. Renal injury in anti-GBM disease is amplified by the activation of complements and protease after the binding (Sheerin et al., 1997; Baricos et al., 1991). The release of reactive oxygen species by neutrophils is also probably an important pathogenic mechanism of tissue injury.

The histogenesis and origin of cellular crescents, which are cap-like multilayered accumulations of proliferating cells, have remained controversial. Although early ultrastructural studies suggested that crescents are formed by proliferating epithelial cells (Morita et al., 1973; Min et al., 1974), subsequent histochemical studies with antibodies against leukocytes identified the presence of monocytes-macrophages in cellular crescents (Atkins et al., 1976; Thomson et al., 1979). It was demonstrated that epithelial cells predominated in crescents of patients during the early phases of disease; later phases were characterized by rupture of the basement membrane of Bowman's capsule and subsequent infiltration of cellular crescents, predominantly by macrophages (Boucher et al., 1987). The composition of cellular crescents may change during the progression of disease after the inciting glomerular injury (Ophascharoensuk et al., 1998).

The main stimulus to the migration of macrophages and neutrophils is probably the exudation of fibrin in Bowman's space caused by the disruption of the GBM and Bowman's capsule (Tipping et al., 1988). Several possible causes of acute renal injury in anti-GBM disease were identified, including the functional roles of a number of macrophage proinflammatory mediators, such as IL-1 (Lan et al., 1993), TNF-alpha (Lan et al., 1997; Le Hir et al., 1998) and matrix metalloproteinase (Kaneko et al., 2003). In epithelial crescent formation in the glomerulus, thrombin generated by coagulation (He et al., 1991) and growth-factor cytokines (IL-1 and IL-2) released by monocytes and platelets (Adler et al., 1990) stimulate the migration of epithelial cells. Moreover, interleukin-12 (Kitching et al., 1999) and interferon-γ (Timoshanko et al., 2002) are also involved.

3.4 Environmental and genetic factors

Environmental factors are thought to play a role in triggering the disease. In the first case of Goodpasture's syndrome, intercurrent infection amplifies the intensity of inflammatory responses and can aggravate disease and so make it clinically apparent. There are a number of case reports of clusters of patients with anti-GBM disease (Perez et al., 1974), which may implicate an infective agent; however, no clear viral association has been identified. Group A type 12 streptococcal cell membrane shares some cross-reactivity with the human glomerular basement membrane, generating another hypothesis: that infection may initiate anti-GBM antibody production (Blue & Lange, 1975).

Goodpasture's syndrome has been noted to occur more frequently in smokers (Salama et al., 2001). Lazor et al. (2007) reported that 89% of their patients with Goodpasture's syndrome were active smokers. In another study, alveolar hemorrhage was present in 100% of patients who smoked and in only 20% of nonsmokers with Goodpasture's disease (Donaghy & Rees, 1983). No significant difference in circulating anti-GBM antibody titers was found between smokers and nonsmokers, suggesting that cigarette smoking may increase the permeability of lung capillaries and thus expose alveolar basement membranes to circulating anti-GBM antibodies (Donaghy & Rees, 1983; Klasa et al., 1988). Other inhaled substances may also be associated with anti-GBM disease, including cocaine (García-Rostan y Pérez et al., 1997; Lazor et al., 2007), hard metals such as inert tungsten carbide and cobalt (Lechleitner et al., 1993), smoke inhalation (Klasa et al., 1988) and possibly volatile hydrocarbon solvents (Beirne & Brennan, 1972; Bombassei & Kaplan, 1992). In particular, hydrocarbon exposure may influence the development of alveolar hemorrhage (Churchill et al., 1983; Bonzel et al., 1987). Another environmental factor, alemtuzumab, which is a humanized anti-CD52 monoclonal antibody, recently was identified as a cause of anti-GBM disease (Clatworthy et al., 2008).

Genetic factors appear to play a role in susceptibility to anti-GBM disease. As a genetic factor of anti-GBM disease, the human leukocyte antigen (HLA) complexes are known to influence susceptibility to anti-GBM disease. A strong association with HLA-DR2 specificity has been confirmed (Rees et al., 1978). In HLA genotyping, DRB1*1501 (the serologically defined HLA-DR2 gene) and DRB1*1502 (HLA-DR15 gene) allele at the DRB1 locus is associated with anti-GBM disease in Caucasians (Fisher et al., 1997), Chinese (Yang et al., 2009) and Japanese (Kitagawa et al., 2008). The strongest association was with HLA DRB1*1501 but, when the effect of this gene was excluded, subsequent analysis revealed an increased frequency of DRB1*04 and DRB1*03 and a decreased frequency of DRB1*07 and DRB1*01 (Phelps & Rees, 1999). Other genetic influences of anti-GBM disease have been identified, including the immunoglobulin heavy chain Gm locus that encodes the constant region of the IgG heavy chain (Rees et al., 1984), polymorphisms of FCGR genes that encode the Fc receptor for IgG (FcγR) (Zhou et al., 2010a, 2010b) and kallikrein genes (Liu et al., 2009).

4. Clinical symptoms

General malaise (fatigue), weight loss, fever, arthralgia or myalgia may be the initial features of anti-GBM disease in a pattern similar to but much less prominent than that in systemic vasculitis. Symptoms relating to anemia may also occur even in the absence of significant hemoptysis.

Authors	Year	Nation	symptoms					
			fatigue	fever	dys-pnea	hemo-ptysis	macro hematuria	oligo-anuria
Shah et al. *	2002	Various	15% (8/54)	28% (15/54)	26% (14/54)	65% (35/54)	7% (4/54)	17% (9/54)
Lazor et al.	2007	France & Switzerland	64% (18/28)	43% (12/28)	79% (22/28)	75% (21/28)	36% (10/28)	18% (5/28)
Merkel et al.	1994	Germany	40% (14/35)	28% (10/35)	14% (5/35)	51% (18/35)	20% (7/35)	N.A.
Daly et al.	1996	Ireland	N.A.	N.A.	N.A.	25% (10/40)	35% (14/40)	50% (20/40)
Williams et al.	1988	United Kingdom	N.A.	N.A.	10% (1/10)	10% (1/10)	10% (1/10)	60% (6/10)
Proskey et al. *	1970	United States	51% (29/56)	22% (12/56)	57% (32/56)	82% (46/56)	12% (7/56)	N.A.
Wilson et al.	1973	United States	34% (17/50)	14% (7/50)	32% (16/50)	46% (23/50)	42% (21/50)	10% (5/50)
Briggs et al.	1979	United States	22% (4/18)	11% (2/18)	44% (8/18)	50% (9/18)	56% (10/18)	N.A.
Walker et al.	1985	Australia	N.A.	N.A.	N.A.	62% (13/21)	N.A.	62% (13/21)
Teague et al. *	1978	New Zealand	68% (19/28)	26% (7/27)	78% (21/27)	86% (25/29)	43% (12/28)	N.A.
Li et al.	2004	China (Hong Kong)	N.A.	N.A.	N.A.	40% (4/10)	N.A.	40% (4/10)
Cui et al.	2005	China	N.A.	N.A.	N.A.	59% (57/97)	27% (26/97)	52% (50/97)
Hirayama et al.	2008	Japan	53% (25/47)	57% (27/47)	6% (3/47)	15% (7/47)	19% (9/47)	28% (13/47)

The frequencies of patients with each symptom are expressed as percentages. *All investigated patients had Goodpasture's syndrome. Abbreviations: N.A., not available.

Table 2. Investigations of clinical symptoms in anti-GBM disease at the initial presentation.

The principal clinical features relate to the development of renal failure due to RPGN or alveolar hemorrhage (Table 2). Hemoptysis is the predominant symptom of alveolar hemorrhage. Alveolar hemorrhage may cause severe impairment of oxygenation, so intensive care and artificial ventilation are sometimes needed. The mild lung symptoms are only dry cough and shortness of breath. Although one-third to two-thirds of patients with anti-GBM disease demonstrate alveolar hemorrhage in general, in our survey, 23.4% (11/47) of patients with anti-GBM disease suffered from alveolar hemorrhage (Hirayama et al., 2008). A minority of patients exhibited macrohematuria. Anirua or oliguria was seen in 17-62% of patients at presentation, and these findings suggested a poorer prognosis (Levy et al., 2001; Hudson et al., 2003).

5. Laboratory examinations

In general, all patients with anti-GBM disease had microscopic hematuria on urinalysis. Proteinuria is modest, but can be heavier when the disease has a more subacute course. In our survey (Hirayama et al., 2008), the mean 24-hour excretion of urinary protein in renal-limited anti-GBM disease was 2.1±3.0 g and that of Goodpasture's syndrome was 3.7±3.2 g.

Authors	Year	Nation	Urinary protein (g/day)	Serum creatinine (mg/dL)	ESRD (%)
Shah et al.	2002	Various	N.A.	6.62 (N.A.)	35% (27/78)
Herody et al.	1993	France	N.A.	N.A.	55% (16/29)
Lazor et al.	2007	France & Switzerland	1.2 (0 – 35.0)	1.27 (0.61 – 21.47)	41% (11/28)
Andrassy et al.	1991	Germany	6.4 (0 – 15.3)	12.8 (6.1 – 16.5)	67% (2/3)
Merkel et al.	1994	Germany	N.A. (0.2 – 3.5)	11.41 ± 5.64 (0.19 – 22.96)	71% (20/28)
Daly et al.	1996	Ireland	N.A.	5.1 ± 6.8 (N.A.)	50% (20/40)
Segelmark et al.	2003	Sweden	N.A.	8.94 (5.44 – 12.34)	46% (36/79)
Savage et al.	1986	United Kingdom	N.A.	N.A.	64% (69/108)
Williams et al.	1988	United Kingdom	N.A.	11.80 ± 4.67 (1.60 – 18.37)	70% (7/10)
Levy et al.	2001	United Kingdom	N.A.	3.59 (0.6 – 10.9)	55% (39/71)
Briggs et al.	1979	United States	2.6 ± 0.5 (1.9 – 3.5)	5.94 ± 7.11 (0.8 – 30.0)	33% (6/18)
Johnson et al.	1985	United States	4.3 ± 5.2 (0 – 22.0)	4.87 ± 6.93 (0.9 - 25.0)	12% (2/17)
Jennette	2003	United States	1.67 ± 3.35 (0.20- 16.20)	9.7 ± 7.2 (0.8 - 50)	N.A.
Walker et al.	1985	Australia	1.4 (0.4 – 5.4)	6.56 (1.24 – 32.35)	45% (10/22)
Simpson et al.	1982	New Zealand	N.A.	5.37 ± 5.22 (0.68 – 19.80)	10% (2/20)
Teague et al.	1978	New Zealand	N.A.	N.A.	14% (4/29)
Li et al.	2004	China (Hong Kong)	N.A.	6.96 ± 6.41 (1.19 – 22.09)	50% (5/10)
Cui et al.	2005	China	N.A.	N.A.	71% (69/97)
Hirayama et al	2008	Japan	2.4 ± 3.0 (0.1 – 12.2)	7.29 ± 4.19 (1.00 – 16.80)	60% (28/47)

Amounts of urinary protein and serum creatinine levels are expressed as means ± standard deviation or medians with ranges. Frequency of end-stage renal failure at presentation is expressed as a percentage. To convert serum creatinine in mg/dL to μmol/L, multiply by 88.4. Abbreviations: ESRD, end-stage renal disease; N.A., not available.

Table 3. Investigations of renal findings in anti-GBM disease at the initial presentation.

Unfortunately, most patients with anti-GBM disease had renal failure at the time of diagnosis, and the number of patients needing dialysis was not a few (Table 3). In our survey (Hirayama et al., 2008), the mean serum creatinine (s-Cr) level in renal-limited anti-GBM disease was 7.07±4.21 mg/dl, while that in Goodpasture's syndrome was 7.99±4.31 mg/dl. Hemodialysis therapy had already been initiated in 59.6% (28/47) of the anti-GBM disease patients before the start of immunosuppressive treatments.

Anemia was observed in most patients with anti-GBM disease, and the mean hemoglobin concentration in renal-limited anti-GBM disease was 8.8±1.7 g/dl, while that in Goodpasture's syndrome was 7.5±1.1 g/dl. The mean erythrocyte sedimentation rate (ESR) in renal-limited anti-GBM disease was 105±44 mm/h, and that in Goodpasture's syndrome

was 82±45 mm/h. The mean serum C-reactive protein (CRP) level in renal-limited anti-GBM disease was 8.5±7.2 mg/dl, and that in Goodpasture's syndrome was 8.2±8.1 mg/dl. In comparison with other forms of RPGN, such as micropolyangiitis (MPA) and Wegener's granulomatosis (WG), there was no difference in inflammation markers such as leukocyte count, ESR and serum CRP. However, in patients with anti-GBM disease, the mean level of s-Cr at the time of diagnosis was higher than that in patients with MPA (4.54±3.13 mg/dl) or WG (3.84±3.24 mg/dl). Therefore, early diagnosis of anti-GBM disease is very important.

Although obvert hemoptysis may not be immediately present in patients with Goodpasture's syndrome and alveolar hemorrhage may not be immediately obvious in radiological examinations, an elevated alveolo-arterial oxygen difference ($AaPO_2$) can be a sensitive indicator of alveolar hemorrhage. An elevated red blood cell count in bronchoalveolar lavages, as detected by bronchoscopy, is useful information for the diagnosis of alveolar hemorrhage, but lung biopsy does not contribute to this diagnosis (Lazor et al., 2007).

The diagnosis of anti-GBM disease is dependent on the detection of anti-GBM antibodies in either the circulation or the kidney tissue. These serum antibodies are usually detected using an enzyme-linked immunosorbent assay or radioimmunoassay method. The antibodies have not been reported to occur in the absence of disease, and false negatives are rare when appropriate checks are performed. In our survey (Hirayama et al., 2008), 91.5% (43/47) of patients with anti-GBM disease were diagnosed via the detection of serum anti-GBM antibodies.

In serological examinations, other autoantibodies were not usually detected. However, in our survey (Hirayama et al., 2008), anti-nuclear antibodies were detected in 11.8% of renal-limited anti-GBM disease and in 27.3% of patients with Goodpasture's syndrome. Anti-DNA antibody was not detected in renal-limited anti-GBM disease, but it was detected in 22.2% of patients with Goodpasture's syndrome. Moreover, anti-neutrophil cytoplasmic antibodies (ANCA) were detected in 12.8% (5/39) of patients with anti-GBM disease; a perinuclear pattern was detected in all five anti-GBM disease patients with ANCA, and a cytoplasmic pattern was detected in one. Anti-GBM antibody and ANCA coexisted in 15 - 50% of cases of anti-GBM disease described in the previous literature (Jayne et al., 1990; Bosch et al., 1991; Yang et al., 2005; Rutgers et al., 2005; Levy et al., 2004). Other studies revealed that patients with double-positive antibodies were predominantly MPO-ANCA, older and male (Jayne et al., 1990; Bosch et al., 1991; Yang et al., 2005; Rutgers et al., 2005). In our survey (Hirayama et al., 2008), the age at onset of patients with double-positive antibodies was higher (mean age, 52.6 years), but female-dominant (male : female = 1 : 4). The prognosis of patients with double-positive antibodies varied; the renal and patient survival rates of patients with double-positive antibodies were reported to be either better (Jayne et al., 1990; Bosch et al., 1991), not significantly different (Yang et al., 2005), or worse (Rutgers et al., 2005; Levy et al., 2004) than those of patients with anti-GBM antibody alone. In our survey (Hirayama et al., 2008), the prognosis of patients with double-positive antibodies was poor; two died and the remaining three required maintenance hemodialysis. Alveolar hemorrhage was observed in two of five patients with double-positive antibodies, and three of them had interstitial pneumonitis.

6. Imaging examinations

Kidneys were usually normal-sized or enlarged due to inflammation. In our survey (Hirayama et al., 2008), ultrasonography showed that 61.0% of patients with anti-GBM

disease had kidneys of normal size, while atrophic kidneys were observed in 12.2% of patients and enlarged kidneys were observed in 26.8%. There were no specific morphological abnormalities on any type of renal imaging examinations.
In cases with Goodpasture's syndrome, shadows usually involve the central lung fields with peripheral and upper-lobe sparing on chest radiography or computed tomography (Figure 2). Although the shadows are generally symmetrical, they can be markedly asymmetrical.

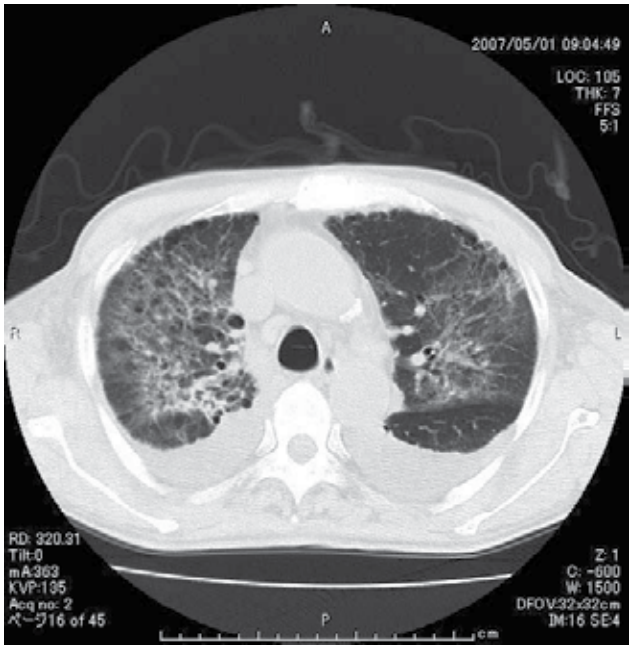

Fig. 2. Chest computed tomography in a patient with Goodpasture's syndrome.

Symmetrical shadows involved the central lung fields with peripheral sparing. Bilateral pleural effusions due to hypervolemia in acute kidney injury were also observed.

7. Pathological findings

A renal biopsy is essential in suspected anti-GBM disease both to confirm the diagnosis and to assess the renal prognosis. Glomerular fibrinoid necrosis and crescent formation with linear staining of the glomerular capillary walls for IgG are the histological hallmarks of anti-GBM disease.

7.1 Light microscopic findings

The histological pattern of disease starts with mesangial expansion and hypercellularity. It progresses to focal and segmental glomerulonephritis with infiltration by inflammatory cells, accompanied by segmental necrosis with prominent breaks in the GBM. Later, glomeruli develop an extensive crescent formation composed of parietal epithelial cells and macrophages in association with the destruction of the GBM (Figure 3). The crescents are usually at the same stage of evolution.

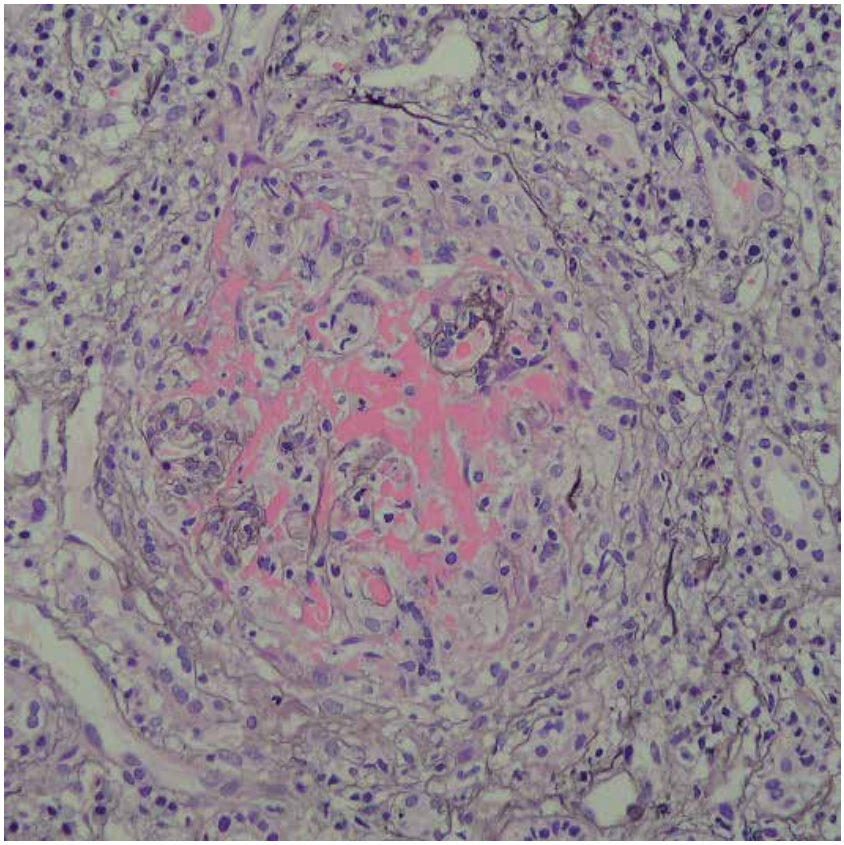

Fig. 3. Periodic acid-Schiff methenamine silver (PAM)−stained glomerulus in a patient with anti-GBM disease.

The disruption of the capillary walls, segmental necrosis and cellular crescent formation are observed. Rupture of the basement membrane of Bowman's capsule and periglomerular infiltration of inflammatory cells are also observed. Interstitial edema with infiltration of inflammatory cells is revealed.

Various degrees of crescent formation are observed in more than 90% of patients with anti-GBM disease. In Europe, the United States and Asian-Pacific, including Japan, the mean percentage of glomeruli showing crescent formation ranged from 40% to 100%, and about 70% to 100% of patients with anti-GBM disease had more than 50% crescentic glomeruli (Figure 4). Anti-GBM disease is pathologically the most severe form of glomerulonephritis (Holdsworth et al., 1985; Jennette, 2003, Hirayama et al., 2008).

Although tubules are usually normal, epithelial flattening is revealed in the severe acute phase. In the chronic phase, tubules in the area of severe injury undergo atrophy and some disappear. Acute tubulitis sometimes occurs if there is a linear staining of tubular basement membranes for IgG. Interstitial edema with infiltration of inflammatory cells is predominant in the acute phase, whereas interstitial fibrosis is revealed in the chronic phase. Interstitial infiltrates are composed of neutrophils, eosinophils, lymphocytes, monocytes and macrophages. If Bowman's capsules are disrupted, inflammatory cells infiltrate around glomeruli and have a granulomatous appearance. Acute inflammation of renal vessels,

except for glomerular capillaries, is not typical for anti-GBM disease, unless the case has concurrent ANCA (Bosch et al., 1991).

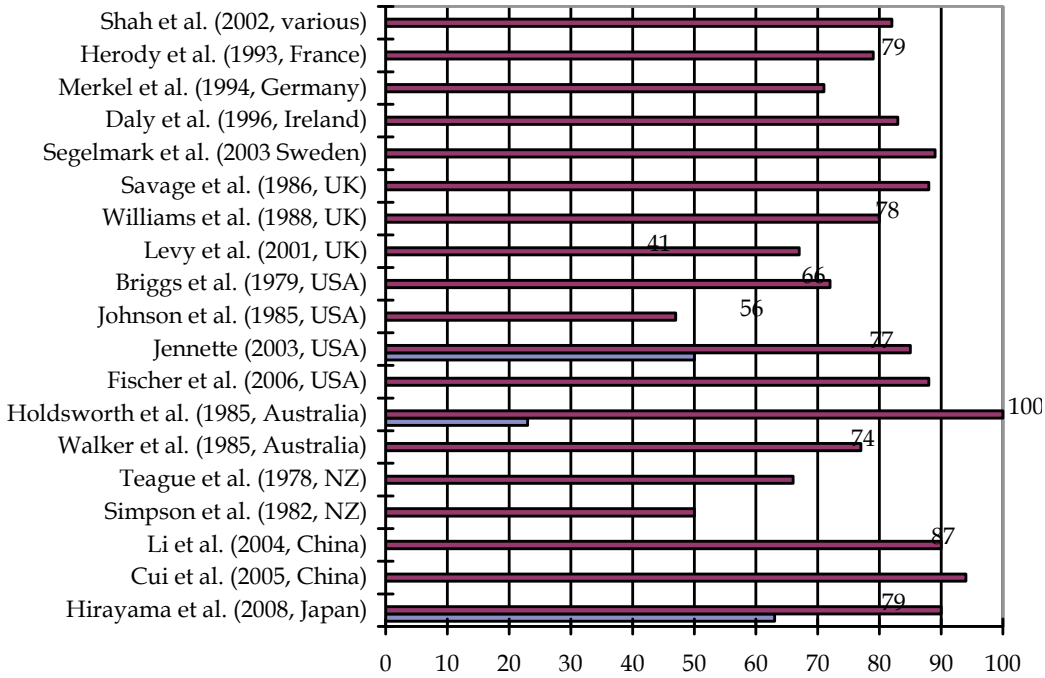

Fig. 4. Previous investigations of crescent formation in anti-GBM disease and ANCA-associated vasculitis.

Each bar shows the frequency of patients with 50% or more crescents in anti-GBM disease (purple) and ANCA-associated vasculitis (blue). The numbers show the mean percentage of glomeruli showing crescent formation in anti-GBM disease. Abbreviations: UK, United Kingdom; USA, United States; NZ, New Zealand.

7.2 Immunofluorescence findings

The immunohistologic feature of anti-GBM disease is linear staining of the glomerular capillary walls for IgG (Figure 5). IgG1 is the predominant IgG subclass in staining of the glomerular capillary walls (Bowman et al., 1987; Segelmark et al., 1990). Linear staining for IgM and IgA is less common, but rare cases with anti-GBM disease have linear staining only for IgA and circulating IgA-class anti-GBM antibodies in the absence of IgG-class anti-GBM antibodies in the serum or staining in glomeruli (Border et al., 1979; Gris et al., 1991; Borza et al., 2005). Granular or discontinuous linear staining for C3 is observed in most cases with anti-GBM disease, but glomerular staining for C3 is negative for some cases (Wilson and Dixon, 1973). Irregular staining for fibrin is observed in portions of glomerular necrosis and cellular crescents.

Linear staining of tubular basement membranes for IgG sometimes occurs (Lehman et al., 1975; Andres et al., 1978).

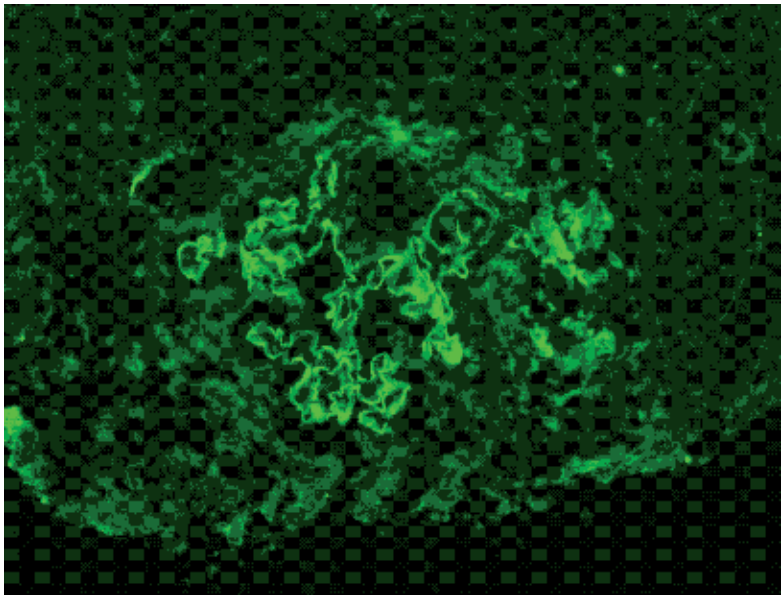

Fig. 5. Direct immunofluorescence for IgG in a patient with anti-GBM disease.

Linear staining for IgG along glomerular capillary walls is observed, but staining for IgG at the part of the cellular crescent is not.

7.3 Electroscopic findings

The rupture of GBM with variable degrees of endothelial swelling and lucent expansion of the subendothelial zone is common urtrasturactural findings in the acute phase of anti-GBM disease. Rupture of Bowman's capsule, focal effacement of epithelial foot processes and accumulation of epithelial cells and macrophages in Bowman's spaces are also observed. Occasionally, neutrophils are identified in capillaries, especially at sites where GBM is disrupted. Those findings are also observed in pauci-immune crescentic glomerulonephritis, but electron-dense deposits are absent, unlike the case with immune complex-type crescentic glomerulonephritis.

8. Treatments

As the pathogenesis of anti-GBM disease became clear, treatment regimens were designed to remove the circulating pathogenic anti-GBM antibodies by therapeutic plasma exchange, attenuate the pathogenic antibody-mediated glomerular inflammatory responses by administration of corticosteroids and suppress further production of these pathogenic antibodies by the use of immunosuppressive agents.

8.1 Therapeutic plasmapheresis

To remove the circulating pathogenic anti-GBM antibodies, therapeutic plasma exchange is recommended as the initial treatment. The effectiveness of therapeutic plasmapheresis for improving renal function has been reported. In the most commonly used regimens, plasma exchange of 4 L of plasma for 5% human albumin was performed daily for 14 days or until

the circulating anti-GBM antibodies were no longer detected (Lockwood et al., 1976). In the presence of alveolar hemorrhage, 300-400 ml of fresh-frozen plasma was given at the end of each treatment.

To reduce the replacement of plasma, anti-GBM antibody removal has been modified. Immunoadsorption to remove circulating IgG immunoglobulins without the need for protein substitution during daily treatments may also be beneficial in Goodpasture's disease. Anecdotal case reports suggest that it may be an alternative to plasmapheresis in patients with severe renal failure (Laczika et al., 2000). There was a case report of Goodpasture's syndrome that we treated with double filtration plasmapheresis combined with immunosuppression therapy (Nagasu et al., 2009). In that therapy, the removal efficiency for the anti-GBM antibody was 24 to 60% for each procedure.

8.2 Corticosteroids

To attenuate the pathogenic antibody-mediated glomerular inflammatory responses, corticosteroid is also a key element of this treatment. According to the most commonly used regimens, oral dosing of prednisolone at 1 mg/kg/day ideal body weight (maximum 80 mg daily) continues for at least 2 weeks, after which the dose is reduced every second week to 30 mg by 8 weeks. After that, the dosages of prednisolone are tapered to 2.5-5.0 mg/week and maintained at 7.5-10 mg/kg/day. Oral corticosteroids have generally been continued for at least 6 months. Intravenous administration of methylprednisolone 10 mg/kg (500-1000 mg) once daily for 1-3 days has been advocated for patients with severe alveolar hemorrhage or very rapid deterioration of renal function (Johnson et al., 1985).

8.3 Immunosuppressive agents

To further suppress the production of pathogenic anti-GBM antibodies, a combination of immunosuppressive agents is usually given. Among these immunosuppressive agents, cyclophosphamide is usually administered. According to the most commonly used regimens, the oral dose is 2-3 mg/kg/day (this is rounded down to the nearest 50 mg; reduced to 2 mg/kg/day in patients over 55 years) for 3 months. This administration is stopped if white blood cell counts fall below 4,000/μL. In such cases the agent is restarted at a lower dose once the white blood cell counts return above 4,000/μL. Intravenous cyclophosphamide (IVCY) is not usually administered, but it may be useful for a refractory case of the standard therapy (Baumgartner et al., 1995).

Although azathioprine is sometimes used as maintenance therapy, it alone does not provide adequate immunosuppression to modify the disease.

8.4 Therapeutic options for refractory diseases

There is very little study on the treatment of refractory anti-GBM disease. Cyclosporine is controversial; at 6 mg/kg/day it was effective for an anti-GBM disease patient treated with corticosteroid, cyclophosphamide and plasma exchange (Querin et al., 1992), whereas it was not useful (Pepys et al., 1982). Small numbers of case reports of successful outcomes with mycophenolic acid or mycophenolate mofetil in patients unresponsive to or intolerant of standard therapy have been published (Garcia-Canton et al., 2000; Kiykim et al., 2010; Malho et al., 2010). Rituximab, a chimeric monoclonal anti-CD20 antibody, was effective for a case of relapsed anti-GBM disease that was resistant to standard treatment (Arzoo et al., 2002). In that case, rituximab (375 mg/m^2) was administered once a week for 6 consecutive weeks; the symptoms completely resolved and anti-GBM antibody titers were decreased from 51 U/mL

to the undetectable range. However, these treatments cannot yet be recommended as a first-line therapy because no randomized controlled trials have been carried out.

9. Prognosis

Most patients without treatment died shortly after diagnosis of anti-GBM disease; the survival rate at 12 months was 4%, and the renal survival rate was 2% (Benoit et al., 1963). Although mortality has improved by the introduction of intense immunosuppression, renal survival remains very poor because of the delayed diagnosis of anti-GBM disease or delayed initiation of induction therapies.

9.1 Outcomes

The prognosis for patients with anti-GBM disease is poor; the survival rate at 6-12 months was 67-94%, but the renal survival rate was 15-58% in Europe, the United States, China and Japan (Table 4).

Authors	Year	Nation	Treatment	N	AH (%)	1-year survival (%)	
						Patient	Renal
Herody et al.	1993	France	OCS+CYC+AZA	29	50	93	41
Lazor et al.	2007	France & Switzerland	OCS+CYC+PE	24	100	100	58
Merkel et al.	1994	Germany	OCS+CYC+PE	35	57	89	29
Daly et al.	1996	Ireland	IS+PE	40	67	98	20
Segelmark et al.	2003	Sweden	OCS+CYC+PE	79	24	66	25
Peters et al.	1982	United Kingdom	IS+PE	41	56	76	39
Savage et al.	1986	United Kingdom	IS+PE	108	52	78	20
Levy et al.	2001	United Kingdom	OCS+CYC+PE	71	62	77	53
Proskey et al.	1970	United States	IS	56	100	77	23
Wilson et al.	1973	United States	IS	53	60	53	13
Beirne et al.	1977	United States	IS	29	54	42	17
Briggs et al.	1979	United States	IS(+PE)	18	61	84	22
Johnson et al	1985	United States	OCS+CYC	9	78	89	22
			OCS+CYC+PE	8	100	100	75
Walker et al.	1985	Australia	IS+PE	22	62	59	45
Teague et al.	1978	New Zealand	IS+PE	29	100	64	31
Simpson et al.	1982	New Zealand	no treatment	8	100	63	25
			OCS+AZA	4	100	100	50
			OCS+CYC+PE	8	100	100	63
Li et al.	2004	China (Hong Kong)	IS+PE	10	40	70	15
Cui et al.	2005	China	IS+PE	97	58	92	22
Hirayama et al	2008	Japan	OCS+CYC	21	14	86	24
			OCS+CYC+PE	22	36	68	14

Abbreviations: N, number of patients; AH, alveolar hemorrhage; IS, immunosuppressants (including methylprednisolone pulse therapy, oral corticosteroids, cyclophosphamide or azathioprine); PE, plasma exchange; OCS, oral corticosteroids; CYC, cyclophosphamide; AZA, azathioprine.

Table 4. Investigations of treatments for anti–GBM antibody disease.

Renal function improves in 15-75% of patients with anti-GBM disease through the combination of plasma exchange with corticosteroids and immunosuppressive agents, whereas the renal survival rates of anti-GBM disease patients treated with immunosuppressive agents alone ranged from 2-22%. Improvement of renal function is usually evident within days of the start of plasma exchange. However, it should be emphasized that this regimen has never been properly assessed by a prospective randomized controlled trial because of the rarity and acuteness of the condition. The only reported randomized controlled trial was very small and used lower doses of both plasma exchange and cyclophosphamide than those that are generally used in practice.
Although the effectiveness of treatment using therapeutic plasma exchange combined with immunosuppressive agents to improve renal function has been reported, only half of patients with anti-GBM disease had been treated with plasma exchange in our survey (Hirayama et al., 2008). Therefore, there was no significant difference in the renal survival rates between anti-GBM antibody disease patients treated with and without plasma exchange (P = 0.683 by the Log-rank Mantel-Cox test). Moreover, there was no significant difference in mortality between anti-GBM antibody disease patients treated with and without plasma exchange (P = 0.109).

9.2 Predictors of survival

The best predictors of renal survival are s-Cr at the initiation of treatment and the mean percentage of crescent formations. Renal function improves coincidentally with the introduction of plasma exchange in about 80-95% of patients with s-Cr levels less than or equal to 5.7-6.8 mg/dL (500-600 μmol/L), but in far fewer of those with higher s-Cr levels or those who require dialysis. Unfortunately, most patients with anti-GBM disease had renal failure at the time of diagnosis, and the mean percentage of crescent formation was high in anti-GBM disease patients. Therefore, in most patients with anti-GBM disease, the diagnosis may have been made too late to improve renal function by combination therapy.

9.3 Relapse/recurrence

Relapses of anti-GBM disease are rarely observed, in contrast to most other autoimmune kidney diseases. The anti-GBM antibodies seem to disappear spontaneously after 12-18 months (Levy et al., 1996). However, several reports demonstrated recurring cases with anti-GBM disease (Adler et al., 1981; Hind et al., 1984; Klasa et al., 1988; Levy et al., 1996). In our survey (Hirayama et al., 2008), relapse or recurrence was also rare in patients with anti-GBM disease (13.9%) in comparison with patients with ANCA-associated vasculitis, such as WG (29.4%) and MPA (29.3%). Therefore, remission induction therapy is more important in anti-GBM disease. The mean time to recurrence is estimated to be 4.3 years (range, 1-10 years), and that late recurrence may occur with a frequency of 2-14%. During relapses, circulating anti-GBM antibodies often reappear. The combination of plasmapheresis and immunosuppressive agents as re-remission induction therapy is also successful in relapsing cases (Levy et al., 1996).

10. Conclusion

Anti-GBM disease is a rare but well-characterized glomerulonephritis. It occurs across all racial groups but is most common in Caucasians. Although the effectiveness of treatment using therapeutic plasma exchange combined with immunosuppressive agents to improve

renal function has been reported, the prognosis for patients with this disease is poor. To improve the prognosis, it may be necessary to detect this disease in earlier stages and to treat it without delay.

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16

Mixed Hematopoietic Chimerism Allows Cure of Autoimmune Glomerulonephritis: Its Potential and Risks

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1. Introduction

Patients with severe autoimmune lupus glomerulonephritis that is resistant to immunosuppressive therapy need alternative treatment. Recently, bone marrow transplantation (BMT) has been proposed as a potential therapy for refractory autoimmune disease. BMT involves the administration of hematopoietic stem cells, which are self- renewing and capable of giving rise to all mature hematopoietic cell types and possibly some non-hematopoietic cell types. The etiologic and pathogenic bases of many autoimmune diseases ultimately reside in the self-renewing hematopoietic stem cell population. Therefore, the effects of BMT as a treatment for and/or preventive measure against these autoimmune diseases have been investigated extensively (Sykes&Nicolic, 2005). Studies in animal models have shown that the transfer of hematopoietic stem cells can reverse the autoimmune state. The induction of fully allogeneic bone marrow (BM) chimerism, however, is fraught with difficulties. Each of the various methods of inducing fully allogeneic BM chimerism through hematopoietic cell transplantation (HCT) requires a different set of conditions, such as host T cell depletion, donor myeloablation, major histocompatibility complex (MHC) fully matched donor BM, or lethal dose of total body irradiation (TBI). Meeting these conditions is usually a burden on the recipient. Moreover, fully allogeneic BM chimerism is always associated with risks of graft versus host disease (GVHD) and immunodeficiency, which make it less practical for clinical application.

Accordingly, the induction of mixed allogeneic BM chimerism has been proposed as a treatment for autoimmune disease. Mixed chimerism refers to a state in which allogeneic donor hematopoietic cells coexist with recipient cells in host bone marrow.

In this paper, the advantages of inducing mixed BM chimerism are summarized and a process for inducing peripheral/central tolerance is introduced. Several mechanistic pathways which are thought to be involved in reversing the autoimmune state are then described. Based on our original data, we propose one possible mechanism in which newly developed donor T cells, which have been positively selected in the host thymus and restricted host MHC, are able to regulate auto-reactive B cells through T cell receptor (TCR)/MHC interaction. Finally, we discuss the potential risks associated with fully MHC-

mismatched allogeneic mixed chimerism. This information will help to determine the role that HCT can play in the treatment of autoimmune glomerulonephritis.

2. Bone marrow mixed chimerism

2.1 What is the advantage of mixed chimerism?

Mixed chimerism refers to a state in which allogeneic donor hematopoietic cells coexist with recipient cells in host bone marrow, whereas fully allogeneic chimerism refers to a state in which donor hematopoietic cells completely replace recipient cells.

It is known that fully allogeneic chimeras transplanted from a donor with fully mismatched MHC usually reject donor BM, or experience severe GVHD. Even if donor BM cells were safely engrafted in host BM, the resulting fully MHC-mismatched chimeras would develop immunodeficiency. In fully allogeneic chimeras, all mature T cells are supposed to be restricted to the host MHC type, irrespective of their own genetic background. This occurs because thymocytes, the precursors of mature T cells, are positively selected for weak reactivity to the self-peptide/MHC complex in the host thymus; this positive selection is mediated only by thymic cortical epithelial cells and not by bone marrow-derived cells. Therefore, in the periphery, all TCRs have certain affinity to host MHC molecules but not to donor MHC molecules. Thus, if the donor MHC is fully mismatched with the host MHC, there are no peripheral T cells which can interact with peripheral B cells differentiated from donor hematopoietic stem cells which generate donor-type MHC. This is the cause of deficiency in humoral immunity in fully MHC-mismatched allogeneic chimerism (Janeway et al., 2001).

In mixed chimerism, on the other hand, TCR/MHC interactions are at least partially maintained, because B cells differentiated from recipient hematopoietic stem cells are still being generated. Moreover, during intrathymic development, thymocytes that have high affinity to self MHC molecules are deleted from the repertoire in a process known as negative selection. Thymocytes from both the recipient and the donor mature on the thymic epithelium expressing MHC molecules with the recipient haplotype. Nevertheless, the repertoire of T cells, which react with high affinity to MHC molecules with the donor haplotype, eliminated in mixed chimera. This implies that bone marrow-derived cells must be able to induce negative selection. Actually, negative selection in the thymus can be mediated by several different cells. The most important of these are the BM-derived dendritic cells and macrophages. In mixed chimera, the dendritic cells and macrophages differentiated from both donor and host hematopoietic stem cells are located in the thymus, where they eliminate T cells with strong reactivity to self-peptides on both donor and host MHC; thus donor- and host-specific tolerance to each other is established.

To summarize, mixed chimerism offers several advantages over full chimerism as a means of treating autoimmune disease:

1. Mixed chimeras exhibit superior immune-competence across complete MHC barriers. Mixed chimeras possess certain populations of antigen presenting cells (APCs) and B cells which express host-type MHC molecules in the periphery, whereas mixed chimeras exhibit normal humoral and cellular immune responses.
2. In mixed chimeras, dendritic cells and macrophages differentiated from both the recipient and the donor hematopoietic stem cells locate to the thymus where they delete both host-reactive and donor-reactive T cells through negative selection, resulting in a

peripheral T cell repertoire that is tolerant toward both donor and host cells. Therefore, GVHD, one of the most important complications of allogeneic BMT, is not seen in mixed chimeras.

3. Mixed chimerism can be achieved through non-myeloablative regimens, which are generally less toxic than the myeloablative regimens necessary to induce full BM chimerism (this point will be discussed in detail in the next section).

2.2 How is mixed chimerism induced?

As explained above, once specific tolerance is established, the state of mixed chimerism is thought to be stable. The difficulty in establishing stable mixed chimerism lies in blocking the first attack of host peripheral T cells on donor bone marrow stem cells until "tolerized" T cells are renewed in the host thymus. Because host T cells play a dominant role in the rejection of allografts, several methods of deleting host T cells through the injection of various lymphocyte-deleting antibodies along with either total body irradiation or immunosuppressive drugs have been attempted (Tomita et al, 1996. Nikolic et al, 2000). These regimens enabled BM engraftment but were a burden on recipients and frequently made them susceptible to infection. The toxicity of the necessary conditioning regimens has precluded the use of this approach in clinical transplantation.

Another method of inducing allogeneic tolerance involves the temporary inhibition of co-stimulatory interaction between APCs and T cells by injecting blocking antibodies. This method works because T cell activation without proper co-stimulation can induce a state of antigen-specific non-responsiveness (Fig.1).

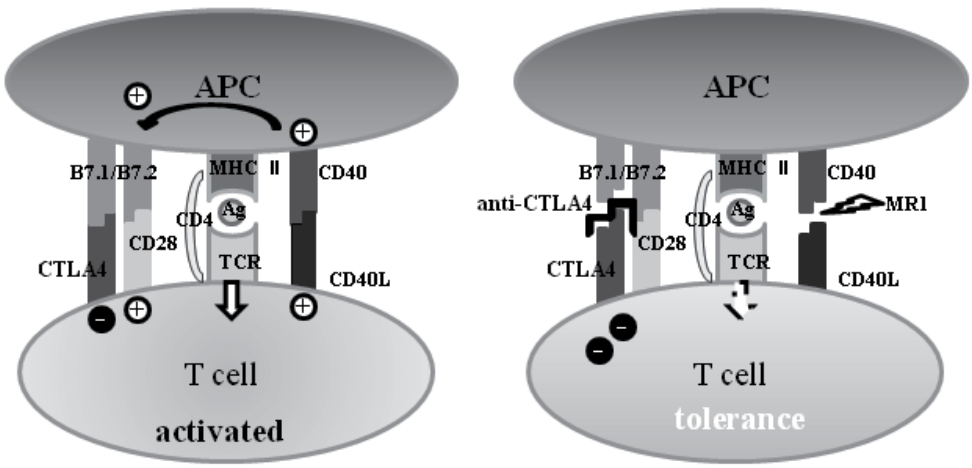

Fig. 1. Activation of naïve T cells requires co-stimulation. Binding of the peptide/MHC complex by the TCR and the CD4 or CD8 co-receptor transmits the first signal to the T cell. Activation of naïve T cells requires a second signal, namely, the ligation of B7 molecules (B7.1/B7.2) and CD28, which stimulates the clonal expansion of naïve T cells. Binding of CD40L by CD40 plays a central role effector function in the full differentiation of T cells. This ligation also activates APCs to express B7 molecules (left). Stimulation of CTLA4 with anti-CTLA4 Ab and/or Blocking CD40/CD40L by MR1 induces antigen-specific T cell tolerance (right).

Recently, Takeuchi et al. have shown that administration of MHC-mismatched donor bone marrow to mice receiving 3Gy TBI one day before BMT and a single injection of Hamster- anti-mouse CD40L monoclonal antibody (MR1, hybridoma) intraperitoneally (i.p.) with BMT permitted the induction of permanent mixed chimerism and tolerance without T cell depletion (Y. Takeuchi et al., 2004). This regimen is quite simple and less toxic than the alternatives, because 3Gy TBI is nonlethal and does not require MHC matching. Therefore we have adopted this regimen for treatment of autoimmune disease in systemic lupus erythematosus (SLE) model mice ("BXSB"mice) and investigated the effect of induction of fully MHC-mismatched bone marrow mixed chimerism (E. Takeuchi & Y. Takeuchi, 2007).

3. Treatment of autoimmune glomerulonephritis in BXSB lupus mice

3.1 BM mixed chimerism can be induced in BXSB mice

BXSB mice spontaneously develop autoimmune disease with features similar to human SLE. The disease is associated with auto-antibodies to self-antigens (Ags) including double strand (ds) DNA, single strand (ss) DNA, anti-platelet antibodies (Abs) and anti-erythrocyte Abs, with accompanying splenomegaly and lymphadenopathy. Immune complex-mediated nephropathy is the hallmark disease associated with the BXSB genotype. Histopathological changes are evident by 10 weeks of age, leading to end-stage renal disease and 70% mortality by 40 weeks of age. We sought to determine whether the simple regimen described above was also effective for the induction of long-term mixed chimerism in BXSB mice. Twenty million normal bone marrow cells from MHC-matched (B6/GFP: H-2^b) or -mismatched (BALB/c: H-2^d) donors were injected with 2.0mg MR1 (i.p.) to seven-week old BXSB mice (H-2^b) that had received a nonlethal dose of 4Gy TBI one day prior to BMT. We increased the TBI dose for BXSB mice from 3 to 4Gy because BXSB mice are more resistant to engraftment than normal recipients are. This regimen allowed the induction of multi-lineage mixed chimerism in 70-90% of host BXSB mice.

As shown in Table 1 and Fig.2, long-term stable chimerism was observed in MHC-mismatched chimeric mice. No clinical signs of GVHD were seen during the observation period.

	CD4 Tcell	CD8 Tcell	B cell	macrophage
20wks after BMT	73.9 ± 11.3	46.9 ± 10.8	51.0 ± 11.8	94.9 ± 4.02
40wks after BMT	78.1 ± 13.1	56.1 ± 17.6	68.6 ± 14.4	85.2 ± 7.75

Table 1. The percentage of donor cells among PBL of chimeric mice. (n=9)

To confirm the establishment of donor-specific tolerance, chimeric BXSB mice also received skin grafts from a BM donor and a third party (C3H/HeN, H-2^k) one day after BMT. All chimeric BXSB mice accepted the donor skin, but rejected the third-party skin grafts within 20 days (Fig.3), indicating that chimeric BXSB mice acquired donor-specific tolerance without immune-deficiencies (E. Takeuchi, 2011).

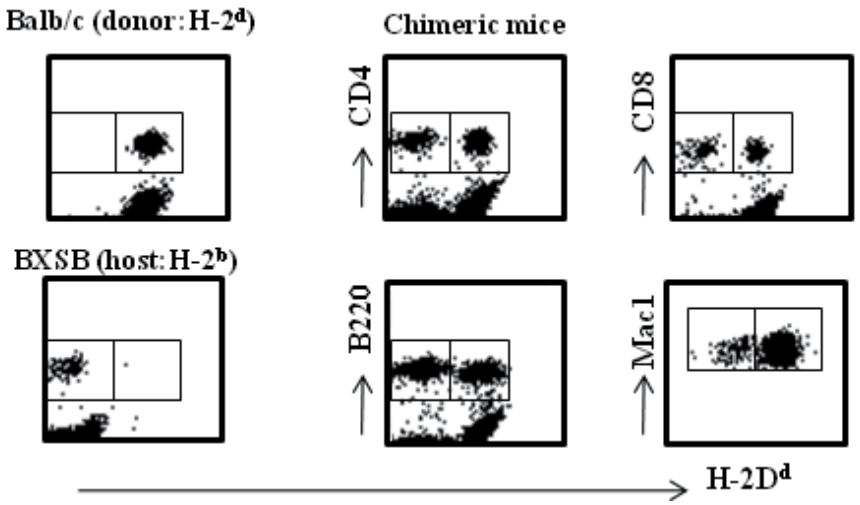

Fig. 2. An example of chimerism in peripheral blood lymphocytes (PBL).
The percentage of BALB/c (H-2D^d) donor cells present among PBL of various lineages was analyzed through two-color FACS. These data were obtained 24 weeks after BMT.

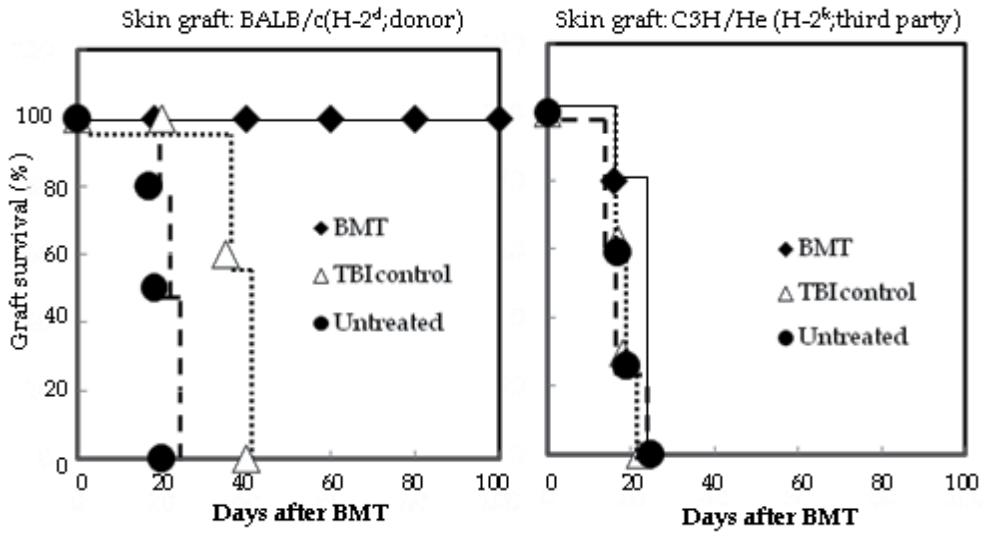

Fig. 3. Donor-specific skin graft tolerance. Donor and third-party skin was grafted 1 day post-BMT. Chimeric mice receiving MHC-mismatched BALB/c BM (◆BMT: n=9) accepted BALB/c skin grafts permanently, while third-party skin was rejected. Mice treated with TBI and anti-CD40L Ab (△: n=5) and mice receiving no treatment (●:n=5) rejected both donor and third-party skin.

These results indicate that, with regard to reciprocal tolerant between donor and host, T cells in stable mixed chimeric mice do not reject additional tissue grafts transplanted from the same donor. In lupus patients who suffer from renal disorders, and who are treated by means of kidney transplantation, the induction of specific immunologic tolerance to donor

antigens would prevent both chronic graft rejection and the side effects associated with chronic, nonspecific immunosuppressive therapy.

3.2 Induction of mixed chimerism suppressed lupus nephritis in BXSB mice

Even when transplanted kidneys are engrafted stably, when pre-existing lupus goes untreated, renal disorder will eventually recur. It is also known, however, that the induction of mixed chimerism reverses the autoimmune state. To evaluate the effect of inducing MHC-matched or MHC-mismatched mixed chimerism, individual kidneys were harvested from experimental mice and tissue sections were stained with periodic acid-Schiff (PAS) for histopathologic examination. None of the donor mouse strains were prone to autoimmune disease (Fig.4A). In both the fully MHC-matched and the fully MHC-mismatched chimeric mice groups, lupus glomerulonephritis was significantly ameliorated compared with that in untreated BXSB mice, as revealed by pathological analysis conducted more than 40 weeks after BMT (Figs.4D and E).

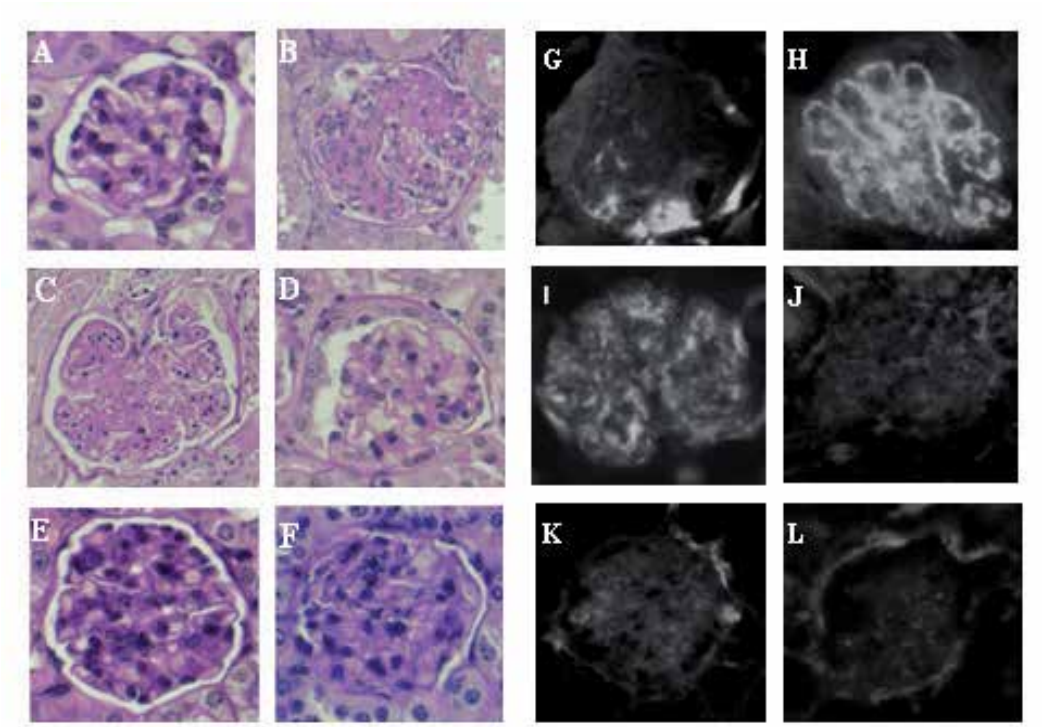

Fig. 4. Kidney sections stained with PAS (left panel) and IF with anti-mouse complement C3 (right panel). Kidneys were harvested from normal control mice or 47-50-week-old (40-43 weeks after BMT) BXSB mice with or without the indicated treatments. C57BL/6 control mice (A and G), untreated BXSB mice (B and H), irradiated BXSB mice (C and I), BALB/c BMT chimeric mice (D and J), GFP/B6 BMT chimeric mice (E and K), BALB/c×GFP/B6 F1 BMT chimeric mice (F and L).

Both untreated (Fig.4B) and irradiated BXSB mice (Fig.4C) exhibited severe glomerulonephritis with PAS-positive deposits. BXSB mice that had received TBI+MR1

exhibited histopathology similar to BXSB mice that had received only TBI (data not shown). For semiquantitative histologic analyses, more than 30 glomeruli from each kidney section were examined. Glomerulonephritis was scored on a scale of 0-4, based on the intensity and extent of histopathological changes [0; no glomerular lesions. 1; minimal thickening of the mesangium. 2; noticeable increase in both mesangeal and glomerular capillary cellularity. 3; same as 2 with the addition of superimposed inflammatory exudates and capsular adhesions. 4; obliteration of the glomerular architecture (>70% of glomeruli)]. Mean renal scores of both MHC-matched and –mismatched chimeric mice were significantly better than those of untreated or irradiated control BXSB mice (BALB/c BMT: 0.97±0.74, GFP/B6 BMT: 0.07±0.26 vs. untreated BXSB: 2.96±0.72, TBI: 1.85±0.77, TBI+MR1: 2.47±3.4, >30 glomeruli from each kidney section in three mice of each group were evaluated. $p<0.05$). We also evaluated immune-complex mediated glomerulonephritis through immunofluorescence (IF) staining with anti-mouse complement C3 in 30 glomeruli per renal section (Fig.4. right panels). Untreated mice and irradiated mice exhibited the peripheral loop pattern (Figs. 4H and I), while almost all glomeruli in the sections from chimeric mice showed negative staining (Figs. 4J and K). Because the cause of death in BXSB mice is most often renal failure, the improvement of their glomerulonephritis may have contributed to the prolongation of their life-spans. These results indicate that the induction of mixed chimerism significantly inhibited the development of lupus-like disease.

It should be noted, however, that the induction of mixed chimerism in BXSB mice could not completely eliminate auto-reactive host lymphocytes because our regimen retains certain stem cells and lymphocytes belonging to the host. This naturally leads to the question of how donor cells reverse the host autoimmune state, which is discussed in the next section.

4. How does BM chimerism reverse the autoimmune state?

4.1 Hypothesis

We and several other groups have shown that the induction of bone marrow mixed chimerism is an effective treatment for and/or means of prevention against the development of autoimmune disease. Previous studies have debated the mechanisms that may be responsible for the reversal of the autoimmune state in BM chimerism, but the mechanism of the exclusion of self-reactive lymphocytes has not yet been conclusively identified. Preceding studies have argued about the mechanisms underlying the reversal of the autoimmune state in BM chimerism. In several studies which reversed destructive autoimmune type I diabetes (NOD mice with induced mixed chimerism), the suppression of autoimmune disease was attributed to reciprocal clonal deletion or to anergy induction of T lymphocytes of recipient and donor origin (Mathieu et al., 1997. Nikolic et al., 2004). Other mechanisms have also been proposed, including induction of peripheral anergy, a change in the Th1/Th2 profile, correction of abnormal secretion of cytokines and positive selection of regulatory T cells in the thymus. Among proposed hypotheses, we have focused on the role of cognate TCR/MHC interactions in the pathogenesis of autoimmune disease in BXSB mice (E. Takeuchi et al., 2011)

4.2 The induction of MHC-mismatched chimerism does not suppress anti-DNA Abs

During the development of their lupus-like autoimmune disease, BXSB mice are known to produce auto-antibodies to self-antigens including dsDNA. We measured serum anti-

dsDNA antibody (anti-DNA Ab) levels by means of ELISA to evaluate whether auto-reactive Abs were eliminated by the induction of mixed chimerism. Actually, the anti-DNA Ab levels in both MHC-matched and MHC-mismatched chimeric mice were lower than those in untreated or irradiated BXSB mice. Meanwhile, anti-DNA Ab levels in fully MHC-matched mixed chimeric mice (GFP/B6 BMT) were not statistically different from those in normal control B6 mice, but those in fully MHC-mismatched mixed chimeric mice (BALB/c BMT) were significantly higher than those in normal controls. This tendency was even more pronounced when anti-DNA IgM levels in the above groups were compared. There were no significant differences in anti-DNA IgM levels between MHC-mismatched chimeric mice and untreated or TBI+MR1 mice, even though total anti-DNA levels were much lower in chimeric mice than in other groups.

These data indicated that anti-DNA Ab producing cells were still present in the BXSB chimeric mice, though they stopped switching iso-types from IgM to IgG in MHC-mismatched chimera. Only in MHC-matched chimeric mice could the expansion of anti-DNA Ab production be suppressed down to a normal level; the induction of fully MHC-mismatched chimerism did not completely suppress or eliminate anti-DNA-producing B cells.

4.3 Selective suppression of auto-reactive antibodies in chimeric mice

In order to distinguish the contributions of donor-type and host-type B cells to anti-dsDNA antibody production, we determined IgM allotypes [IgMa: BALB/c (donor), IgMb: BXSB (host)] in the serum of fully MHC-mismatched (BALB/c→BXSB) chimeric mice 20 weeks after BMT. At this point, the percentage of allogeneic donor B cells in the chimeric mice was 51.0±11.8%, indicating that allogeneic donor and host B cells had contributed equally to the immune response. Surprisingly, however, we found that the majority of the anti-DNA IgM was IgMa (allogeneic donor-type), whereas IgMb (host-type) anti-DNA antibody production was suppressed (Fig. 5A).

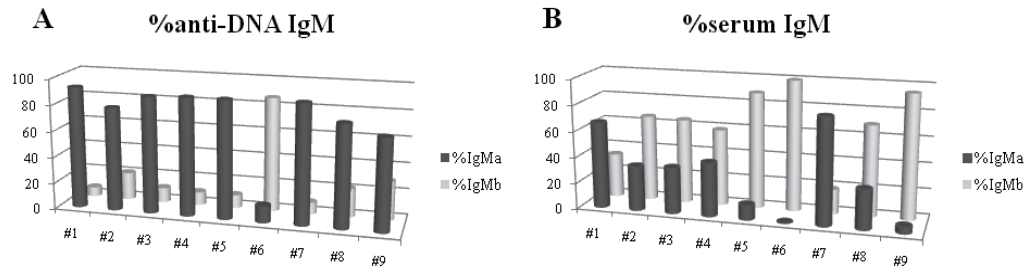

Fig. 5. The ratio of host-type IgM to donor-type IgM. (A) Serum anti-DNA IgM in BALB/c BMT BXSB was measured in each sample by means of ELISA. The percentages of anti-DNA IgMa (donor-type IgM: dark bar) and IgMb (host-type IgM: light bar) add up to the total anti-DNA IgM. (B) Total IgM in each BALB/c BM- transplanted BXSB mouse was measured individually. The percentages of serum IgMa (donor-type IgM: dark bar) and IgMb (host-type IgM: light bar) add up to the total IgM.

The majority of the total serum IgM, on the other hand, was host-type IgMb (Fig. 5B), suggesting that the production of "normal" serum Ig is dependent on host MHC-restricted T cells. Total serum IgM levels in fully MHC-mismatched chimeric mice were not significantly

different from those in other groups (data not shown). These results indicated that normal B cells derived from donor BALB/c mice, rather than genetically lupus-prone host-type B cells, were responsible for anti-DNA antibody production in these chimeric BXSB mice. Thus the regulation of auto-antibody production appears to be under MHC-restriction of the host type. Additionally, to confirm which set of B cells (donor-type or host-type) could react with foreign antigens, sheep red blood cells (SRBC) were administered intraperitoneally to five BALB/c chimeric BXSB mice. Three days after immunization, serum anti-SRBC Ab was detected through flow cytometry. All chimeric mice produced antibodies that were reactive with SRBC. As expected, almost all of the detected anti-SRBC Ab was IgMb (host-type), not IgMa (allogeneic donor-type) (data not shown). In mixed chimeras, all peripheral T cells are supposed to be restricted to host MHC, because of positive selection in the host thymus. T cells in fully MHC-mismatched chimeric mice should therefore be capable of cognate interaction with host-type B cells but not with donor-type B cells. Accordingly, only host-type B cells were activated by antigens through cognate interaction with helper T cells. Donor-type B cells remained "silent" because they could not interact properly with T cells. Interestingly, however, our results indicated the possibility that not only "proper" activation against foreign antigens, but also suppression of auto-reactive antibody production were regulated through TCR/MHC cognate interactions.

4.4 Do T cells survey auto-reactive antibody production?

Based on these data, we drew the following conclusions: 1. Allogeneic BM chimerism ameliorates autoimmune disease, but fully MHC-mismatched chimerism fails to suppress the production of anti-DNA antibodies. 2. In MHC-mismatched mixed chimeras, anti-DNA antibodies are produced by donor-type B cells rather than host-type B cells. 3. In MHC-mismatched chimera, TCRs are restricted to host-type MHC. Accordingly, T cells can recognize only host B cells but not donor B cells. To tie theses conclusions together, we propose a possible T cell surveillance system of mixed chimerism, as depicted in Fig.6.

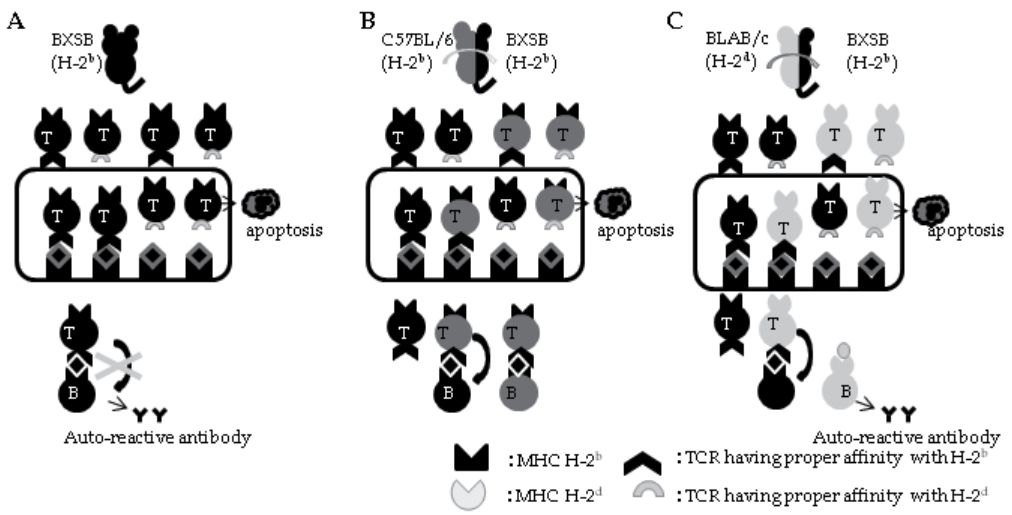

Fig. 6. The proposed of T cell surveillance model.

T cell precursors derived from BM differentiate to mature T cells in the host thymus. Through a process known as positive selection, all T cell populations can interact with self-MHC with proper affinity. Since these processes are performed by MHC molecules expressed on thymic epithelial cells, the TCR repertoire in mixed chimera is restricted to host MHC. We and others speculate that under genetically normal conditions, T cell-mediated trimming of autoantibody production may occur through cognate interactions between TCR and MHC+peptide presented on B cells (Rathmell et al., 1995, Shinohara et al., 1997). In the case of the BXSB mouse, it is known that T cells have certain defects which might play an important role in the pathogenesis of autoimmunity (Wofsy, 1986). We also speculate that the pivotal defect of BXSB may be a genetic defect in this surveillance function of T cells (Fig.6A). In the case of fully MHC-matched chimerism, T cells derived from donor BM can take this place of defective host T cells. Auto-reactive B cells derived from both donor and host BM can be regulated or trimmed by donor T cells through TCR/MHC interactions (Fig.6B). In MHC-mismatched chimerism, immature T cells are positively selected on the basis of their weak reactivity with self-peptides presented exclusively on H-2^b MHC molecules, since thymic epithelial cells express only host MHC. Therefore, in the periphery, all T cells recognize antigens presented by APCs on H-2^b MHC molecules (BXSB: host type MHC). Yet, B cells expressing H-2^d MHC (BALB/c: donor type MHC) are still generated from donor BM stem cells as this process is genetically determined. T cells developing in the BXSB thymus should be incapable of cognate interactions with these "wrong" MHC molecules expressed on donor B cells. We speculate that the failure of cognate interaction with T cells might be the reason why auto-reactive antibody levels rose in MHC fully-mismatched chimeric mice (Fig.6C).

The present study did not address the question of how anti-self B cells were initially triggered. We also induced fully MHC-mismatched BM chimerism in normal B6 mice (BALB/c→B6) as opposed to lupus BXSB mice. The anti-DNA Ab levels seen in BALB/c→B6 chimeric mice were slightly higher than those in normal B6 mice, but, much lower than those seen in BALB/c→BXSB chimeric mice (data not shown). This means that the induction of fully MHC-mismatched chimerism in normal mice may carry a risk of autoantibody production, though uncertain factors in host BXSB mice drives a non-physiological priming of B cells.

4.5 BMT from haplo-identical donor effectively suppressed auto-antibody production

If there is indeed a host-type MHC restriction in the suppression of auto-reactive antibody production, BMT from a donor with partially identical MHC that is sufficient to maintain cognate interaction should be equally as effective as BMT from a fully MHC-matched GFP/B6 mouse. To test this hypothesis, BM cells taken from BALB/c (H-2^d) × GFP/B6 (H-2^b) F1 mice with haplo-identical MHC (H-$2^{b/d}$) were transplanted to BXSB (H-2^b) mice. In this case, all B cells, even those differentiated from donor BM, contained at least one H-2^b allele. As shown in Figs.4F and L, lupus glomerulonephritis in F1 chimeric mice was alleviated to a degree comparable to that seen in MHC-matched GFP/B6 chimeric mice. Serum anti-DNA Ab in F1 chimeric mouse group was decreased to level comparable to that seen in fully MHC-matched GFP/B6 chimeric mice (data not shown).

The survival rates in both the GFP/B6 chimeric mouse group and the F1 chimeric mouse group, which were higher than that in the BALB/c chimeric mouse group, indicated that BMT from F1 mice is also effective as a treatment for lupus-like disease in BXSB mice (B6

BMT: 100%, F1 BMT: 80%, vs BALB/c BMT: 70%, TBI+MR1: 60%, TBI: 20%, untreated: 20% survival, 50weeks after BMT). These results suggest that the maintenance of TCR/MHC cognate interaction with all B cells is important in regulating auto-reactive Ab production, and that reconstitution of the T cell surveillance system may reverse the autoimmune state of lupus-like disease in the BXSB mouse. Moreover, our results indicate one possibility for clinical application: BMT between parent and child, both parent→child and child→parent, may be able to reverse the autoimmune state of SLE effectively.

5. Clinical application and unknown risks of mixed chimerism

This paper has demonstrated that the maintenance of TCR/MHC interaction with all B cells is important in regulating auto-reactive Ab production. However, we and several other groups have reported that the induction of fully MHC-mismatched chimerism is certainly effective as a treatment for autoimmunity. How does the induction of fully MHC-mismatched mixed chimerism suppress autoimmune disease? One answer to this question is demonstrated by the results of an immunohistochemical experiment in which we staind for several isotypes of immunoglobulin.

As depicted in Table 2, linear staining patterns with IgG and/or IgM were definitely observed on the glomeruli of kidney sections taken from MHC-mismatched chimeric mice; the same sections were negative for C3 depositions, however. Linear staining with both IgG and C3 was observed on the glomeruli taken from untreated mice.

		C3	IgG				IgM
treatment				IgG1	IgG2a	IgG3	
Untreated	1	+	-	-	-	-	-
	2	+	M	-	-	M	M
	3	+	-	-	M	-	M
	4	+	+	-	-	+	+
	5	+	+	+	M	-	+
BALB/c BMT	1	M	+	-	-	-	+
	2	-	-	-	-	-	+
	3	-	-	-	-	-	
	4	-	+	-	-	-	+
	5	-	-	-	-	-	+

+: linear staining, -: negative staining M: mesangeal staining

Table 2. Immunofluorescence staining with several isotype Ig of glomeruli.

This indicates that antibodies deposited in fully MHC-mismatched chimeric mice, mainly IgM, did not activate the complements effectively. As a result, lupus glomerulonephritis is milder in chimeric mice than in untreated mice. We presume that class-switching from IgM to other isotypes does not occur on donor anti-dsDNA IgM-producing B cells, since TCR/MHC cognate interactions were disrupted.

Given that T cells could neither activate nor suppress B cells, B cells are expected to be inactive or "silent". In fact, we have confirmed that, when fully MHC-mismatched

chimeric mice are immunized with foreign antigen (sheep red blood cells, SRBC), antigen-specific antibodies (anti-SRBC IgM) are mainly produced by host B cells (as mentioned in 4.3). In rare cases, however, especially when immunization with the same antigen is repeated several times, donor B cells accidentally respond and produce specific antibodies. A T cell that is specific for one peptide on an MHC molecule may cross-react with peptides presented by other allogeneic MHC molecules. By these accidental interactions, donor B cells are activated and start to produce specific antibodies. During activation and proliferation, B cells undergo variable-region somatic hypermutation and change their antigen affinity, resulting in the generation of variant immunoglobulins, some of which are thought to bind to the original foreign antigen with higher affinity. However, the potential disadvantage of this process is that some of the antibodies could be auto-reactive.

To test this hypothesis, we induced an auto-cross-reactive antibody (Ab) by introducing a foreign antigen with a homolog to an auto-antigen into a BXSB lupus mouse strain of mixed chimerism with several combinations of donor BM. The titer of auto-cross reactive foreign Ab plateaued at low levels in normal mice and MHC-matched/haplo-identical chimeric mice, but rose higher in BXSB and fully MHC-mismatched chimeric mice (unpublished data). These results indicate that the induction of fully MHC-mismatched chimerism may carry a risk of secondary auto-antibody production.

Under normal conditions, these auto-reactive B cells may be anergic. In lupus patients, however, non-physiological factors may prime auto-reactive B cells. For example, it has been reported that circulating B cell activating factor (BAFF) is elevated in the serum of human patients with lupus, and that the overexpression of BAFF in mice promotes TLR-induced production of auto-antibodies through a T cell independent process (Groom et al., 2007). Under autoimmune conditions, normal B cell may have the potential to run off the rails. The maintenance of TCR/MHC interaction may be a "rein" by which immune-response is controlled in chimeras.

Because several mechanisms have been suggested as drivers of autoimmune disease, further study is necessary to identify each mechanism's role. Nevertheless, our results showing the specific suppression of auto-reactive antibody production suggest the existence of a surveillance system that trims auto-reactive B cells after priming. The reconstitution of this surveillance system through the induction of BM mixed chimerism can be an effective treatment for lupus disease. Moreover, the induction of BM mixed chimerism with haplo-identical donor BM, which maintains cognate T/B interaction with both donor and host cells, can be equally effective as fully MHC-matched donor BM. These results may support the clinical application of BMT as a treatment for lupus disease.

6. Conclusions

Induction of BM mixed chimerism can be useful for treatment of refractory lupus glomerulonephritis. Elucidation the mechanism through which mixed chimerism reverses the autoimmune state is necessary for clinical application.

In this paper, we suggested the existence of T cell surveillance system through TCR/MHC interaction. Through TCR/MHC interaction, T cell-mediated trimming of auto-antibody production may occur under normal condition. The induction of bone marrow mixed chimerism may reverse the auto-immune state through reconstruction of the T cell

surveillance system and the maintenance of TCR/MHC interaction with all B cells is important in regulating auto-antibody production.

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17

Differential Diagnosis of the Pulmonary-Renal Syndrome

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1. Introduction

The pulmonary-renal syndrome involves the combination of diffuse alveolar hemorrhage and a rapid progressive glomerulonephritis (RPGN). It is usually a systemic vasculitis that can led through a vast vasculitic process to life-threatening injury to the involved organs lung and kidney [Niles 1996, Salant 1987, Boyce 1986, Gallagher 2002 & De Groot 2005].
In the differential diagnosis other diseases of acute renal and lung injury with alveolar hemorrhage without RPGN have to be discussed.

2. Pulmonary-renal syndrome

The *diffuse alveolar hemorrhage* is defined by the triad of hemoptysis, diffuse alveolar infiltrates and low hematocrit. However, the clinical presentation is variable (slight cough, progressive dyspnea, manifest hemoptysis) and these symptoms don`t have to occur simultaneously [Hauber 2007]. Slowly protracted courses through to fulminant organ failure are described.
A *rapid-progressive glomerulonephritis (RPGN)* is manifested by a rapidly progressive renal function loss (a few days to few weeks) and the presence of a nephritic sediment with deformed erythrocytes of a glomerular origin and possibly red cell casts.

2.1 Pathophysiology

The underlying cause of a pulmonary-renal syndrome is usually a systemic vasculitis of the small pulmonary and renal vessels. These vasculitides have a heterogeneous pathogenesis - there are three different pathophysiological mechanisms of injury [Niles 1996, Salant 1987 & De Groot 2005]:

1. mediated by anti-neutrophil-cytoplasmic antibodies (ANCA),
2. immune-complex mediated vasculitis of small vessels or
3. by antibodies against the glomerular basement membrane (Goodpasture Syndrome).

In the kidney a *RPGN* is caused by damage of the capillaries and basal membranes with leakage of erythrocytes, followed by an influx of macrophages, fibrinogen and the formation of extracapillary cell proliferation (so called crescents) [Salant 1987].
In the lungs, a *diffuse alveolar hemorrhage* is caused by a pulmonary capillaritis [Hauber 2007].
In the case of ANCA-associated systemic vasculitis the detection of ANCA is possible in ~ 80% of patients. Besides the correlation of ANCA titers with disease activity, there is

evidence of a pathogenetic role of ANCA. Myeloperoxidase (MPO) and proteinase 3 (Pr3) are detected in the cytoplasm of non-stimulated neutrophils. It is assumed that cytokines (e.g. TNF, interleukins) raise the expression of Pr3 and MPO on the cell surface of granulocytes and thus a reaction of these antigens with ANCA is possible. This process leads to activation of granulocytes and release of adhesion molecules to the interaction of leukocytes with vascular endothelial cells. Finally cell necrosis and apoptosis contribute to vascular inflammation process [Bosch 2006].

2.2 Diagnosis

2.2.1 Basic steps

As with any systemic vasculitis the diagnosis of pulmonary-renal syndrome is made in three steps:

1. *Adequate evaluation and networking* of existing and past patient's symptoms.
2. *Establishing the diagnosis* by laboratory, technical and biopsy examinations.
3. Differential diagnosis of vasculitis.

2.2.2 Imaging

The value of imaging refers to the extent of pulmonary capillaritis resulting in *diffuse alveolar hemorrhage*: in a conventional X-ray or in a computer tomography of the chest confluent or mixed interstitial-alveolar infiltrates are found (Fig. 1).

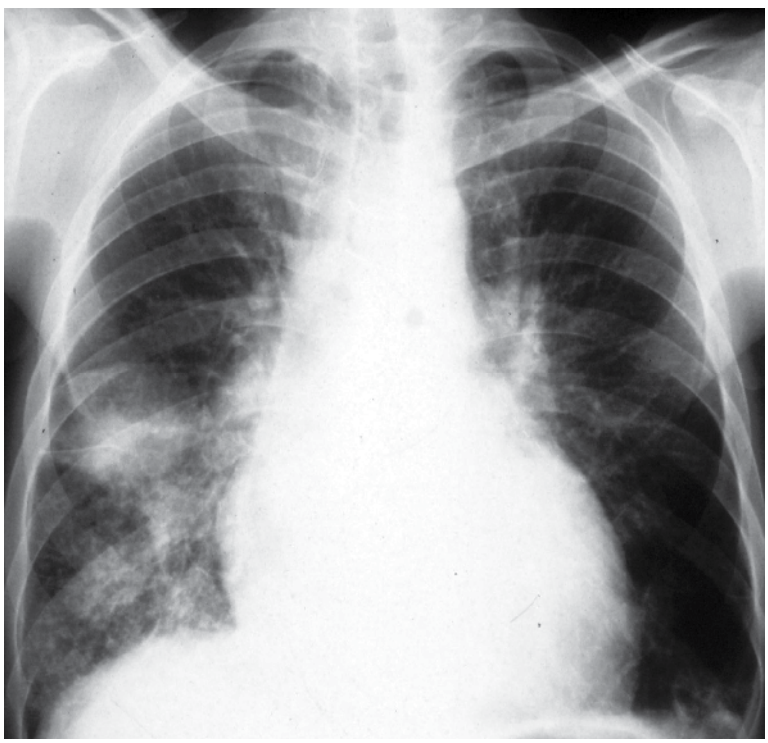


Fig. 1. Diffuse alveolar hemorrhage in chest x-ray in Wegener's granulomatosis.

In a RPGN sonographically enlarged kidneys presents with a wide parenchym area.

2.2.3 Serology

Antibodies against the glomerular basement membrane (GBM) can be found typically in the rare Goodpasture's syndrome (Tab. 1).

The above briefly described heterogeneous pathogenesis of small vessels vasculitis results in the immunological classification considering serological / immunological parameters such as anti-neutrophil-cytoplasmic antibodies (ANCA) by immunfluorescence-optical findings (perinuclear or cytoplasmtic fluorescence) or ELISA against the target antigen proteinase 3 or myeloperoxidase (Tab. 1) [Bosch 2006]. In addition, the eosinophils, IgE and the extended autoimmunserology: anti-nuclear factor (ANA), anti-ds-DNA, C3, C4 and cryoglobulins can be determined.

Disease	Proteinase-3-Antibody	Myeloperoxidase (MPO-)-Antibody	ANCA negative	Anti-GBM-Ab
Wegener`s Granulomatosis	70 %	20 %	10 %	<10%
Microscopic Polyangiitis	30 %	60 %	10 %	<10%
Churg-Strauss-Syndrome	10 %	60 %	30 %	<10%
Goodpasture Syndrome	<10%	<30%	70%	95%

Table 1. ANCA-Sensitivity.

The rapid availability of these antibodies has improved the time to establish an early diagnosis, which is prognostically relevant [Saxena 1995].

2.2.4 Renal biopsy

The diagnosis of RPGN is done by renal biopsy: in light microscopy there is a glomerulonephritis with crescent formation in the Bowman's capsule compartment (extracapillary proliferation) in more than 50% of the glomeruli. The further work is carried out by immunohistochemistry and electron microscopy.

In immunohistology, the type of immunoglobulins and the deposition pattern (capillary, mesangial, granular, linear along the glomerular basement membrane) differ. Only in Goodpasture syndrome, linear deposits are found along the glomerular basement membrane. In case of an ANCA triggered form immune deposits are missing (pauci-immune RPGN). In contrast, in immune-complex vaculitis there can be found a different picture, usually with granular deposition of IgG, IgM, IgA or complement.

2.2.5 Bronchoscopy

The diagnosis of *diffuse alveolar hemorrhage* includes the clinical picture and a brochoscopy with a bronchoalveolar lavage and the microscopic detection of siderophages. Especially in the case of diffuse infiltrates in imaging without hemopytsis a bronchoscopy can be helpful and a definite diagnosis can be established [Hauber 2007].

3. Differential diagnosis of the pulmonary-renal syndrome

As already stated the pulmonary-renal syndrome is usally caused by a systemic small vessels vasculitis (Tab. 2), these can be categorized [Niles 1996, Salant 1987, De Groot 2005, Jennette 1994 & Falk 1997]:

- morphological criteria (size of the infesting vessels, presence or absence of granulomas),
- etiological criteria (idiopathic or secondary forms) and
- immunological criteria (ANCA-associated vasculitis, immune-complex vasculitis or caused by anti-basement antibodies).

3.1 ANCA-associated small vessel vasculitis

The Chapel Hill Consensus Conference classification defines [Jennette 1994]:

1. Wegener's granulomatosis,
2. microscopic polyangiitis and
3. Churg-Strauss syndrome.

Renal involvement is present in many systemic diseases, especially in the small vessel vasculitis – pointed out by Gallo in the New England Journal of Medicine: "The kidney is often a window on systemic disease" [Gallo 1991].

The suspicion of a pulmonary-renal syndrome in an ANCA-associated systemic vasculitis can often be taken from a careful history and thorough clinical examination with detection of other vasculitic signs (eye inflammation, intractable rhinitis / sinusitis, skin rashes, arthralgia, myalgia or polyneuropathy) (Fig. 2).

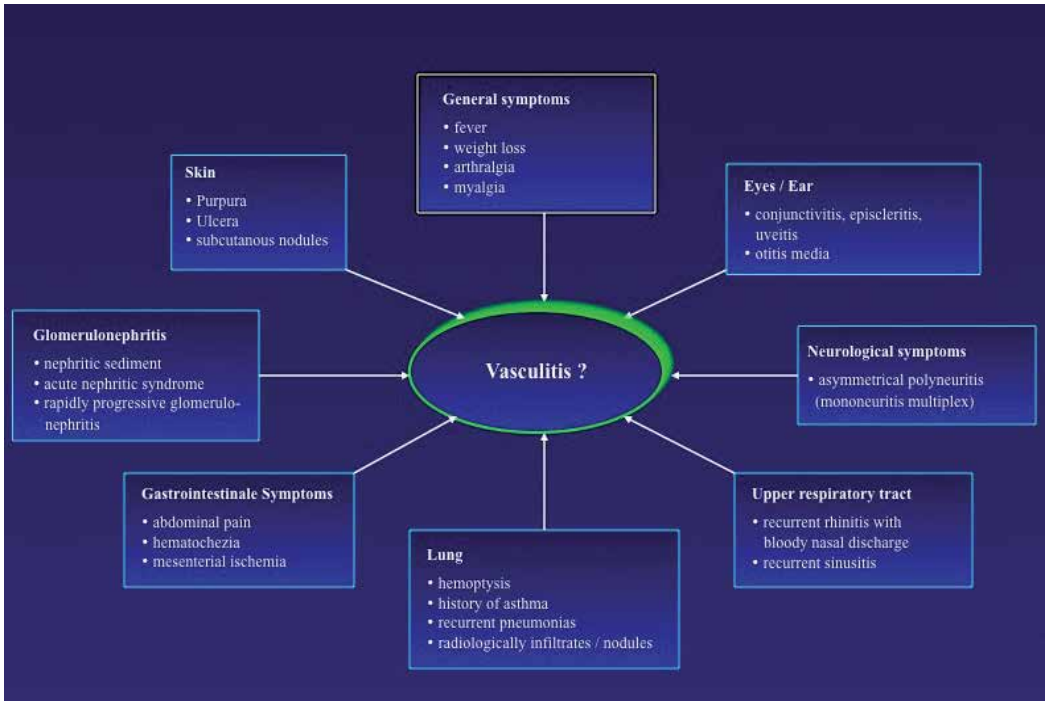


Fig. 2. General symptoms and signs of organ involvement in systemic small vessels vasculitis.

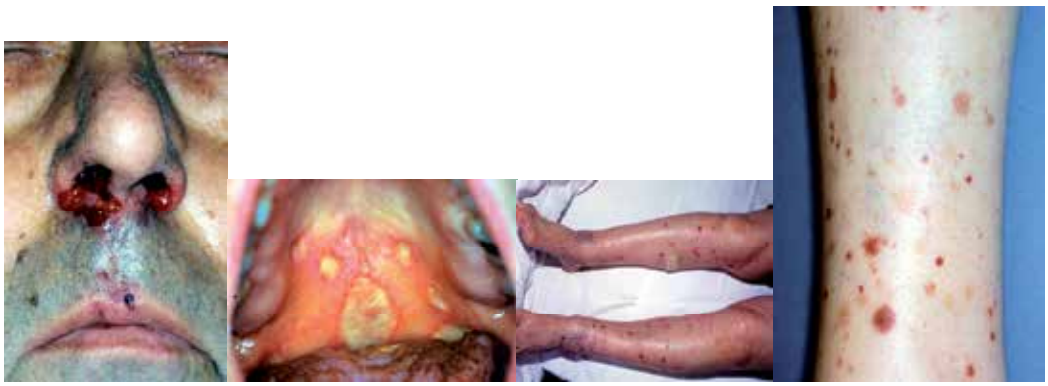
	ANCA-vasculitis				
	WG	**MP**	**CSS**	**GP**	**SLE**
Clinical presentation					
Vasculitic general symptoms	+	+	+	-	+
Granulomatous inflammation	+	-	+	-	-
Eye involvement	+	(+)	(+)	-	(+)
Recurrent asthma bronchiale	-	-	+	-	-
Pulmonary-renal syndrome possible	+	+	(+)	+	(+)
Skin (purpura, necrosis)	+	+	+	-	+
Gastrointestinal symptoms	(+)	(+)	(+)	-	(+)
	WG	**MP**	**CSS**	**GP**	**SLE**
Laboratory workup					
ANCA	**80 - 90 %**	**80 - 90 %**	**50 - 70 %**		
Pr3-antibody	**~ 70 %**	**~ 30 %**	**~ 10 %**	**< 30 %**	**-**
MPO-antibody	**~ 20 %**	**~ 60 %**	**~ 60 %**		
Eosinophilia	-	-	+	-	-
Reduced complement levels	-	-	-	-	+
Anti-ds-DNA	-	-	-	-	+
Anti-GBM-Ab	-	-	-	**95%**	-
Histology/Immunohistology					
Leucocytoclastic vasculitis	+	+	+	-	+
Granulomatous inflammation	+	-	+	-	-
Eosinophil granulomatous inflammation	-	-	+	-	-
Kidney Biopsy					
Light microscopy	**necrotising intra- and extracapillary proliferative GN**			**necrotising intra- and extracapillary proliferative GN**	**Lupus-nephritis**
Immunohistology	**pauci-immune GN without immune-complex deposits**			**linear IgG-deposits in the glomerular basement membrane**	**granular deposits of IgG, IgM, IgA and complement factors**

WG = Wegener's Granulomatosis. MP = microscopic polyangiitis. CSS = Churg-Strauss syndrome. GP = Goodpasture's Syndrome SLE = systemic lupus erythematosus. ANCA = antineutrophil cytoplasmic antibodies. PR-3 = proteinase-3. MPO = myeloperoxidase. IC = immune complex. GBM = Glomerular basement membrane. ds = double strand.

Table 2. The most important differential diagnoses of pulmonary-renal syndrome with clinical features, laboratory and histological findings.

3.1.1 Wegener's granulomatosis

Wegener's granulomatosis is a necrotizing vasculitis of the small and medium-sized vessels, associated with granulomas inflammation of the upper and lower respiratory tract and the frequent finding of glomerulonephritis. In active disease in about 90% of cases c-ANCA are directed against proteinase 3 (Tab. 1 and 2). Figure 3 shows the predominant symptoms in image and Figure 4 in number (at onset and during disease) [Hoffmann 1992].



a bloody rhinitis b aphthae c / d vasculitic skin involvement

Fig. 3. Wegener's granulomatosis.

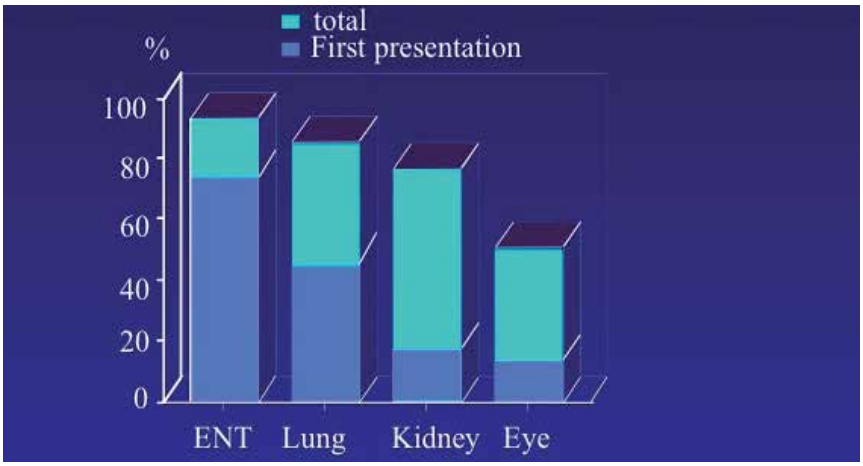


Fig. 4. Organ involvement in Wegener's granulomatosis.

3.1.2 Microscopic polyangiitis

Microscopic polyangiitis is characterized by a necrotizing vasculitis of small vessels with minimal or missing immune deposits and an inflammation of the pulmonary capillaries. Typically, there are p-ANCA directed against myeloperoxidase (Tab. 1 and 2) [Jennette 1994 & Falk 1997]. Wegener`s granulomatosis and Microscopic polyangiitis shows comparable organ involvement, but the symptoms of the upper respiratory tract are usally milder in Microscopic polyangiitis, because there is no granulomatous inflammation. Compared to Wegener's granulomatosis disease recurrence is rare in patients with Microscopic polyangiitis.

3.1.3 Churg-Strauss syndrome

The Churg-Strauss syndrome is characterized by recurrent asthma attacks and allergic rhinitis, an intermittently or permanently detectable eosinophilia (> 1500/mm^3) and necrotizing granulomas and / or necrotizing arteritis with a Wegner's granulomatosis-like presentation. The serological diagnosis is less clear: c-ANCA or anti-PR3-Ab detected only rarely, p-ANCA and anti-MPO-Ab can be detected in up to 60% of cases (Tab. 1 and 2) [Hoffmann 1992].
The Churg-Strauss syndrome can be distinguished clinically (asthma attacks, eosinophilia) from Wegener`s granulomatosis or Microscopic polyangiits. Renal involvement is seen in approximately 25% of patients.

3.2 Goodpasture syndrome

The Goodpasture's syndrome is a rare disease with an incidence of 0.1-1 per million per year and affects mainly caucasian males. It is characterized by hemoptysis and / or radiological evidence of pulmonary infiltrates and a RPGN, which usually develops after hemoptysis. There is the very reliable detection of anti-GBM antibody, the identified antigen is the C-terminal end of the alpha-3-chain of type IV collagen. The immunohistological workup of the renal biopsy shows linear IgG deposits in the glomerular basement membranes (Tab. 1 and 2) [Goodpasture 1919, Salama 2001, Pusey 2003 & Hudson 2003].
Compared to the ANCA-associated small vessel vasculitidies (Wegener`s Granulomatosis, Microscopic polyangiitis and Churg-Strauss syndrome) there are no other, general vasculitic symptoms in patients with Goodpasture syndrome.

3.3 Immune-complex vasculitis of small vessels

Immune-complex vasculitidies like
1. systemic lupus erythematosus,
2. cryoglobulinemic vasculitis and
3. Purpura Schoenlein-Hennoch [Markus 1989]

are important differential diagnostic considerations of the pulmonary-renal syndrome.
They can be distinguished by the previously described extended-serological diagnostic workup.

3.4 Further differential diagnoses

If this diagnostic workup is unremarkable, there are several more differential diagnosis:
1. antiphospholipid syndrome with vasculitis and / or pulmonary embolism
2. mixed connective tissue diseases (systemic sclerosis, polymyositis)
3. thrombotic thrombozypenic purpura.
4. infectious diseases involving kidney and lung (e.g Hanta-virus, cytomegalie-virus, Legionella, Mycoplasma, Leptospirosis, tuberculosis, sepsis)

Moreover, a primary renal disease can lead to a pulmonary disease and mimic the image of a pulmonary-renal syndrome:
1. acute renal failure with pulmonary edema and uremic hemoptysis
2. thromboembolism in nephrotic syndrome: renal vein thrombosis and/ or pulmonary embolism
3. immunosuppression in renal disease and a pneumonia.

Conversely, a primary pulmonary disease can lead to renal disease and can mimic the image of pulmonary-renal syndrome:

1. Infection of the respiratory tract with prerenal renal failure and / or postinfectious glomerulonephritis or hematuria in IgA nephropathy
2. Lung cancer with immune-complex nephritis.

4. Conclusions

Since early treatment with the mentioned diseases is critical, the diagnosis has to be established quickly - the fast antibody-diagnostic and diagnostic imaging have a central role.

5. Acknowledgment

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18

RPGN - Clinical Features, Treatment and Prognosis

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1. Introduction

Rapidly progressive glomerulonephritis (RPGN) is one of the nephrology emergencies which needs special attention. RPGN is a clinical description which determines by symptoms and signs of glomerulonephritis (GN); edema, hypertension and gross hematuria, and evidence of acute renal failure(severe decrease in glomerular filtration rate presents as oliguria or anuria, and increased serum levels of BUN and creatinine). Definite diagnosis of the disorder is based on kidney biopsy's findings. Early diagnosis and appropriate treatment plays a critical role in renal saving and preventing permanent glomerular damage.

This chapter will focus on clinical manifestations, therapeutic protocols and prognostic factors in patients with different subtypes of RPGN.

Rapidly progressive glomerulonephritis (RPGN) is defined as a syndrome with abrupt or insidious onset of hematuria, proteinuria, anemia, and rapidly progressing acute renal failure(ARF), and special findings on light microscopy examination of kidney biopsy's specimen; crescentic lesions which usually involved most glomerular architectures (Hirayama, et al., 2008; Rutgersetal., 2004). It also characterized by rapid loss of renal function (GFR<50% within 3 months)with histological findings of crescent lesions which usually involves>50 % of glomeruli (Couser, 1988).

RPGN can be primary or secondary. Secondary forms occur in any form of severe glomerulonephritis including membranoproliferative GN, IgA nephropathy, post infectious GN, and systemic lupus erythematous (SLE). Primary RPGN is an autoimmune disease which is divided into three immunopathologic categories:(Rutgerset al., 2004; Haas .& Eustace, 2004):

Type 1 RPGN: glomerulonephritis with antibodies directed against the glomerular basement membrane (GBM) (anti-GBM mediated GN)

Type II RPGN: immune-complex induced glomerulonephritis

Type III RPGN: Antineutrophil cytoplasmic antibody associated glomerulonephritis (ANCA-associated glomerulonephritis or pauci-immune GN)

RPGN type 1 and 2 are responsible for 10-20 % and 40 % of all cases respectively. **RPGN type 2** can be found in different forms of systemic disease such as post infectious GN(PSGN), Ig-A nephropathy, Henoch–Schönlein purpura (HSP), SLE, membranous GN(MGN) or membrano- proliferative GN(MPGN). A few cases of idiopathic immune-complex-mediated RPGN have been reported (Jindal, 1999).

Interestingly different forms of RPGN share similar clinical features including hematuria, proteinuria, edema, hypertension and symptoms of ARF .patients with Anti –GBM anti - body or pauci-immune RPGN (ANCA-associated vasculitis) may have pulmonary hemorrhage and hemoptysis (Jindal, 1999). In pauci-immune RPGN the initial symptoms are non-specific; often fatigue, fever, night sweats and artheralgias are first clinical manifestations (Jindal, 1999).

2. Histopathology characteristics of RPGN and laboratory findings

Variable clinical manifestations and non-specific histologic changes complicate diagnosis and classification of vasculitis .To confirm the diagnosis light microscopy and immuno - fluorescence examinations of kidney biopsy shouldbe accompanied by appropriate serologic tests, includingANCA (Vizjak et al., 2003).

2.1 Light microscopy findings

Histopathologically, RPGN is characterized by avasculitis which involves glomerular capillaries, and results in formation of cellular crescents within most glomeruli (Hricik et al., 1998). The hallmark histologic lesions are crescents; a morphologic expression of severe glomerular injury. In severe glomerular injury rupture of the glomerular capillaries allows inflammatory mediators to spill into Bowman's space, resulting in epithelial cell proliferation and invasion of monocyte and macrophage to Bowman's space (Couser, 1988; Jennette & Falk, 1998; Jennette &Thomas, 2001). Crescents are divided into cellular, fibro cellular or fibrous types. Halmarks of irreversible glomerular or tubulointerstitial injuries are glomerular sclerosis, fibrous or fibro cellular crescents, and interstitial fibrosis.The lesions usually are seen in various stages of activity or resolution.Necrotizing inflammation in small cortical arteries is reported in 10 % of biopsy specimens .Inflammation of medullary vasa rectae with papillary necrosis is another finding that may be found(Lionakiet al., 2007).

In acute pauci-immune glomerulonephritis(RPGN type III) fibrinoid necrosis accompanies crescents. These lesions occur at the same frequency irrespective of the presence or absence of associated vasculitis (DAgati et al., 1986). Acute lesions range from focal segmental fibrinoid necrosis affecting less than 10 %of glomeruli to severe diffuse necrotizing and crescentic glomerulonephritis that may injure all glomeruli.Periglomerular granulomatous inflammation may occur, but is not specific for pauci-immune glomerulonephritis (Lionakiet al., 2007).

3. Histopathology characteristics on immunoflurecent microscopy

Anti-GBM glomerulonephritis characterized by linear staining for IgG and usually C3 along the glomerular capillary. Immune complex-mediated glomerulonephritis, which is found in severe forms of various types of glomerulonephritis such as PSGN, IgA nephropathy, and lupus nephritis, characterized by granular glomerular staining for one or more immuno-globulins and/or complement components, and pauci-immune glomerulonephritis characterized by mild or absent glomerular tuft staining for immunoglobulins and/or complement (Rutgerset al., 2004) .

Anti-neutrophil cytoplasmic antibodies(ANCAs) associated glomerulonephritis are usually pauci immune; however, immunofluorescence microscopy often reveals a low level of

staining (less than +2, in the 0–4 scale (Harriset al., 1998). **Figure 1** presents histologic findings in light and immunoflurecent microscopy.

3.1 Electron microscopy findings

On electron microscopy examination absence of electron-dense immune complex deposits (type I RPGN), multiple electron-dense deposits (type II RPGN), and few or no electron-dense deposits (typeIII RPGN) are main findings (**figure 2)**(Haas, M.&Eustace, 2004).

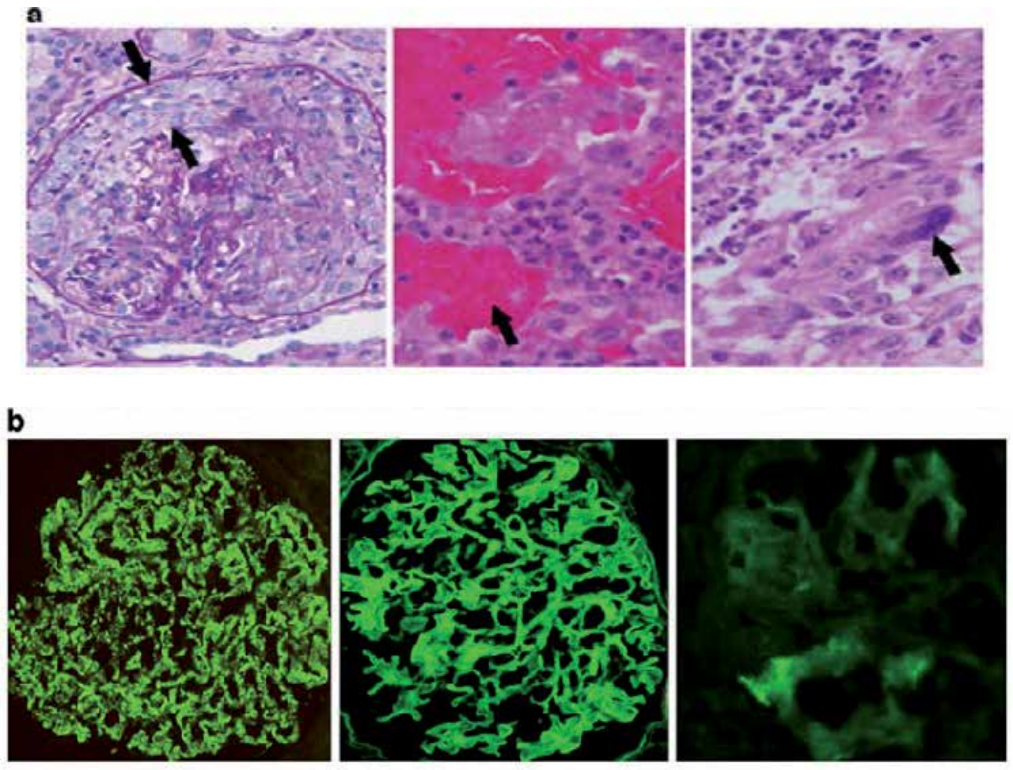

Fig. 1. Histopathologic findings in light and immunoflurecent microscopy (Lionakiet al., 2007) **a/left**; light microscopic demonstration of ANCA-associated necrotizing GN (with a crescent), arrows; (alveolar capillaritis with intra-alveolar hemorrhage); arrow; **middle** (and pulmonary necrotizing granulomatous inflammation with a multinucleated giant cell) arrow; **right b/middle**; Immunofluorescence microscopy can separate crescentic glomerulonephritis into anti-GBM with linear IgG staining, (**left**) immune complex with granular staining, (**right**); or pauci-immune categories with little or no immunoglobulin staining

4. Anti-neutrophil cytoplasmic and anti-GBM antibodies

Anti-neutrophil cytoplasmic antibodies(ANCAs)are characteristic markers of small vessel vasculitides; Wegener'sgranulomatosis(WG), Microscopic polyangiitis(MPA), Churg-Strauss Syndrome(CSS), and idiopathic pauci-immune necrotizing glomerulonephritis for them the term ANCA associated vasculitides(AAV) has long being used(Jennette et al., 1989).

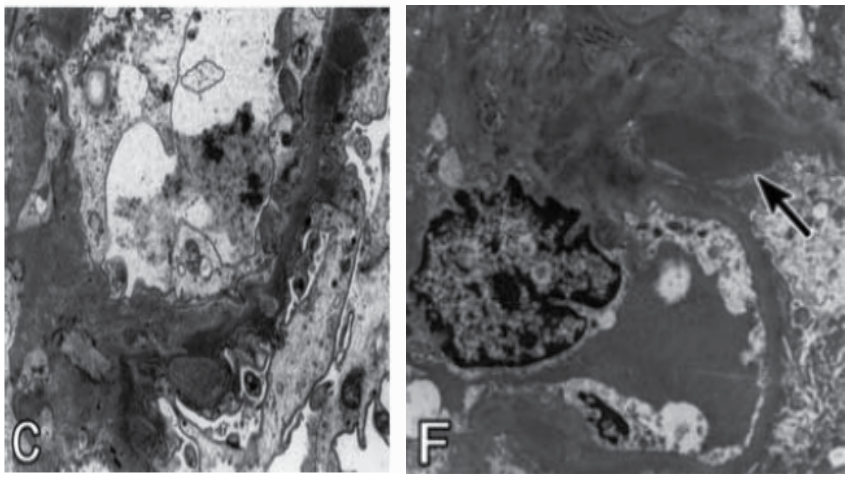

Fig. 2. Electron microscopy findings (Haas, M. &Eustace, 2004**)**
C: Electron microscopy showing multiple sub-epithelial electron-dense deposits, some appearing partially resorbed, with extension of the glomerular basement membrane (GBM) around the deposits (uranyl acetate and lead citrate stain, original magnification ×6300**)**
F: Electron microscopy showing a large subepithelial deposit in a "notch" area (arrow), as well as mesangial deposits (uranylacetate and lead citrate stain, original magnification×3800).

The standard approach for detection of ANCA is indirect immunofluorescence (IIF) technique followed by antigen-specific quantitative assays .Myeloperoxidase (MPO) and proteinase3 (PR3) are major ANCA antigens (Falk .&Jennette, 1988; Goldschmeding et al., 1989; Niles et al., 1989).
In patients with RPGN, there are two major sub classes of ANCA, namely perinuclear (p -ANCA) and cytoplasmic (c -ANCA) (Guillevinet al., 1999) .The main epitope of p-ANCA is myeloperoxidase(MPO), and that of c-ANCA is proteinase-3 (PR3) (Asarodet al., 2000). MPO-ANCAis a useful serum marker for MPA and idiopathic pauci-immune crescentic GN, and PR3-ANCA is regarded as a serum marker for Wegener's granulomatosis and MPA (Asarodet al., 2000). Majority (approximately80- 85%) of cases of pauci-immune crescentic glomerulonephritis are ANCA positive(Haueret al., 2002;Jennette, 2003). Greater than 95% of cytoplasmic ANCA are PR3-ANCA and >95 %of perinuclear ANCA are MPO-ANCA (Lionakiet al., 2007). The c and p-ANCAs are mostly directed against the azurophilic granule proteins proteinase 3 (PR3)and myeloperoxidase (MPO), respectively .Detection of c and especially p-ANCAs are not equivalent to the presence of PR3-and MPO-ANCA. It is recommended to detect ANCA by an antigen-specific ELISA (Savigeet al., 2000; Cohen Tervaert et al., 1991).
Anti-GBM antibodies which are directed to the non-collagenous part ofthe α 3 chain of type IV collagen, can also be evaluated byboth IIF and ELISA(Rutgerset al., 2004) .The ANCA-GBM dot-blot is a qualitative assay that uses nitrocellulose strips on which purified antigens are blotted at preset spots .MPO and PR3 antigens that are used in these tests are produced from human leukocytes .The GBM-ANCA dot-blot assay has been revealed reactivities that had not been detected by ELISA(**Table 1)** (Rutgers et al., 2004).

	MPO-ANCA	PR3-ANCA	Anti-GBM antibodies
Sensitivity	80–86%[a]	92–95%[a]	100%
Specificity	100%	100%	91–94%[a]
Inter-observer effect	5%	1%	8–24%[b]

Table 1. Characteristics of the GBM-ANCA Dot-Blot Assay (Rutgerset al., 2004)

5. RPGN, Pulmonary–renal syndrome (PRS), and ANCA-associated vasculitis (AAVs)

The ANCA-associated vasculitis(WG, MPA, and CSS) are a group of rare autoimmune conditions characterized by the development of necrotizing vasculitis .They share a number of clinical features and are therefore treated using similar treatment protocols.

The AAVs are rare with an annual incidence of 20/million in Europe, with WG as the most common and CSS the least frequent (Ntatsaki et al., 2010).In far-east, MPA is more common than WG .It's thought that they arise from interaction between an environmental factor and a genetically predisposing agent(Ntatsaki et al., 2011).

Pulmonary–renal syndrome (PRS) is defined as combination of diffuse alveolar hemorrhage (DAH) and glomerulonephritis(Savageet al., 1997;Seo& Stone, 2004;Jennette& Falk, 2003).

This syndrome is caused by different diseases, including various forms of primary systemic vasculitis especially WG and MPA, ANCA-associated systemic vasculitis(AAV), Good pasture's syndrome, SLE, and infection-associated or drug induced glomerulonephritis (Westmanm et al., 1997;Brusselle, 2007).

Immunologic injuries or non-immunologic mechanisms are involved in pathogenesis of PRS .Immunologic mechanisms such as production of anti-GBM antibodies, ANCA, immune complexes mediated injuries and non-immunologic mechanisms such as thrombotic microangiopathy(Rondeauet al., 1989)have been suggested .Pulmonary involvement in the majority of cases is the result of small-vessel vasculitis that involves arterioles, venules and alveolar capillaries (necrotic pulmonary capillaritis). These lesions are clinically expressed with DAH(Levy. &Winearls, 1994). In the majority of cases the underlying renal pathology is a form of focal proliferative glomerulonephritis (Jayne et al., 2000), with fibrinoid necrosis, as well as micro vascular thrombi, and extensive crescent formation accompanies glomerular tuft disease (Walters et al., 2010).

According to results of ANCA pulmonary renal syndrome can be categorized into two sub-groups:

1. ANCA-positive Pulmonary–renal syndrome
2. ANCA-negative Pulmonary–renal syndrome

Circulating ANCAs are detected in the majority of pulmonary–renal syndromes(The Wegener's Granulomatosis Etanercept Trial [WGET] Research Group2005; Dickersinet al., 1994.)Three major systemic vasculitis syndromes are associated with positive ANCAs consists of Wegener's granulomatosis, microscopic polyangiitis and Churg–Strauss syndrome (The Wegener's Granulomatosis Etanercept Trial[WGET]Research Group, 2005), and idiopathic pulmonary–renal syndrome(Bolton&turgill, 1989).

Majority of cases of pulmonary-renal syndromes are related to ANCA associated vasculitis (Brusselle, 2007).Pulmonary–renal syndrome in ANCA-negative systemic vasculitis is very rare and has been reported in Behçet's disease, HSP, IgA nephropathy and in mixed cryo–globulinaemia and rarely in thrombotic thrombocytopenic purpura(TTP)(Naseri & Zabolinejad, 2008).

ANCA-positive Pulmonary–renal syndrome : More than 80 %of patients with necrotizing pauci immune small-vessel vasculitis have circulating ANCA (Jennette .&Falk, 1997). The main clinicopathological expressions of AAV are WG, MPA, CSS and renal-limited vasculitis (RLV). The highest incidence is for WG in northern Europe (Haugebergm etal., 1998; Watts et al., 2000, 2008; Laneet al., 2000; Reinhold-Keller et al., 2005), while the incidence of MPA/RLV is higher in Japan (Fujimotoet al., 2006).

Wegener's granulomatosis (WG): Friedrich Wegener in 1939 was the firsti nvestigator to recognize a group of diseases characterized by extra vascular necrotizing granulomatous inflammation of the respiratory tract with vasculitis and/or glomerulonephritis (Schultz &Tozman, 1995). In 1983, Fauci et al reported details of 85 patients with Wegener's granulomatosis followed over a 21-year period. Their diagnosis was based on upper and lower respiratory tract complications, renal disease, and variable involvement of other organs with disseminated vasculitis .Tissue biopsies confirmed the characteristic clinical findings (Fauciet al., 1983).Pathologically Wegener's granulomatosis was characterized by small-vessel necrotizing vasculitis and granulomatous inflammation involving mostly the upper and lower respiratory tracts and the kidneys (Sugimoto et al., 2007).

Diffuse alveolar hemorrhage is the most serious complication in small-vessel vasculitis. Respiratory symptoms including cough and hemoptysis.CXR or chest CT-scan may reveal diffuse lung infiltrate.Clinical manifestations, pathologic and serologic findings play important role in the diagnosis of WG. 75- 90 %of patients with active disease have PR3-ANCAs (Langford, 2005;, Ozaki, 2007). The role of PR3-ANCA in the pathogenesis of the disease is not clear, but in vitro evidence suggests that PR3-ANCA can directly or indirectly damage endothelial cells (Preston et al., 2002).With the recognition of association between ANCAs and Wegener'sgranulomatosis, the concept of Wegener's granulomatosishas been modified and recently a less restrictive definition has been proposed, termed ''Wegener's vasculitis.'' This term includes ANCA-positive patients with clinical presentations of WG such as sinusitis, pulmonary infiltrates, nephritis, and documented necrotizing vasculitis, but without biopsy-proven granulomatousin flammation. Both classic Wegener's granulomatosis and Wegner's vasculitis are different manifestations of the same disease process.The term ''Wegener'ssyndrome "is a more generic term suggested by the working classification of ANCA-associated vasculitides(Tervaert, &Stegeman, 2003).

Diagnostic criteria have been reported by the research group of intractable vasculitis, ministry ofHealth, Labor, and Welfare {MHLW} of Japan for definite diagnosis of AAV including WG (**Table 2**) (Ozaki, 2007).Necrotizing granulomatous lesions commonly affect the ear, nose and throat (E), lung (L), and kidney(K).Systemic symptoms in WG are classified as the following :

1. E Symptoms: nasal symptoms; purulent rhinorrhea, epistaxis, and a saddle nose, eye symptoms; ophthalmic pain, visual disturbance, and exophthalmia, ear symptoms; otalgia and otitis media, and throat symptoms; pharyngeal ulcer, hoarseness, and laryngeal obstruction.

2. lung (L)symptoms: bloody sputa, cough, and dyspnea
3. Kidney(K)symptoms :hematuria, proteinuria, rapidly progressive renal failure, edema, and hypertension

1. Symptoms
(1) E symptoms
Nose (purulent rhinorrhea, epistaxis, and saddle nose)
Eyes (ophthalmic pain, visual disturbance, and exophthalmia)
Ears (otalgia and otitis media)
Throat (pharyngeal ulcer, hoarseness, and laryngeal obstruction)
(2) L symptoms
Bloody sputa, cough, and dyspnea
(3) K symptoms
Hematuria, proteinuria, rapidly progressive renal failure, edema, and hypertension
(4) Others due to vasculitis
(a) General symptoms: fever (38°C or higher, 2 weeks or longer), weight loss (6 kg or more for 6 months)
(b) Local symptoms: purpura, polyarthritis/polyarthralgia, episcleritis, mononeuritis multiplex, ischemic heart disease, gastrointestinal bleeding, and pleuritis
2. Histological findings
(1) Necrotizing granulomatous vasculitis with giant cells at the sites of E, L, and/or K
(2) Necrotizing crescentic glomerulonephritis without immune deposits
(3) Necrotizing granulomatous vasculitis of arterioles, capillaries, and venules
3. Laboratory findings
(1) Positive PR3-ANCA (or C-ANCA by an indirect immunofluorescence)
<Diagnosis>
1. Definite WG
(1) Positive for 3 or more of the symptoms, including E, L, and K symptoms
(2) Positive for 2 or more of the symptoms, and positive for either of the histological findings
(3) Positive for 1 or more of the symptoms, positive for either of the histological findings, and positive PR3-ANCA/C-ANCA
2. Probable WG
(1) Positive for 2 or more of the symptoms
(2) Positive for 1 of the symptoms, and positive for either of the histological findings
(3) Positive for 1 of the symptoms, and positive PR3-ANCA/C-ANCA

Table 2. Diagnostic criteria for Wegener's granulomatosis (Ozaki, 2007)

Biopsies of the nasal mucosa, lung, and kidney reveal necrotizing granulomatous vasculitis and necrotizing crescentic glomerulonephritis without immune deposits(Ozaki, 2007). WG with E, L, and K involvement is classified as the generalized form, while when there is no kidney involvement, E only, L only, or E +L, the term of limited form is used. The therapeutic strategies are different for each forms (Ozaki, 2007).

Microscopic polyangiitis (MPA)

Renal and pulmonary symptoms are characteristic in MPA, and interstitial pneumonitis and pulmonary hemorrhage are common clinical features .MPO-ANCA is positive in 50-75% of patients and biopsy of the lung and kidney reveals necrotizing vasculitis of arterioles, capillaries, and venules with few immune deposits necrotizing and crescentic GN (Ozaki; 2007). Granulomatous inflammation and asthma are not seen in MPA (Jennette &Falk, 1997).

Table 3 presents diagnostic criteria for microscopic polyangiitis(Ozaki, 2007).

Allergic granulomatous angiitis (AGA) or Churg-Strauss syndrome(CSS)

Churg and Strauss was firstly described allergic granulomatous angiitis(Churg, & Strauss, 1951). The disease is characterized by presence of asthma, eosinophilia, and necrotizing granulomatous inflammation .Clinical manifestations of small-vessel vasculitis; palpable purpura of the lower extremities, mononeuritis multiplex, abdominal pain, and gastro - intestinal bleeding develop several years after the onset of asthma.Positive MPO-ANCA are seen and skin biopsy shows necrotizing vasculitis of small vessels with massive eosinophilic infiltration and extravascular granulomatosis(Ozaki, 2007). **Table 4** presents diagnostic criteria for CSS(Ozaki, 2007).

1. Symptoms
(1) Rapidly progressive glomerulonephritis
(2) Pulmonary hemorrhage
(3) Other organ symptoms: Purpura, subcutaneous hemorrhage, gastrointestinal bleeding, and mononeuritis multiplex
2. Histological findings
(1) Necrotizing vasculitis of arterioles, capillaries, and venules, and perivascular infiltration of inflammatory cells
3. Laboratory findings
(1) Positive MPO-ANCA
(2) Positive CRP
(3) Proteinuria, hematuria, elevation of BUN and serum creatinine
<Diagnosis>
1. Definite MPA
(1) Positive for 2 or more of the symptoms, and positive histological findings
(2) Positive for 2 or more of the symptoms including the symptoms (1) and (2), and positive MPO-ANCA
2. Probable MPA
(1) Positive for 3 of the symptoms
(2) Positive for 1 of the symptoms, and positive MPO-ANCA

Table 3. Diagnostic criteria for microscopic polyangiitis(Ozaki, 2007)

SLE and AAV

Systemic lupus erythromatosis is an autoimmune disorder .Variety of autoantibodies are present in SLE patients including ANCA which have been reported in 3-69 %of cases(Molnaretal., 2002;Edgar et al., 1995; Pradhan et al., 2004)**.**Some studies(Chinet al., 2000; Nishiyaet al., 1997) have reported p-ANCA in 37.3-42 %of patients with lupus nephritis, mainly those who have diffuse proliferative GN(DPGN), and minority of patients without renal involvement. In Pradhan et al's study predominant ANCA pattern was p-ANCAwhile c-ANCApattern was not found in any patient(Pradhan et al., 2004).Their study revealed that ANCA can be used as a serological marker to differentiate vasculitides in lupus nephritis cases from SLE without nephritis.

1. Symptoms
   (1) Bronchial asthma and/or allergic rhinitis
   (2) Eosinophilia
   (3) Symptoms due to vasculitis
      (a) General symptoms: fever (38°C or higher, 2 weeks or longer), weight loss (6 kg or more for 6 months)
      (b) Local symptoms: mononeuritis multiplex, gastrointestinal bleeding, purpura, polyarthritis/polyarthralgia, and myalg (muscle weakness)
2. Characteristic clinical course
   (1) Symptoms (1) and (2) precede the development of (3)
3. Histological findings
   (1) Granulomatous or necrotizing vasculitis of small vessels with marked infiltration of eosinophils
   (2) Extravascular granulomas

<Diagnosis>

1. Definite
   (1) Positive for 1 or more of the symptoms (1) and (2), and positive for either of the histological findings (Definite AGA)
   (2) Positive for 3 of the symptoms, and the characteristic clinical course (Definite Churg-Strauss syndrome)
2. Probable
   (1) Positive for 1 of the symptoms, and positive for either of the histological findings (Probable AGA)
   (2) Positive for 3 of the symptoms, but not the characteristic clinical course (Probable Churg-Strauss syndrome)

Table 4. Diagnostic criteria for Churg-Strauss syndrome (Ozaki, 2007)

Organ involvement	ANCA serology			Other autoantibodies				
	anti-MPO (12/59)	anti-LF (10/59)	anti-CG (8/59)	ANA (59/59)	anti-dsDNA (45/59)	anti-ssDNA (40/59)	anti-nRNP (38/59)	anti-Sm (16/59)
Skin (50)	4	0	1	48	42	40	38	16
Renal (41)	12	10	8	34	29	28	28	12
DPGN with crescents (21)	5	3	4	19	16	17	15	3
FPGN with crescents (14) *	5	3	2	12	10	10	9	4
RPGN with crescents (4)	2	4	1	2	2	1	2	3
MPGN with crescents (2)	0	0	1	1	1	0	2	2
Joint (35) **	0	6	3	26	18	20	35	12
Serositis (16)	0	6	2	14	14	12	10	10
Haematological (8)	0	0	2	4	4	5	2	2
GI tract (8)	0	0	2	6	6	4	2	2
CNS (8)	0	0	3	3	3	4	2	2

Table 5. Correlation of organ involvement with ANCA serology and other autoantibodies in SLE patients(Pradhan et al., 2004)

Atypical or X-ANCA has been reported among SLE patients by Savige et al, This antibody showing specificities tocathepsin G and lactoferrin (Savige et al., 1996). **Table 5** shows correlation of organ involvement with ANCA serology and other autoantibodies in SLE patients (Pradhan et al., 2004).

6. Treatment of RPGN

Untreated RPGN typically progresses to end-stage renal disease over a period of weeks to a few months. Early diagnosis and initiation of appropriate therapy is essential to minimize the degree of irreversible renal injuries. The therapy of most patients involves pulse methyl-prednisolone followed by daily oral prednisone, oral or intravenous (IV) cyclophosphamide, and in some cases plasmapheresis. Empiric therapywith IV methylprednisolone should be begun in patients with severe disease with adding of plasmapheresisespecially if the patient has hemoptysis (Appel et al., 2010), If a renal biopsy performs soon after initiating empiric therapy the histological abnormalities will not alter.

6.1 Treatment of ANCA associated vasculitis (AAV)

Pauci-immune crescentic glomerulonephritis is a severe form of glomerular inflammation, which if left untreated, usually progresses to end-stage renal failure in weeks or months, AAV is responsible for 80 %of cases(Sakaiet al., 2002).The evidence based studies for the management of the AAV is well established and the strategy of induction, consolidation and maintenance therapy is accepted. Guidelines have been designed by both the British Society for Rheumatology (Lapraiket al., 2007) and European league against rheumatism (Mukhtyaret al., 2009) on the management of the AAVs. The AAVs are conventionally treated with a strategy of remission induction followed by maintenance therapy using glucocorticoids combined with cyclophosphamide during induction and azathioprine (AZA) for maintenance (Ntatsaki et al., 2011). Current standard treatment is combination of cyclophosphamide and steroids, but the optimal doses, routes of administration, and duration of therapy remain poorly defined (Hotta, et al., 2005). As immunosuppressive therapies increases the risk of infection, therefore one of the most important aspect of successful treatment srategies should be sufficient attenuation of inflammation without serious immunosuppression which leading to life-threatening infection.

Corticosteroids

For the induction of remission corticosteroid regimen is recommended which includea daily intravenous pulse of methylprednisolone (15 mg /kg)for 3 days, followed by oral prednisone (1mg/kg/day) for 3 weeks, which then tapered progressively (Pagnouxet al., 2008). In pauci-immuneRPGN and MPA usefulness of combination of methylprednisolone pulse therapy for 3 days, oral corticosteroid of 1mg/kg/day for 1 month, and cyclophos -phamide of 2 mg/ kg/day for 6 to 12 months has been confirmed in several studies (Salama, et al., 2002; J indal, 1999; Hoffman, 1997; Guillevin et al., 1991).

Cyclophosphamide(CYC)

Different therapeutic agents have been used in treatment of AAV.Standard therapy for AAV is treatment with combination of cyclophosphamide and prednisolone(Pagnouxet al., 2008). After induction with daily oral or pulse intravenous cyclophosphamide therapy, relapse rates of 15% at 12 months(Haubitzet al,. 1998), and 38% at 30 months(Guillevinet al., 1997)

have been reported.In long-time follow-up 50 %of patients experience relapse within 5 years (Hoffman et al., 1992).Treatment with cyclophosphamide is effective, but also very toxic (Hoffmanet al., 1992).Repeated treatment with cyclophosphamide increases adverse effects. To avoid cyclophosphamide -related toxicity, alternative induction treatments are needed (Stassen et al., 2007).

Mycophenolate mofetil (MMF): MMF is considered as a potent immuno suppressive drug with favorable side effects, so it has been considered as an alternative to cyclophosphamide treatment in patients with AAV (Stassen et al., 2007;Pesavento et al., 1999; Haidinger et al., 2000; Joy et al., 2005;Koukoulaki, & Jayne, 2006). MMF is a drug which usually is well tolerated. In patients with auto-immune diseases such as SLE, MMF is effective in inducing remission with short-term efficacy which is comparable with cyclophosphamide (Chanet al., 2000, 2005; Ong et al., 2005; Ginzler etal., 2005). Induction treatment with MMF and oral steroids consisted of oral MMF 1000 mg twice daily and oral prednisolone 1 mg/kg once daily (maximum 60 mg). If patients are still in remission after 1 year, MMF is tapered by 500 mg every 3 months and Prednisolone is tapered after 6 weeks by 10 mg every2 weeks until a dose of 30 mg was reached, and by 5 mg every2 weeks until 10 mg. Next, the dose is reduced2.5 mg every month (Stassen et al., 2007). Stassen et al reported that combination of oral steroids and MMF induced complete and partial remission in 78% and 19 %of patients respectively(Stassen et al., 2007), Therefore they suggested oral steroids with MMF in patients with relapses of AAV intolerant to cyclophosphamide therapy.Bone marrow suppression is an uncommon side effect of MMF treatment which can result in anemia, leucocytopenia or thrombocytopenia in a number of patients .Fortunately this side effect responds to temporary dose reduction in nearly all patients(Stassen et al., 2007).

Methotrexate(**MTX**)

For remission maintenance in patients who are intolerant of AZA or relapse while taking it other alternative agents such as MTX or MMF have been recommended (Ntatsaki et al., 2011). Methotrexate is an alternative drug for cyclophosphamide which is currently studied (De Grootet al., 2005; Sneller et al., 1995; Stoneet al., 1999). According to one randomized (De Groot et al., 2005) and two uncontrolled studies (Sneller et al., 1995; Stoneet al., 1999) MTX effectively induced remission in 90 %and 71–74 %of patients respectively, but relapses occurred more frequently than when cyclophosphamide was used (70%vs. 47%)(De Groot et al., 2005). MTX is excreted by kidney, therefore should be used with caution in those with renal impairment (Ntatsaki et al., 2011; M etzler et al., 2007).

Groot et al conducted an unblended, randomized, controlled trial study todetermine whether MTX could replace treatment with cyclophosphamide and oral corticosteroids(De Grootet al., 2005).They found that MTX can replacecyclophosphamide for initial treatment of early AAV, but it was less effective for induction of remission in patients with extensive disease or those with pulmonaryinvolvement .In addition the relapse was more common in patients who treated with MTX than those who received cyclophosphamide.Patients were randomized to receive either MTX 7.5 mg/week increasing to 20 mg/week at 8 weeks or LEF loading dose 100 mg/day for 3 days, followed by20 mg /day until Week 4, then 30 mg/day for 2 years .Their study showed no differences in efficacy or safety between two treatments.

Pagnoux et al(Pagnouxet al 2008)compared AZA with MTX in maintaining remission in WG and MPA patients who had achieved remission with intravenous pulse of cyclophos - phamide. They found no significant difference in adverse events and relapses rates either during the 12-month treatment phase or subsequent follow-up between two groups.

Intravenous immunoglobulins(IVIG)

ANCAs can induce cytokine-primed neutrophils undergo degranulation and respiratory bursts during which they release toxic oxygen species and lytic enzymes (Falk et al., 1990). Anti-idiotype antibodies with inhibitory effects on ANCA in vitro have been found in a pooled human gamma globulin preparation (Pall et al., 1994.)Evidence showed that IVIG acts in different phases of theimmune response including neutralization of circulating pathogenic antibodies, Fc receptor modulation, blockade or suppression of antibody-dependent cell toxicity, natural killer cell function, auto antibodyproduction and complement activation, and acceleration of neutrophil apoptosis (Kazatchkine & Kaver i, 2001; Tsujimoto et al., 2002).

Beneficial effect of high-dose intravenous immunoglobulin in ANCA–associated vasculitis has been approved by different studies (Ito-Iharaet al., 2006; Tuso et al., 1992; Jayneet al., 1993; Richteret al., 1995). Ito et al evaluated IVIG monotherapy (400 mg/kg/day for 5 days)in AAV patients. Their study showed that IVIG decreased the leukocyte count, C- reactive protein level, Birmingham Vasculitis Activity Score and improved the systemic symptoms (Ito-Iharaet al., 2006). Other studies suggested that IVIG induces remission in 40–82% of patients (Richter et al., 1995; Jayne et al., 1991; Levy et al., 1999; Jayne et al., 2000). IVIG appears to have an important place in the management of ANCA-RPGN, but it's indications have not been determined. Because IVIG is an expensive drug additional studies on its cost-effectiveness and rational introduction into clinical practice are needed(Hotta et al., 2005).

Anti-B-cell therapy(Rituximab)

Beneficial effects of Rituximab which is a monoclonal antibody against anti-CD20 have been reported in several case series(Gottenberg et al., 2005; Omdal et al., 2005; Speckset al., 2001), and uncontrolled studies(Eriksson, 2005; Keogh et al., 2005a, 2005b; Smith et al., 2006).The main problem of treatment with rituximab is that relapses commonly occurred after 6–9 months(Keogh et al., 2005a, 2005b; Smith et al., 2006).

Apheresis therapies

Results of clinical trial of apheresis therapies, either plasmapheresis or cytapheresis in AAV are disappointing. These studies showed no benefit(Glöckner et al., 1988; Cole et al., 1992; Zäuneret al., 2002), benefits just in dialysis dependent patients(Pusey et al., 1991)or benefits on preserving the renal function (Furutaet al., 1998; Hasegawaet al., 2005).

Pusey et al .Found that plasma exchange is of added benefit in dialysis-dependent patients because in patients who were initially dialysis-dependent, renal function was more likely to have recovered when treated with plasma exchange plus drugs rather than drugs alone (Pusey et al., 1991).Report from the European community group suggested that adding plasma exchange to immuno suppressive therapy was not beneficial if there was severe tubular atrophy and fewer than 33 %of the glomeruli were normal(De Lind van Wijngaarden et al., 1998).

In contrast to AAV in anti-GBM RPGN, the beneficial effect of plasma exchange has been well established .It might be attributable to the direct role of anti-GBM antibody in the pathogenesis of anti-GBM antibody RPGN, while in AAV no direct role for ANCA have been established (Hotta et al., 2005). The main advantage of Apheresis therapies is that no severe infectious episodes have been noted (Nagaseet al., 1998; Sawadaet al., 2003). Japan nation wide survey of RPGN (Yamagataet al., 2004) recommends cytapheresis in patients with aggressive forms of RPGN (rapid deterioration of renal function like the PR3-ANCA-

associated RPGN, or pulmonary renal syndrome complicated by severe inflammation, or relapses with high MPO-ANCA titer).

Anti-TNF-alpha

Insights into the role of Th1 cytokines in the pathogenesis of WG have led to trial therapies with antagonists of tumor necrosis factor-alpha (TNFα)and inhibitors of monocyte function, such as interleukin-10(Kamesh et al., 2002) .Etanercept(Enbrel ;soluble receptors), infliximab (Remicade;human-mouse chimeric antibody against TNF), and adalimumab (Humila; human anti-TNF antibody)are biological antagonists of TNF(Ozaki, 2007).Etanercept have been reported ineffective in maintaining remission and a higher rate of malignancy have been noted in patients who have received the drug (Wegener's granulomatosis etanercept trial [WGET] research group, 2005).In an open label study, infliximab was added to standard immuno suppressive therapy in 16 patients with acute AAV at first presentation or relapse and in 16 with persistent disease despite multiple immuno suppressive regimens, 88% of patients achieved remission within a mean of 6.4 weeks(Boothet al., 2004) .

Anti-T cell antibodies :Different studies have been reported that active systemic vasculitis is mediated in part by T cell-induced injury .This finding has led to the evaluation of anti-T cell antibodies in patients with Wegener's granulomatosis who are resistant to cytotoxic therapy (Lockwoodet al., 1993; Hagenet al., 1995; Schmittet al., 2004).

In one study, the administration of a combination of two humanized monoclonal antibodies led to long-lasting remission in four patients with different forms of refractory vasculitis (Lockwoodet al., 1993).In other study 15 patients with refractory disease received anti-thymocyte globulin(ATG)which resulted in a partial or complete remission in 9 and 4 patients, respectively (Schmittet al., 2004).The role of these experimental therapies remains to be determined.

Intravenous azathioprine

High dose intravenous azathioprine has been tested for treating a variety of immune-mediated diseases .In one report, four patients with WG who had not responded to oral cyclophosphamide were treated with monthly infusions of azathioprine) (Benensonetal., 2005).Two reached remission of disease, one of whom developed renal involvement during relapse, which responded to retreatment.

15-Deoxyspergualin

15-deoxyspergualin (gusperimus), a drug with anti-proliferative effect on antigen-stimulated B cells, has been evaluated in a small number of patients who didn't respond to cyclophosphamide or had contraindications to the use of cyclophosphamide .The administration of 15-deoxyspergual in 20 patients resulted in complete or partial remission in six and eight respectively(Bircket al., 2003).All patient experienced transient leucopenia with each treatment cycle. In another study seven patients treated with 15-deoxyspergualin and glucocorticoids, all reached complete or partial remission .The main problem was that to maintain remission prolonged treatment up to 4 years was required(Schmittet al., 2005).In addition serial monitoring of white blood count is required to avoid excessive leucopenia.

Radiation therapy

Radiation therapy has been evaluated in patients with WG and air way involvement (Eagletonet al., 1979; Neviani et al., 2002).The use of ionizing radiation for non-malignant

disease is controversial .Current data do not support its use in systemic disorder like Wegener's granulomatosis (Stone et al., 2010).

Stem cell transplantation: High-dose, myeloablative chemotherapy with stem cell transplantation has been used for the treatment of refractory severe vasculitis. There are few case reports of successful treatment in patients with WG (Kötteret al., 2005). More studies are required to determine the role of stem cell transplantation in the management of resistant AAV.

Prophylaxis against Pneumocystis carinii pneumonia(PCP)

Opportunistic infections especially Pneumocystis carinii pneumonia are potentially fatal complications of immuno suppressive therapy in RPGN and AAV .The estimated incidence of PCP is approximately 6 percent(Ognibeneet al., 1995).Different approaches to prophylaxis against PCP infection during initial immunosuppressive therapy have been suggested: trimethoprim-sulfamethoxazole one single strength (80 mg/400 mg)tablet daily or one double strength tablet (160 mg/800 mg)three times per week or Atovaquone in patients who are allergic to sulfonamides or do not tolerate trimethoprim-sulfamethoxazole.During treatment with methotrexate and glucocorticoids, the addition of trimethoprim-sulfamethoxazole increases the risk of pancytopenia, therefore Atovaquone is suggested for prophylaxis in such patients..It has been recommended to continue PCP prophylaxis in maintenance immuno suppressive therapy phase until the CD4-positive T cell count exceeds 300/microL(Mansharamaniet al., 2000).patients who have received trimethoprim-sulfamethoxazole for prophylaxis during induction, should continue the prophylaxis when azathioprine is used and switch to atovaquone when methotrexate is replaced for maintenance therapy.

Some patients have low CD4-positive T cell counts for prolonged periods after the cessation of cyclophosphamide and require prolonged PCP prophylaxis; in addition glucocorticoids should be tapered to the lowest possible dose .If patients develop neutropenia when receive prophylaxis, the drug should be switched to atovaquone (Stoneet al., 2010).

Management of RPGN and AAV in pregnancy: As with active disease in non-pregnant patients, prednisone alone is relatively ineffective, and to induce remission combined therapy with cyclophosphamide is needed.The major challenges of treatment during pregnancy are potentially serious adverse effects which can occur with both MMF and cyclophosphamide. In addition, insufficient data about the safety of rituximab is available. High risk of skeletal and palatal defects, as well as malformations of the limbs and eyes has been noted in case of fetal cyclophosphamide exposure during the first trimester.Although fetal risk is much smaller during the second and third trimesters, pancytopenia and impaired fetal growth can occur .MMF fetal exposure increases the risk of miscarriage and congenital malformation such as cleft lip and palate .As a result, some consider MMF to be contraindicated in pregnancy.The safer immuno suppressive drugs that have been effective in WG and MPA include glucocorticoids, azathioprine, cyclosporine and tacrolimus. Alternatives that could be considered include cyclophosphamide or rituximab in the second or third trimester once organogenesis is complete(Stoneet al., 2010).

6.1.1 Prognosis of RPGN and ANCA associated vasculitis

RPGN If left untreated typically progresses to end-stage renal disease over a period of weeks to a few months .Patients with fewer crescents may present slowly progressive

course(Baldwin et al., 1987) .Despite various immuno suppressive therapy protocols mortality of ANCA positive RPGN is still high, and the major cause of death is infectious complications(Booth et al., 2003; De Lind van Wijngaardenet al 1998).Outcome of AAV depends on patient 's age, degree of renal impairement at presentation and presence of pulmonary involvement. During the first 6 months of treatment mortality is very high as a result of aggressive course of the disease and toxic effects of early immuno suppressive treatments(Sakai et al 2002;Boothet al., 2003). Although the introduction of steroids and cyclophosphamide pulse therapy have improved the overall mortality of AAV, the 2-year mortality rate is still high (20 % in 2-year follow-up)(Booth et al., 2003;Hogan et al., 1996;Falk et al 2000;Franssen et al 2000). Serum creatinine, dialysis dependency, and percentage of non-crescentic glomeruli at diagnosis have been considered as the best predictors of disease outcome(Bajema et al., 1999; Levy et al., 2001; Slotet al.2003).The prognosis for patients with anti-GBM antibody disease is poor(Hirayama, et al., 2008), and renal survival and mortality rates of 20.9%and 23.3 %at 6 months after onset have been reported respectively. Early diagnosis and starting treatment without delay might improve the prognosis(Hirayama, et al., 2008.)A large nationwide survey of RPGN in Japan showed that mortality correlates with age, severity of renal dysfunction, presence of pulmonary involvement, and high C-reactive protein level(Sakaiet al., 2002). The mortality rate of Japanese patients was higher than in European or American patients because of the high incidence of lethal infection (Hotta et al., 2005).

Japan Nationwide Survey of RPGN noted that 6-month renal and patients' survival for PR3-ANCA-associated RPGN were 88.2 %and 92.3% respectively, while for MPO-ANCA they were 69.9 %and 74.2 %respectively(Yamagata et al., 2005).patients' survival is very low in MPA if the disease presents as pulmonary renalsyndrome (Gallagheret al., 2002 Lauque et al., 2000; Niles et al., 1996).Introduction of immuno suppressive agents considerably has improved the outcome of AAV over the past 30 years .WG and MPA if left untreated have a rapidly progressive and usually fatal course(Ntatsaki et al., 2011). Walton reported a mean survival of 5 months in patients with WG, and mortality rate of 82 % and 89 %in 1 and 2 year follow-up respectively (Walton, 1958).Standard treatments significantly have improved prognosis in WG and MPA, and some studies have reported 5-year survival rates of 81 %for MPA and 87 % for WG (Eriksson et al., 2009).in European Vasculitis Study, there was 11.1 %mortality at 1 year (Littleet al., 2010.)High age at presentation, severe renal involvement (high serum creatininelevel at presentation), pulmonary involvement, high ESR and high scores of disease activity and damage were poor prognostic factors (Holle et al., 2011).

Suzuki et al 's study confirmed that ANCA-associated vasculitis is the most serious etiologies of RPGN(Suzukiet al., 2010).In nationwide survey byYamagata and Koyama (Koyamaet al., 2009; Yamagata et al., 2005), and Suzuki's study main causes of patients' death were infectious complications, including DIC which was mainly linked to pneumonia by opportunistic pathogens(Pneumocystis carinii, Candida albicans, and cytomegalovirus).

Researchers hope they will find new immuno suppressive drugs with highest efficacy, lowest side effects and high safety during pregnancy to improve patients' survival and quality of life .It's a dream that undoubetly will be achieved in next years.

7. Conclusion

RPGN is a nephrology emergency which needs special attention.If the disease left untreated typically progresses to end-stage renal disease over a period of weeks to a few

months.When there is a strong clinical suspecsion special immuno suppressive treatment should be started as soon as possible (preferably after kidney biopsy) .Despite various immuno suppressive therapy protocols mortality of ANCA positive RPGN patients is still high, also prognosis of anti-GBM antibody disease is poor.acctually treatment of RPGN and AAV are serious challenges in nephrology medicine which needs more clinical trial studies in larger groups of patients.

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Part 4

Glomerular Disease in Metabolic and Systemic Conditions

19

Diabetic Glomerulopathy

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1. Introduction

Diabetic nephropathy is the most common cause of end-stage renal disease (ESRD) in adults. In the United States, almost half of patients entering ESRD programs were diabetic, and most of them (≥80%) had type 2 diabetes. This is due to the facts that 1) diabetes, particularly type 2, is increasing in prevalence; 2) diabetes patients now live longer; and 3) patients with diabetic ESRD are now being accepted for treatment in ESRD programs where formerly they had been excluded. The annual cost of caring for these patients, in the United States alone, exceeds $10 billion. The mortality rate of patients with diabetic nephropathy is high, and a marked increase in cardiovascular risk accounts for more than half of the increased mortality among these patients.

The earliest clinical manifestation of renal involvement in diabetes is an increase in albumin excretion (microalbuminuria), a stage termed as incipient nephropathy at which renal histology may be relatively normal or may reveal glomerulosclerosis. Diabetes can cause a variety of pathological abnormalities: isolated glomerular basement membrane thickening, mesangial expansion, nodular intercapillary and/or diffuse glomeruloscelorsis or even advanced diabetic sclerosis. While glomerulopathy is quite the hallmark of diabetic nephropathy, the frequency and functional significance of interstitial lesions in diabetic kidney is now well recognized. Furthermore, the occurrence of non-diabetic glomerulopathy or vasculitis alone or superimposed on diabetic nephropathy is increasingly being documented in literature. The pathogenesis is incompletely understood and is very vigorously being investigated.

Once overt diabetic nephropathy (proteinuria) is present, ESRD can be postponed, but in most instances not prevented, by effective antihypertensive treatment and careful glycemic control. Treatment options currently are limited to obvious pathogenic factors while several innovative therapies are under evaluation. Therefore, in the last decades, there has been intensive research into pathophysiologic mechanisms of early diabetic renal injury, predictors of diabetic nephropathy risk, and early intervention strategies

2. Epidemiology

Type 1 diabetes — The epidemiology of diabetic nephropathy has been best studied in patients with type 1 disease, since the time of clinical onset is usually known. About 0.5% of

the population in the United States and Central Europe has type 1 diabetes. The prevalence is higher in the northern Scandinavian countries and lower in southern Europe and Japan. Approximately 20 to 30 percent will have microalbuminuria after a mean duration of diabetes of 15 years (Orchard TJ 1990). Less than half of these patients will progress to overt nephropathy; microalbuminuria may regress or remain stable in a substantial proportion, probably related to glycemic and blood pressure control.

Prior to the current period of intensive monitoring and treatment, it was suggested that 25 to 45 percent of diabetic patients will develop clinically evident disease (the minimal criterion for which is a persistently positive urine dipstick for protein) (Parving HH, 1998). After so-called macroalbuminuria or clinical grade proteinuria (>300 mg albuminuria per day) develops, the majority of patients will progress to end-stage renal failure.

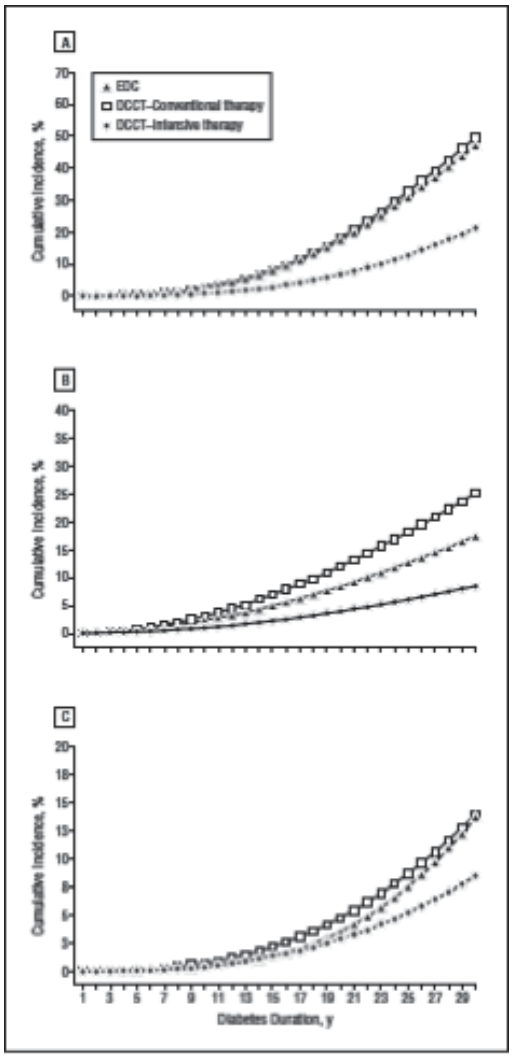


Fig. 1. Estimated cumulative incidences of proliferative retinopathy or worse (A), nephropathy (B) and cardiovascular disease over time.

The overall incidence of end-stage renal disease (ESRD) was also substantial, with reported rates of 4 to 17 percent at 20 years from time of initial diagnosis and approximately 16 percent at 30 years (Nathan DM 2009). A strong predictor of the development of ESRD was the level of glycemic control during the first two decades of IDDM. The risk of ESRD in the group with the poorest glycemic control was almost threefold higher than in the middle group, and fourfold higher than the group with the best glycemic control.

In comparison to these findings, subsequent studies have found that the renal prognosis of type 1 diabetes, including the rate of progression to ESRD, has dramatically improved over the last several decades. In addition to the importance of glycemic control, more aggressive blood pressure reduction and the use of angiotensin converting enzyme inhibitors have been shown to reduce the rate of progression of, though not prevent, diabetic nephropathy (Krolewski M 1996).

Type 2 diabetes is about nine times more prevalent than type 1 diabetes, accounting in part for the greater contribution of type 2 diabetic patients to ESRD incidence. In Caucasians, the prevalence of progressive renal disease has generally been lower in type 2 diabetes than in type 1 disease (Cowie CC, 1989). However, this observation may not apply to all groups with type 2 diabetes, some of whom have had a more ominous renal prognosis. Studies in type 2 diabetic patients from Western Europe and in Pima Indians from Arizona showed rates of progression to nephropathy similar to those of type 1 diabetic patients. The risk of developing ESRD is much higher in black than in white American patients with type 2 diabetes.

As previously described, however, the use of modern therapies lowers the incidence of ESRD, even in groups at extremely high risk such as the Pima Indians. In a subsequent study, for example, the incidence of diabetic ESRD was noted to have declined significantly from the period 1991-1994 to the period 1999-2002 (32 to 15 cases per 1000 patient-years, respectively).

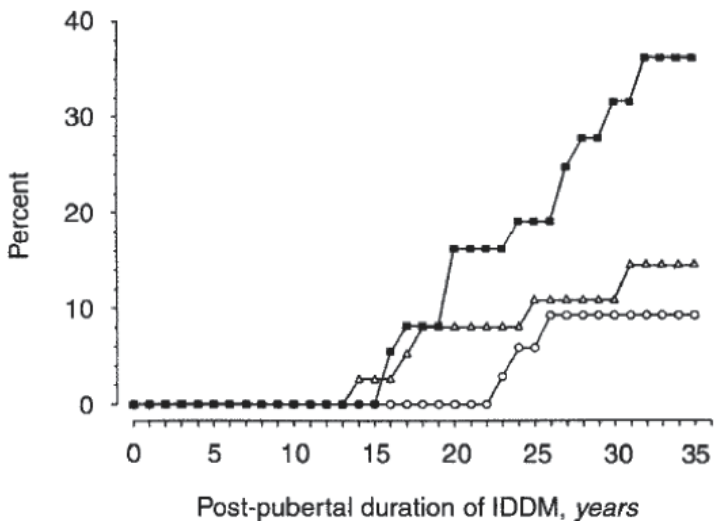


Fig. 2. Cumulative incidence of ESRD according to duration of IDDM and according to tertile of the index of hyperglycemia. Closed rectangles represent the tertile with the highest index of severe hyperglycemia; open triangles represent the middle tertile, and open circles represent the third with the lowest index of hyperglycemia. The differences among the curves are statistically significant, P = 0.017

Data suggest that the renal risk is currently equivalent in the two types of diabetes. Evidence in support of this hypothesis includes the observations in one report that the time to proteinuria from the onset of diabetes and the time to ESRD from the onset of proteinuria were similar in type 1 and type 2 disease (Ritz E, 1999).

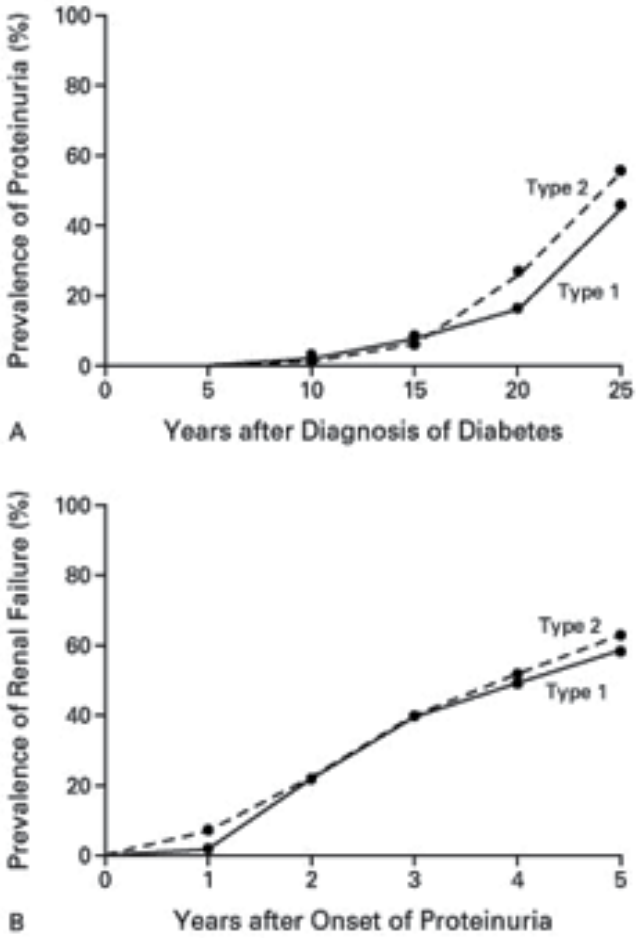


Fig. 3. Cumulative prevalence of persistent proteinuria among patients with type 1 or type 2 diabetes according to the duration of diabetes (Panel A), and cumulative prevalence of renal failure among patients with type 1 or type 2 diabetes according to the duration of proteinuria (Panel B).

Glycemic control, systemic blood pressure levels, and genetic factors seem to be very important in determining diabetic nephropathy risk. Other factors such as lipid levels, smoking habits, and vitamin D intake may also have a role in modulating this risk.
As with type 1 diabetes, some patients with microalbuminuria due to type 2 diabetes, particularly those with good glycemic control, experience regression of microalbuminuria (Araki S, 2005)

3. Natural history and clinical course

The course of renal involvement in type 1 diabetes can be divided in five stages. **Stage I**, present at diagnosis, is that of renal hypertrophy-hyperfunction. At this stage, patients at risk and not at risk of diabetic nephropathy cannot be clearly separated. A 25 to 50 percent elevation in the glomerular filtration rate (GFR) is seen early in the course in up to one-half of patients with type 1 diabetes mellitus, an abnormality that is exaggerated after ingestion of a protein load.

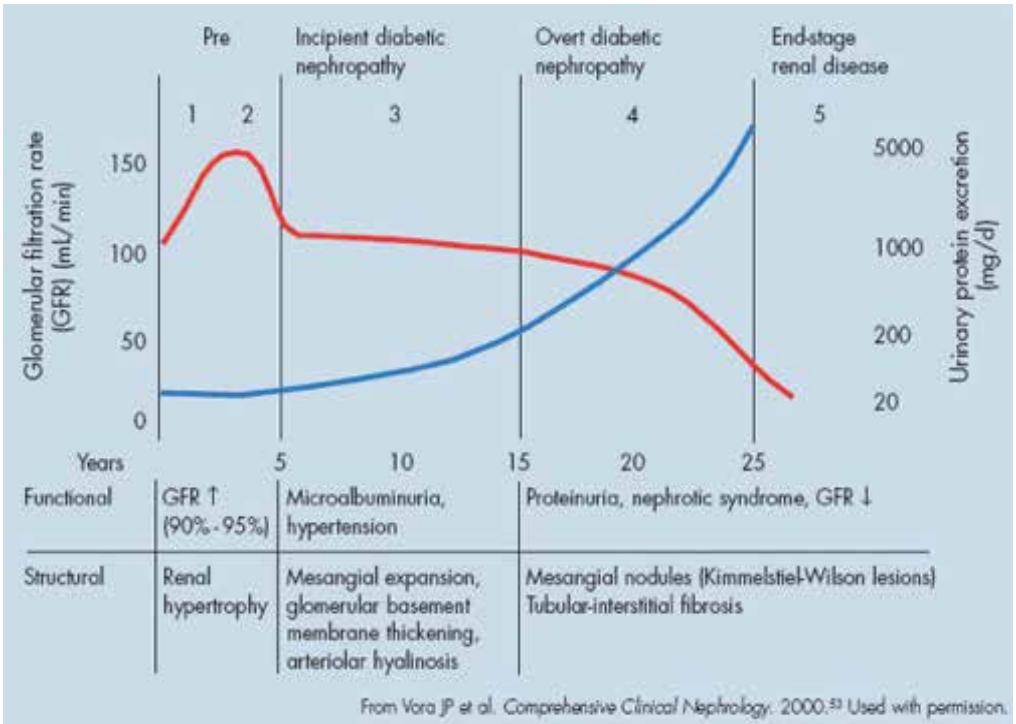


Fig. 4. The five stages of renal involvement in type 1 diabetes

This increase in GFR could be explained by a relative increase in glomerular capillary pressure and/or ultrafiltration coefficient. Glomerular hypertrophy and increased renal size typically accompany the rise in GFR. In an inception cohort study of adult-onset type 1 diabetic subjects, a greater albumin excretion rate within the normal range, male gender, higher mean blood pressure and hemoglobin A1c, and shorter stature were independent predictors of development of microalbuminuria over 18 years of follow-up.

Hyperfiltration also occurs early in the course of type 2 diabetes (Vora JP, 1992). The degree of hyperfiltration and the course of the GFR in type 2 diabetes mellitus was evaluated in more detail in a study of 194 Pima Indians of the Gila River Indian Community in Arizona who have the world's highest incidence of Non-Insulin-Dependent Diabetes Mellitus (NIDDM) (Nelson RG, 1996). The following results were noted:

- In 31 patients with a normal glucose tolerance test, the mean GFR was 123 mL/min
- In 29 patients with impaired glucose tolerance, the mean GFR was 135 mL/min
- In 30 patients with newly diagnosed type 2 disease, the mean GFR was 143 mL/min

- In 70 patients with overt diabetes for more than five years and either normal albumin excretion or microalbuminuria, the mean GFR was 153 mL/min; in 34 similar patients with overt proteinuria, the mean GFR was 124 mL/min

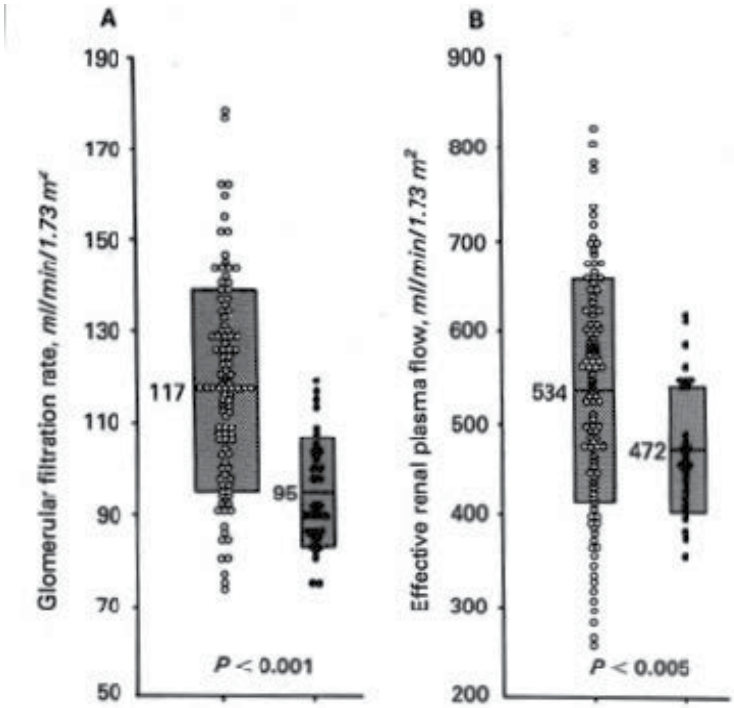


Fig. 5. Glomerular filtration rate and effective renal plasma flow in non-insulin dependent diabetics (NIDDMs) (N= 110) and normal subjects (N = 32).

After four year follow-up, the GFR rose 14 percent in patients with impaired glucose tolerance, 18 percent in newly diagnosed patients, was stable in those with microalbuminuria, and fell 35 percent in those with overt proteinuria. This pattern is consistent with the hypothesis that hyperfiltration causes progressive glomerular damage. However, the base-line glomerular filtration rate in the diabetic subjects predicted neither increasing urinary albumin excretion nor declining glomerular filtration during four years of follow-up, suggesting that hyperfiltration itself is not the principal factor in the development or progression of nephropathy. Higher urinary albumin excretion at base line, however, did predict increasing albuminuria and, in subjects with macroalbuminuria, declines in the GFR; these findings suggest that enhanced protein flux across the glomerular capillary wall contributes to progressive glomerular damage. Proteinuria also predicts the progression of renal disease in patients with nondiabetic renal disease.

Studies in experimental animals indicate that dilatation of the afferent (precapillary) glomerular arteriole plays an important role in the hyperfiltration response, by raising both the intraglomerular pressure and renal blood flow. (Bank N, 1991) A role for hormones is suggested by the ability of a chronic infusion of a somatostatin analogue octreotide to partially reverse both the early hyperfiltration and the increase in renal size in type 1 diabetic patients (Serri O, 1991). There was, however, a fall in the plasma concentration of

insulin-like growth factor I (IGF-1), which is produced in part within the kidney. Although the pathogenetic role of IGF-1 is unproven, it is of interest that infusion of this hormone in normal subjects can replicate the findings seen in diabetics — renal vasodilatation and an elevation in GFR. Similar hemodynamic changes plus renal hypertrophy can be induced by IGF-I in experimental animals.

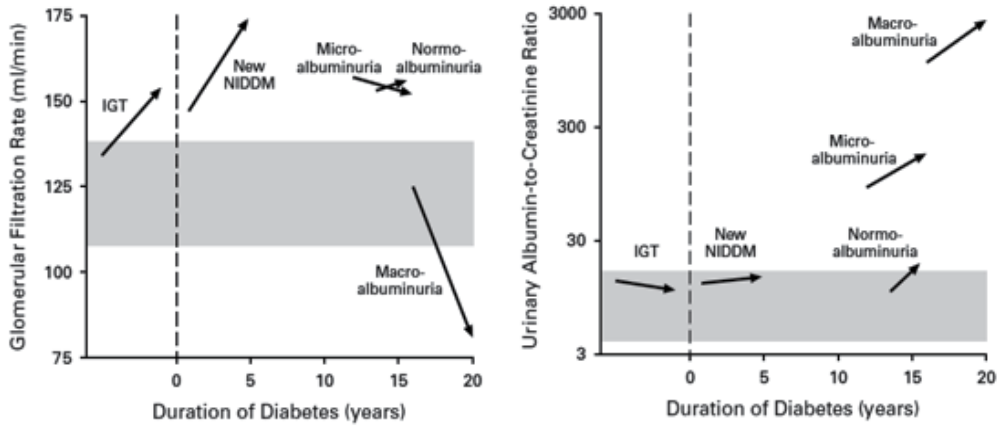


Fig. 6. Changes in the Mean Glomerular Filtration Rate and Median Urinary Albumin-to-Creatinine Ratio from Base Line to the End of Follow-up in Subjects with Impaired Glucose Tolerance (IGT), Newly Diagnosed Non-Insulin-Dependent Diabetes Mellitus (New NIDDM), NIDDM and Normal Urinary Albumin Excretion (Normoalbuminuria), NIDDM and Microalbuminuria, and NIDDM and Macroalbuminuria.

Each arrow connects the value at the base-line examination and the value at the end of follow-up. The dashed line indicates the time of diagnosis, and the shaded area the 25th through 75th percentiles of values in subjects with normal glucose tolerance. Albumin was measured in milligrams per liter and creatinine in grams per liter.

Several other factors directly related to hyperglycemia also may be important, including the intracellular accumulation of sorbitol and the formation of glycosylated proteins (Passariello N, 1993). The enzyme aldose reductase converts intracellular glucose to sorbitol, which then accumulates within the cells. Studies in hyperfiltering humans with type 1 diabetes have shown that the chronic administration of an aldose reductase inhibitor (tolrestat) lowers the GFR toward normal.

Stage II is defined by the presence of detectable glomerular lesions in patients with normal albumin excretion rates and normal blood pressure levels. Normoalbuminuric patients with more severe glomerular lesions might be at increased risk of progression. Patients can remain in stage 2 for the remainder of their lives. However, those in whom nephropathy is destined to progress further will at this stage exhibit a loss of the normal nocturnal blood pressure decline (i.e., night/day ratios >0.9 and non-dipping) as an early diabetic nephropathy indicator that often precedes the development of persistent microalbuminuria.

Microalbuminuria, typically occurring in 2 to 5 percent of patients per year, defines **stage III.** Patients with microalbuminuria are referred to as having incipient nephropathy. Microalbuminuria (a sign of endothelial dysfunction that is not necessarily confined to the kidney) may be present earlier, particularly during adolescence and in patients with poor

glycemic control and high-normal blood pressure levels. Compared with normoalbuminuric patients, patients with persistent microalbuminuria have threefold to fourfold greater risk of progression to proteinuria and ESRD. Current studies indicate that between 20% and 45% of microalbuminuric type 1 diabetic patients will progress to proteinuria after about 10 years of follow-up, whereas 20% to 25% will return to normoalbuminuric levels (Hovind P, 2004) and the rest will remain microalbuminuric. At this stage, glomerular lesions are generally more severe than in the previous stages, and blood pressure tends to be increasing, often into the hypertensive range. Other laboratory abnormalities, such as increased levels of cholesterol, triglycerides, fibrinogen, Von Willebrand's factor, and prorenin, can be detected in some patients. Diabetic retinopathy, lower extremity amputation, coronary heart disease, and stroke are also more frequent in this group.

The normal rate of albumin excretion is less than 20 mg/day (15 μg/min); persistent albumin excretion between 30 and 300 mg/day (20 to 200 μg/min) is called microalbuminuria and, in patients with type 1 diabetes, persistent microalbuminuria may be indicative of early diabetic nephropathy unless there is some coexistent renal disease.

It was initially thought that microalbuminuria precedes the loss of glomerular filtration rate (GFR) in patients with type 1 diabetes. However, some patients with normoalbuminuria or microalbuminuria have significant reductions in GFR prior to the development of macroalbuminuria. Loss of renal function, which was defined as an estimated decrease in GFR of more than 3.3 percent per year, occurred in 9 percent of patients with normoalbuminuria and 16 percent with regression of microalbuminuria. Loss of renal function occurred much more frequently (32 and 68 percent) in patients with stable or progressive microalbuminuria, respectively.

Patients with newly diagnosed type 2 diabetes in which 6.5 percent had microalbuminuria and 0.7 percent had macroalbuminuria at the time of diagnosis. The rate of microalbuminuria at the time of diagnosis of type 2 diabetes may be higher in older patients. There are at least two possible explanations for the presence of microalbuminuria at the time of diagnosis of type 2 diabetes: the patients had previously undiagnosed diabetes or some other disease was responsible for the microalbuminuria. Forty to 50 percent of patients with type 2 diabetes who have microalbuminuria eventually die of cardiovascular disease; this is three times as high a rate of death from cardiac causes as among patients who have diabetes but have no evidence of renal disease.

As with type 1 diabetes, some patients with microalbuminuria and type 2 diabetes regress to normoalbuminuria. At six years, regression occurred in 51 percent, while progression to macroalbuminuria occurred in 28 percent. Several factors (short duration of microalbuminuria, better glycemic and blood pressure control, and the use of ACE inhibitors or angiotensin II receptor blockers) were independently associated with remission.

Stage IV occurs after 10 to 20 years of diabetes and is characterized by the presence of dipstick-positive proteinuria: proteinuria of greater than 300 mg/d. Hypertension is present in about 75% of these patients, and reduced GFR and dyslipidemia are also common. Retinopathy and peripheral and autonomic neuropathy are present in most patients. In addition, the risk for cardiovascular events is extremely high, and asymptomatic myocardial ischemia is frequent. Without therapeutic interventions, GFR declines by about 1.2 mL/min/month in proteinuric type 1 diabetic patients. In type 2 diabetic patients, Once macroalbuminuria is present, creatinine clearance declines at a rate that varies widely from patient to patient; the average reduction is 10 to 12 ml per minute per year in untreated patients. Hypertension and proteinuria may accelerate the decline in the glomerular filtration rate and the progression to end-stage renal disease.

Progression to ESRD (**stage V**) occurs 5 to 15 years after the development of proteinuria. Renal replacement therapy—either dialysis or transplantation is required at this stage.

4. Pathogenesis

Diabetic nephropathy occurs as a result of a complex yet incompletely understood interaction between hemodynamic and metabolic factors (Cooper, M., 2001). Hemodynamic factors that contribute to the development of diabetic nephropathy include increased systemic and intraglomerular pressure, as well as activation of vasoactive humoral pathways including the renin angiotensin system and endothelin (G.M. Hargrove, 2000). These hemodynamic pathways activate intracellular second messengers such as protein kinase C (PKC), Mitogen-activated protein (MAP kinase) (M. Haneda, 1997), nuclear transcription factors such as NF-kB and various growth factors such as the prosclerotic cytokine, TGF-β and the permeability enhancing growth factor, vascular endothelial growth factor, VEGF.
Glucose dependent pathways are also activated within the diabetic kidney and result in enhanced oxidative stress, renal polyol formation (Dunlop ME, 2000) and the accumulation of advanced glycation end products (AGEs). In combination, these pathways ultimately lead to increased renal albumin permeability and extracellular matrix accumulation, resulting in increasing proteinuria, glomerulosclerosis and ultimately tubulointerstitial fibrosis.

5. Hemodynamic pathways

Glomerular hyperperfusion and hyperfiltration are the early signs resulting from decreased resistance in both the afferent and efferent arterioles of the glomerulus. Afferent arteriole seems to have a greater decrease in resistance than the efferent, which in fact may have increased resistance. Many factors have been reported to be involved in this faulty autoregulation, including nitric oxide, prostanoids, vascular endothelial growth factor (VEGF), TGF-β1, and the renin angiotensin system, specifically angiotensin II. These early hemodynamic changes predispose to albumin leakage from the glomerular capillaries and overproduction of mesangial cell matrix, as well as thickening of the glomerular basement membrane and injury to podocytes (Ziyadeh, F 2008). In addition, increased mechanical strain from these hemodynamic changes can induce localized release of certain cytokines and growth factors (Wolf, G.F.N, 2007)

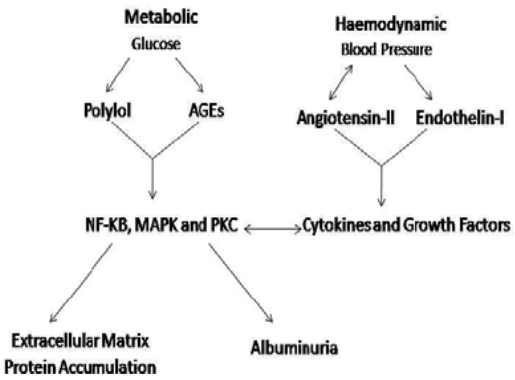


Fig. 7. Interaction of hemodynamic and metabolic pathway, cytokines and intracellular signaling molecules mediating diabetic nephropathy

The action of vasoactive hormones, such as angiotensin II and endothelin are mediator of renal hemodynamic changes. Glomerular hypertension and hyperfiltration contribute to the development of diabetic nephropathy because use of renin–angiotensin blockers preserves kidney function and morphology. Blockade of the renin-angiotensin–aldosterone system antagonizes the profibrotic effects of angiotensin II by reducing its stimulation of TGF-β1 (Hilker, KF, 2005). Support that such profibrotic effects underlie diabetic nephropathy has also been provided by study of an animal model of diabetic nephropathy (Nagai Y, 2005). Transient blockade of the renin–angiotensin system (for 7 weeks) in prediabetic rats reduced proteinuria and improved glomerular structure. Additionally, the administration of an angiotensin converting-enzyme inhibitor to patients with type 1 diabetes and nephropathy appears to reduce serum concentrations of TGF-β1. A correlation exists between decreased levels of TGF-β1 in serum and urine and renal protection, as determined by changes in the glomerular filtration rate.

5.1 Renin-angiotensin system in diabetic nephropathy

The renin-angiotensin system (RAS) has been extensively studied in diabetes. Earlier studies centered on the systemic RAS, and the data obtained have been conflicting, with stimulation, suppression, and no change in the system being reported (Wolf, G, 2007). The factors that influence the systemic RAS in addition to the different stages of disease and species studied may explain many of these divergent findings. However in various diabetic models, increased renal renin content relative to plasma renin levels has generally been found, thus suggesting impaired renal renin release into the circulation. In clinical diabetic nephropathy, there is decreased plasma rennin activity that may be due to nonenzymatic glycation of prorenin with decreased conversion to active renin. Thus, diabetic nephropathy has traditionally been considered a "low renin" state. However, plasma renin activity may not accurately reflect activity of the RAS in the kidney.

Another problem has been the difficulty of accurate measurement of plasma angiotensin-II (Ang II), which is an important issue because discordance can exist between plasma renin and Ang II levels. More recently, the intrarenal RAS has been the focus of extensive study. Abundant evidence indicates the existence of local tissue RASs that are regulated independent of plasma RAS (Ballerma, B.J., 1984). It was reported that glomerular Ang II receptors decrease in the diabetic rat 3 to 4 weeks after induction of the disease. Downregulation of glomerular Ang II receptors implies that intra- renal Ang II generation may be increased. The density of Ang II receptors in the proximal tubules was reported to be reduced in diabetic rats and was accompanied by decreased mRNA expression for the AT1 receptor (Cheng, H.F, 1994). Recently, AT1 receptor density has also been shown to be decreased in mesangial cells when incubated in high-glucose media (Amiri F, G, 1999). ACE activity in whole kidney is low in diabetes.

However, this is probably due primarily to mesangial RAS. Staining for ACE has been found to be enhanced in glomeruli and vasculature of diabetic rats and in patients with diabetic nephropathy (Mizuiri, S., 1998). These data suggest that the term "intrarenal" RAS is oversimplistic, in as much as the vascular RAS (vessels and glomeruli) appears to be regulated differently from the tubulointerstitial RAS. Angiotensin receptor blockers (ARBs) enhance the renal vasodilation in patients with diabetes (despite the presence of low plasma rennin activity), again supporting the concept that the renal vascular RAS is activated in several intrarenal compartments including the glomeruli by several orders of magnitude higher than those found systemically. This shows the existence of both local RAS acting is

independently of the systemic RAS and also is consistent with the finding that in most renal cell culture studies, effects of Ang II are observed at substantially higher concentrations (about 0.01-1.0 mmol/L) than those found in the systemic circulation.

5.2 Vaso-active hormones

Endothelium is an interior covering of blood vessels. There are various biological functions of endothelium and it regulates vascular tone and maintains free flow of blood in vessels (Escandon, J.C, 2001). The luminal surface of every blood vessel, forms a physical and metabolic barrier to circulating elements. The endothelium is an important endocrine organ and releases a number of vasoactive hormones, including endothelin (ET-1) (Ulker, 2003), endothelium-derived hyperpolarizing factor (EDHF: nitric oxide and prostacyclin). Endothelin-1 is a potent vasoconstrictor, Endothelium-derived hyperpolarizing factor is still a controversial subject of vascular biology. Endothelial cells of every blood vessel release nitric oxide and prostacyclin and they form a particular partnership in the regulation of vascular and platelet function.

5.3 Nitric Oxide

Nitric Oxide (NO), originally identified as "endothelial derived relaxing factor," is a ubiquitously utilized signaling molecule that regulates a wide variety of organ and cellular functions, including renal hemodynamics and salt and water regulation. NO is generated enzymatically from the amino acid L-arginine by one of three specific nitric oxide synthases: "neuronal"(NOS1 or nNOS), "endothelial" (NOS3 or eNOS), or "inducible" (NOS2 or iNOS). Many, but not all, of the intracellular signaling pathways activated by NO are mediated by activation of guanylate cyclase, which increases intracellular levels of cyclic guanosine monophosphate.

All three NOS isoforms are present in the mammalian kidney, with both distinct and overlapping patterns of distribution. In normal kidney, NOS1 is highly expressed in the macula densa and glomerular parietal epithelium, as well as in the medulla in the collecting ducts and thin ascending limb. NOS2 is expressed in the endothelium of glomerular capillaries and afferent and efferent arterioles, renal arteries, and descending vasa recta, as well as in proximal tubule and medullary thick ascending limb. NOS3 is also expressed in tubules, including S3 segments of the proximal tubule, medullary thick ascending limb, and collecting duct, in addition to arcuate arteries and vasa recta bundles (Kone, BC, 1997). Both in vivo and in vitro studies have provided conflicting results regarding NOS expression and NO production in diabetes. Most but not all studies in cultured renal cells have determined decreased NO production in response to hyperglycemia (Komers R, 2003).

It has been established for a long time that the principal risk factors that affect the development and progression of diabetic nephropathy includes uncontrolled hyperglycemia, hypertension (systemic and glomerular) and activation of RAAS. All these three factors have been shown to modulate intra renal NO generation either directly or through signaling pathways. The role of NO in affecting the renal structure and function is very complicated and depends on several factors including the stage of diabetic renal disease, isoforms of NOS involved, structures in the kidney, and influence of other factors in diabetic milieu. The complex metabolic milieu in diabetes triggers several pathophysiological mechanisms that simultaneously stimulate and suppress intrarenal NO production. The net effect on renal NO levels depends on the mechanisms that prevail in a given stage of the disease process.

5.3.1 Dual role of Nitric Oxide in diabetic nephropathy

The currently available evidence enables us to reasonably conclude that early diabetic nephropathy is associated with increased renal NO production mediated primarily by constitutively released NO through eNOS or NOS III activation. There is some contribution to this augmented NO production through nNOS (NOS I) derived enhanced synthesis, particularly from macula densa region of the kidney. Together the increased intrarenal NO generation contributes to the development of glomerular hyperfiltration and microalbuminuria that characterize early diabetic nephropathy.

On the other hand, advanced diabetic nephropathy with severe proteinuria, hypertension and renal failure is associated with a state of progressive NO deficiency. As the duration of diabetic state increases, factors that suppress NO bioavailability prevail. Many factors including activation of protein kinase C, activation of TGF-beta, NO quenching by advanced glycosylation end products (AGE) contribute to the NO deficient state – either directly or by inhibiting and/or by post translational modification of activity of NOS isoforms. Other inhibitors of NOS enzyme such as asymmetric dimethylarginine (ADMA) accumulate in diabetic nephropathy and may contribute to progression of DN and such association has also been observed in other microvascular complications such as retinopathy. Most of these changes are mediated by endothelial and partly inducible NOS in the chronic advanced stage of DN. Progressive loss of renal parenchyma also contributes partially to the NO deficiency since kidney is a major source of L-arginine, the sole precursor of NO. These changes and the factors affecting them are discussed well in a review (Prabhakar 2005) and schematically represented in the figure below.

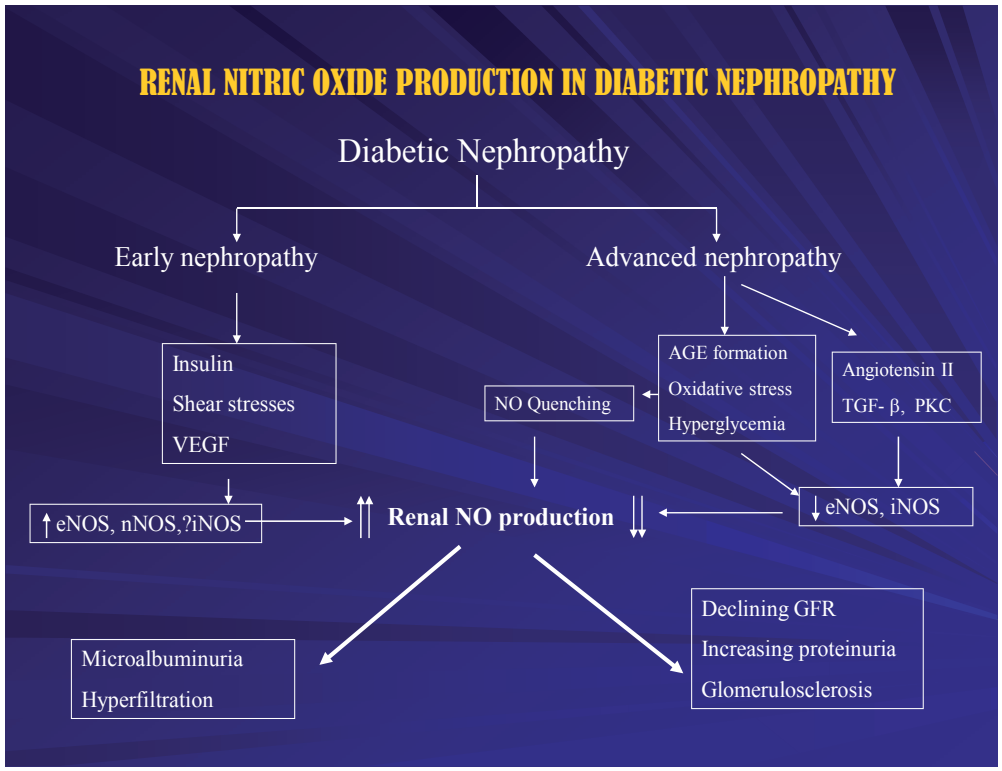


Fig. 8. Role of Nitric Oxide in Diabetic Nephropathy

In a recent study, the natural history of renal manifestations have been described in ZSF_1 rats, a recently developed rodent model of type 2 diabetes who developed obesity and hyperglycemia by 20 weeks of age on a high-carbohydrate diet. They also developed systolic and diastolic hypertension, hypercholesterolemia, profound hypertriglyceridemia, proteinuria, and renal failure. Renal histology demonstrated changes consistent with early diabetic nephropathy, including arteriolar thickening, tubular dilation and atrophy, glomerular basement membrane thickening, and mesangial expansion. Furthermore, renal nitric oxide production was decreased, and homogenates from renal cortices demonstrated reduced expression of renal endothelial and inducible nitric oxide synthases. These changes were associated with increased urinary levels and renal expression of 8-hydroxydeoxyguanosine, an indicator of mitochondrial oxidative stress, as well as with increased renal peroxynitrite formation. Administration of either insulin or the antioxidant alpha-lipoic acid decreased proteinuria and oxidative stress, but only the former slowed progression of renal failure (Prabhakar 2007).

5.4 Prostacyclin

The first step in prostacyclin synthesis is the liberation of arachidonic acid from membrane-bound lipids via the enzymatic actions of phospholipase A2 (PLA2). In endothelial cells, phospholipase A2 activation is a calcium-dependent step. Once liberated, arachidonic acid is available for metabolism by cyclooxygenase (COX). Cyclooxygenase is present in two isoforms: COX-1 and COX-2. Cyclo-oxygenase-1, like NOSI or NOSIII, is constitutively expressed, while COX-2, like NOSII, is induced at sites of inflammation and/or by PAMPs. In healthy endothelial cells, COX-1 is the predominate isoform. Cyclooxygenase has two enzymatic activities: firstly, an oxygenase step forms prostaglandin (PG) G2; and secondly, a peroxidase step, which forms PGH2 from PGG2. Prostaglandin H2 is the substrate for a range of downstream prostaglandin synthase enzymes, including prostacyclin synthetase (PGIS), the actions of which result in the formation of prostacyclin. Endothelial cells are enriched in cyclooxygenase-1(COX-1) and PGIS, which is why, when phospholipase A2 is activated, prostacyclin is the predominant metabolite made. It is important to note that in platelets, which also express predominantly COX-1, thromboxane is the principal product made. This is because platelets express mainly thromboxane synthase with negligible levels of PGIS.

5.5 Endothelin1

Endothelin-1 (ET-1) is a potent vasoconstrictor peptide produced by vascular endothelium from big ET-1 (Xu 1994) via specific cleavage by endothelium converting enzyme (ECE). ET-1 produces its actions by acting on endothelin ETA and ETB receptors (Haynes, 1993). ETA receptor predominates in vascular smooth muscle cells and mediates vasoconstriction in both large and small blood vessels where as ETB receptors on endothelial cells mediate vasodilation through the production of nitric oxide and prostacyclin (Verhaar, MC, 1998). ET-1 is involved in the pathogenesis of cardiovascular disorders such as hypertension and heart failure including diabetic nephropathy (Benigni 1998). Diabetes mellitus induces the renal overexpression of ET-1 in the glomeruli and tubular epithelial cells leading to progression of diabetic nephropathy. It was shown that diabetes-induced elevated level of renal ET-1 might induce glomerular hyperperfusion and damage tubulointerstitium in rats. The diabetes-induced elevated level of renal ET-1 was noted to accelerate the progression of diabetic nephropathy in rats31. It has been documented that ET-1 activates a variety of

signaling systems to induce contraction, hypertrophy, proliferation, and extracellular matrix accumulation in mesangial cells (Sorokin, 2003). The detrimental role of ET-1 in pathogenesis of diabetic nephropathy has been confirmed by the fact that diabetes induced elevated level of renal ET-1 is associated with an expansion of mesangial cells and collagen deposition in the glomeruli of diabetic mice. It has been recently demonstrated that ET-1 mediated activation of ETA receptor induced the renal TGF-β production and inflammation in diabetic rats (Sasser, 2007). Treatment with CPU0213, a dual ETA/ETB receptor antagonist has been found to improve the renal function in rats with diabetic nephropathy by suppressing Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, suggesting that ET-1 contributes to the pathogenesis of diabetic nephropathy via upregulation of NADPH oxidase mediated ROS production in renal cells (Xu, M., 2009). Thus under pathological conditions, prevention of endothelin-mediated various signaling pathways may provide an alternative approach to treat diabetic nephropathy.

5.6 Urotensin

Urotensin II (UII) is an 11-amino acid vasoactive peptide, recently identified as the ligand for a novel G protein-coupled receptor, GPR-14 (renamed urotensin receptor [UT]). In addition to its potentvasoconstrictive actions, UII also has trophic and profibrotic effects, leading to its implication in the pathogenesis of heart failure. However, it has been noted that elevated plasma levels of UII in association with renal impairment and diabetes and diabetic nephropathy. Urotensin-II, an endogenous vasoconstrictor, has been suggested to be involved in the pathogenesis of vascular endothelial dysfunction (VED) (Maguire, J.J., 2002). Urotensin-II increases the activity of NADPH oxidase and plasminogen activator inhibitor-1 (PAI-1) and cause decrease in endothelium dependent relaxation (Watanabe, T., 2006). The overexpression of urotensin-II in endothelial cells cause VED by increasing the expression of type 1 collagen and formation of ROS.

6. Metabolic pathway

Advanced glycosylation end products or AGEs are a chemically heterogeneous group of compounds formed as a result of the ''Maillard reaction'' when reducing sugars react non-enzymatically with amine residues, predominantly lysine and arginine, on proteins, lipids and nucleic acids. While the initial stage of the reaction leading to the formation of reversible glycosylation proteins termed Schiff bases is rapid and glucose dependent, a much slower reaction over a period of days results in the formation of the more stable Amadori product. These early glycosylation products accumulate predominantly on long lived proteins such as vessel wall collagen and crystallines (Brownlee, 2001), undergoing a series of in vivo rearrangements to form irreversible, complex compounds and cross-links, termed AGEs.

Once AGE related cross-links form on proteins, they become resistant to proteolytic degradation (Thomas, 2005). As well as their non-receptor mediated effects; AGES can exert their biological effects through receptor-mediated mechanisms, the most important of which is the receptor for advanced glycation end products (RAGE). RAGE is a signal transduction receptor that belongs to the immunoglobin superfamily and is expressed on a number of cell types including monocytes/macrophages, endothelial cells, renal mesangial cells and podocytes (Yan, S.F., 2004). Binding of AGEs to the RAGE receptor activates a number of pathways implicated in the development of diabetic complications, specifically diabetic

renal disease. These include enhanced cytosolic reactive species formation, stimulation of intracellular molecules such as PKC and NF-kB and the activation and expression of a number of growth factors and cytokines such as TGF-β and VEGF (Wendt, T., 2003). Indeed, strategies to inhibit the formation of AGEs have been shown to ameliorate diabetic nephropathy.

In the initial study by Soulis-Liparota et al. aminoguanidine, an inhibitor of AGE formation, which acts by scavenging intermediates in the advanced glycation pathway attenuated the rise in albuminuria observed in diabetic rodents, while preventing increases in collagen related fluorescence in isolated glomeruli and renal tubules (Soulis-Liparota, T. 1991). Similar results have been obtained with alagebrium, a putative AGE cross-link breaker. In an experimental study, both aminoguanidine and alagebrium attenuated the albuminuria observed in diabetic rodents (Forbes, J.M., 2003). Furthermore, alagebrium was also beneficial when used as part of a delayed intervention protocol, suggesting that it may be useful in both preventing and retarding diabetic nephropathy (Ziayadeh, F.N., 1991).

A subsequent study confirmed and extended these findings. The early (weeks 16–32) and late (weeks 24–32) administration of alagebrium was again shown to reduce albuminuria in a type 1 diabetic rodent model. As a number of previous groups had demonstrated increases in collagen and other extracellular matrix components in experimental diabetic nephropathy, this study also sought to determine the mechanisms surrounding the improvements in microalbuminuria in diabetic rodent kidneys. The compound was shown to reduce diabetes induced increases in the gene expression of TGF-β1, connective tissue growth factor (CTGF) and collagen IV. Early treatment with alagebrium was also associated with significant structural improvement in the kidney including a reduction in the glomerulosclerotic index and tubulointerstitial area, in conjunction with a reduction in AGE peptide fluorescence in serum and the kidney. Furthermore, a reduction in renal accumulation of the specific AGE, carboxymethylysine (CML) and decreased RAGE immunostaining was also seen, providing further evidence that accumulation of AGEs is implicated in renal extracellular matrix accumulation in diabetes.

In the setting of diabetes mellitus and long-term hyperglycemia, nonenzymatic modification of proteins (or lipids) by glucose, or its metabolic products, results in their stable modification and altered function. This process is thought to underlie a major pathogenic pathway leading to tissue injury in diabetes. A major pathway for AGE formation involves triose phosphate intermediaries derived from metabolism of glucose. Triose phosphates build up as intracellular glucose increases and can nonenzymatically form the early glycosylation product methyglyoxal by spontaneous decomposition (Degenhardt 1998 and Frye 1998). Amine-catalyzed sugar fragmentation reactions then modify protein lysine residues directly, forming N- (epsilon) - (carboxymethyl) lysine (CML), a major product of oxidative modification of glycated proteins. Alternatively, reaction of terminal amino groups (e.g., on lysine) with glucose itself may form from early glycation products (i.e., Amadori products) that rearrange to produce stable moieties that possess distinctive chemical crosslinking and biologic properties, designated AGEs (Cohen 2003 and Vlassara 1981). Other glucosederived Amadori products and fructose are thought to be potential precursors of 3-deoxyglucosone (3-DG) in vivo. Fructose generated by the aldose reductase pathway is converted into fructose-3- phosphate by the action of 3-phosphokinase (3- PK). This leads to the generation of 3-deoxyglucosone, a central precursor in the generation of an array of AGEs, in particular, CML-adducts and others (3-Deoxyglucosone, 1999) 3-DG can further react with proteins to form pyrrolines or pentosidine.

AGEs have been suggested to represent a general marker of oxidative stress and long-term damage to proteins in aging, atherosclerosis, and diabetes (Wendt T.M., 2003). Renal CML-AGE is increased in diabetes. Immunolocalization of CML in skin, lung, heart, kidney, intestine, intervertebral discs, and particularly in arteries provide evidence for age-dependent increases in CML accumulation in distinct locations, and acceleration of this process in diabetes (Schleicher, E.D., 1997). Immunostaining and immunoblots of diabetic human kidneys show increased CML in diabetic glomeruli, especially in the mesangial matrix and capillary walls (Nathan, D.M., 2005).

6.1 Oxidative stress

Generally, large amount of reactive oxygen species are generated with in nephron by metabolic activity that is counter balanced by a large number of antioxidant enzymes and free radical scavenging systems. Peroxidation of cell membrane lipids, oxidation of proteins, renal vasoconstriction and damage to DNA are the negative biological effects of reactive oxygen species. Unfortunately, hyperglycemia tips the balance towards production of reactive oxygen species, most of which seem to be generated in the mitochondria (Nishikawa, T. 2007). The metabolism of glucose through destructive alternate pathways, such as via PKC activation and advanced glycation end-product formation, in the setting of hyperglycemia also seems partly dependent on reactive oxygen species. Oxidative stress specifically induced by hyperglycemia even before diabetes becomes clinically apparent. DNA damage marker induced by reactive oxygen species is higher in patients with severe nephropathy (i.e. proteinuria versus microalbuminuria). Diabetic nephropathy is linked with severe oxidative stress. This pathway may be responsible for the decreased bioavailability of nitric oxide in the kidney (Prabhakar 2007).

6.2 Reactive oxygen species

Diabetic nephropathy is characterized by excessive deposition of extracellular matrix (ECM) in the kidney, leading to glomerular mesangial expansion and tubulointerstitial fibrosis. Clinical studies have demonstrated that high blood glucose is the main cause of initiation and progression of diabetic vascular complications including nephropathy. High reactive oxygen species (ROS) induced by glucose upregulates TGF-β1 and extra cellular matrix protein (ECM) expression in the glomerular mesangial cells. Hyperglycemia induced ROS generation and ROS-activated signal transduction cascade and transcription factors and overexpression of genes and proteins in glomerular mesangial and tubular epithelial cells lead to ECM accumulation in diabetic kidney.

6.3 Nephrin

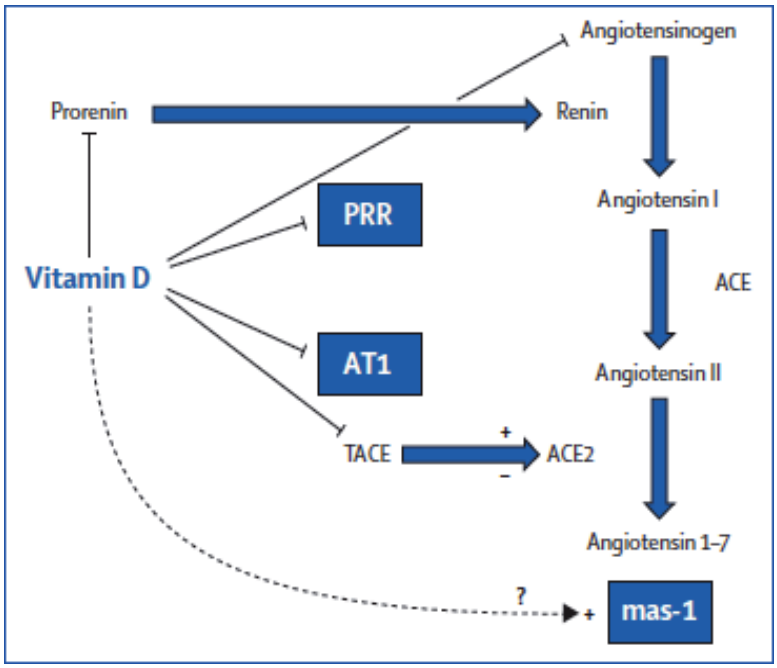
Podocytes (specialized visceral epithelial cells) are important for the maintenance of the dynamic functional barrier (Mundel 2002). Nephrin, a protein found in these cells, is crucial for maintaining the integrity of the intact filtration barrier. The renal expression of nephrin might be impaired in diabetic nephropathy. Patients with diabetic nephropathy have markedly reduced renal nephrin expression and fewer electron-dense slit diaphragms compared with patients without diabetes and minimal nephropathic changes or controls (Benigni. 2004). Furthermore, nephrin excretion is raised 17-30% in patients with diabetes (with and without albuminuria) compared with that in individuals without diabetes.

Thus, nephrin excretion could be an early finding of podocyte injury, even before the onset of albuminuria (Kobayashi, 2006). Treatment with blockers of the renin-angiotensin-

aldosterone system might help protect nephrin expression. In a study of patients with type 2 diabetes, treatment with an angiotensin-converting-enzyme inhibitor for 2 years maintained nephrin expression at control levels compared with that in untreated patients with diabetes. By contrast, the expression of two other important podocyte and slit diaphragm proteins, podocin and CD2AP, was similar in the three groups. Comparable decreases in renal nephrin expression were reported in other studies of diabetic nephropathy.

6.4 Vitamin D

Vitamin D has a role beyond the regulation of calcium metabolism. There is evidence that the vitamin D system is also involved in regulation of the immune system, cell growth, and differentiation. Vitamin D binds to its nuclear receptor, and later to the vitamin D response element of target genes, to regulate gene transcription. It also interacts with pathways that are germane to the development and progression of diabetic complications, including the renin–angiotensin system (RAS), hypertension, inflammation, and albuminuria. Three recent randomized trials have shown that the vitamin D analogue—19-nor-1-α-dihydroxyvitamin D2 (paricalcitol) can reduce proteinuria in patients with chronic kidney disease, including those with diabetes.



ACE=angiotensin-converting enzyme.
AT1=type 1 angiotensin receptor.
PRR=(pro)renin receptor.
ACE2=angiotensin-converting enzyme 2. TACE=tumor necrosis factor α converting enzyme. mas-1=receptor for angiotensin 1–7.

Fig. 9. Interactions between vitamin D and key elements of the renin–angiotensin system

Vitamin D is a negative regulator of RAS (Li YC, 2002). In experimental chronic kidney disease, paricalcitol reduces the renal expression of renin, the (pro)renin–receptor,

angiotensinogen, and the type 1 angiotensin receptor (Freundlich M, 2008). Vitamin D also inhibits tumor necrosis factor α converting enzyme (TACE) (Dusso A, 2010), which regulates the shedding of angiotensin-converting enzyme 2 (ACE2), itself the major enzyme that metabolizes angiotensin II in the proximal tubule. Diabetes is associated with reduced ACE2 expression; therefore inhibition of TACE expression might improve the balance of RAS in the kidney, and might have additional renoprotective effects by inhibition of the TACE-dependent release of other pathogenic mediators.

Vitamin D has several anti-inflammatory actions, including effects on prostaglandin synthesis, inhibition of nuclear factor κB signaling, and innate immunity, all of which have been implicated in diabetic chronic kidney disease. Vitamin D deficiency is associated with raised concentrations of C-reactive protein. In previous studies, paricalcitol reduced C-reactive protein concentrations in patients with chronic kidney disease, which paralleled the decline in albuminuria

Patients with diabetes have increased rates of vitamin D deficiency (Tanaka H, 2009) especially in those with chronic kidney disease in whom the urinary loss of protein-associated vitamin D magnifies reduced activation of vitamin D by the proximal tubule, and reduced expression of the vitamin D receptor. For these patients, it seems rational to replace vitamin D. Selective analogues that restore vitamin D receptor signaling without risking hypercalcemia or hyperphosphatemia might have particular advantages, because of the aberrant calcification of diabetic vessels. Of note, vitamin D replacement reduced proteinuria in about half of diabetic patients with stage 3 or 4 chronic kidney disease in a placebo-controlled trial (Teng M 2003)

7. Histopathology

The pathogenesis of diabetic nephropathy is complex, and renal pathological lesions are diverse. Most likely, there are many pathogenic pathways, which through composite, interactive routes lead to the histological damage that we see in renal biopsies of patients with diabetic nephropathy (Elisabeth J.J, 2011). Though the number of patients with type 2 diabetes in a worldwide context is increasing and is predicted to be 438 million in 2030, paradoxically, diabetic nephropathy is probably becoming the renal disease per se for which the least renal biopsies are performed. In many centers, clinical parameters in the absence of a renal biopsy will diagnose the patient with diabetic nephropathy. Only if unusual signs or symptoms are present, such as sudden onset nephrotic syndrome, will a renal biopsy be performed, mostly with the aim to exclude other causes than diabetic nephropathy for the patient's clinical presentation. This means that in relation to diabetic nephropathy, co-morbidity is often seen by the pathologist in the renal biopsy, and cases in which diabetic nephropathy alone is present become less frequent. In many recent publications, the diagnosis of diabetic nephropathy was based on clinical symptoms, which, in many studies, also formed the gold standard on the evaluation of intervention therapies meant to prevent, slow down, or even reverse the processes causing diabetic nephropathy.

7.1 Histopathological classification system

Up to 2010, the terminology for histopathological lesions in diabetic nephropathy was variable. The new classification launched in 2010 (Thijs W, 2010) distinguishes four classes, essentially characterized by the absence of histological lesions (class I), mesangial changes (class II), nodular lesions (class III), or a predominance of global glomerulosclerosis (class IV).

Class	Description	Inclusion Criteria
I	Mild or nonspecific LM changes and EM-proven GBM thickening	Biopsy does not meet any of the criteria mentioned below for class II, III, or IV GBM > 395 nm in female and >430 nm in male individuals 9 years of age and older[a]
IIa	Mild mesangial expansion	Biopsy does not meet criteria for class III or IV Mild mesangial expansion in >25% of the observed mesangium
IIb	Severe mesangial expansion	Biopsy does not meet criteria for class III or IV Severe mesangial expansion in >25% of the observed mesangium
III	Nodular sclerosis (Kimmelstiel–Wilson lesion)	Biopsy does not meet criteria for class IV At least one convincing Kimmelstiel–Wilson lesion
IV	Advanced diabetic glomerulosclerosis	Global glomerular sclerosis in >50% of glomeruli Lesions from classes I through III

LM, light microscopy.
[a]On the basis of direct measurement of GBM width by EM, these individual cutoff levels may be considered indicative when other GBM measurements are used.

Table 1. Histological classification of Diabetic glomerulopathy

7.1.1 Class I: Glomerular basement membrane thickening

The biopsy specimen shows no or only mild, nonspecific changes by light microscopy that do not meet the criteria of classes II through IV. By direct measurements with EM the glomerular basement membrane (GBM) on average is thicker than 430 nm in males 9 years and older and thicker than 395 nm in females. These cutoff levels are based on a deviation from normal GBM thickness plus 2 standard deviations as recently determined. Light microscopic changes in the GBM and epithelial foot process effacement by EM have no influence on the classification.

Class I incorporates cases with certain degree of chronic and other reactive changes (e.g., changes of arterionephrosclerosis, ischemic type changes, or interstitial fibrosis). Diagnosing DN in cases without characteristic light microscopic glomerular lesions may be difficult, especially when a thicker GBM is also seen with aging or hypertension. The presence of arteriolar hyalinosis may be helpful in these cases, although it is not a prerequisite.

GBM thickening is a characteristic early change in type 1 and type 2 DN and increases with duration of disease. GBM thickening is a consequence of extracellular matrix accumulation, with increased deposition of normal extracellular matrix components such as collagen types IV and VI, laminin, and fibronectin. Such accumulations result from increased production of these proteins, their decreased degradation, or a combination of the two. GBM thickening may already be present in type 1 diabetes patients who are normoalbuminuric. GBM thickening has even been described as a "prediabetic" lesion: In patients with proteinuria and isolated GBM thickening but without overt diabetes, 20% were positive on a blood test for diabetes at the time of biopsy, whereas 44% were diagnosed with diabetes at 6 months, and 70% at 2 years after the biopsy was taken. Long-term glucose control and urinary albumin excretion (UAE) correlate strongly with basement membrane thickness.

7.1.2 Class II: Mesangial expansion, mild (IIa) or severe (IIb)

Class II encompasses those patients classified with mild or severe mesangial expansion but not meeting inclusion criteria for class III or IV and is analogous to the previously used term "diffuse diabetic glomerulosclerosis." Mesangial expansion is defined as an increase in extracellular material in the mesangium such that the width of the interspace exceeds two mesangial cell nuclei in at least two glomerular lobules. The difference between mild and severe mesangial expansion is based on whether the expanded mesangial area is smaller or larger than the mean area of a capillary lumen. If severe mesangial expansion is seen in more than 25% of the total mesangium observed throughout the biopsy, the biopsy is classified as IIb. If this is not the case, but at least mild mesangial expansion is seen in more than 25% of the total mesangium, the biopsy is classified as IIa.

Expansion of cellular and matrix components in the mesangium is a hallmark of type 1 and type 2 DN. It can be detected in some patients within a few years after the onset of type 1 diabetes. When the mesangium expands, it restricts and distorts glomerular capillaries and diminishes the capillary filtration surface.

Various indices have been proposed to describe the amount of mesangial expansion in DN. Mauer et al. define mesangial expansion by mesangial fractional volume or volume density (Vv), defined as the fraction or percentage of the cross-sectional area of the glomerular tuft made up by mesangium, expressed in the formula: Vv (mes/glom). Using this formula, many correlations have been made between mesangial expansion and clinical parameters of DN, particularly showing highly inverse correlations exist between Vv (mes/glom) and GFR. There is also a relationship between Vv (mes/glom) and UAE and blood pressure.

Another index to express mesangial expansion is the so-called "index of mesangial expansion" (IME) for DN. The IME is determined by a semiquantitative estimate of the width of mesangial zones in each glomerulus: grade 0 is normal, 1 is twice normal thickness, 2 is three times normal thickness, and so forth; half grades can also be assigned. The mean of the grades for each glomerulus for IME can thus be determined from a single biopsy. The IME closely correlates with the Vv (mes/glom).

In other classifications, mesangial expansion is defined in more practical ways, such as in the new classification for IgA nephropathy in which it is defined as an increase in the extracellular material in the mesangium such that the width of the interspace exceeds two mesangial cell nuclei in at least two glomerular lobules.

7.1.3 Class III: Nodular sclerosis (Kimmelstiel–Wilson lesions)

If at least one convincing Kimmelstiel–Wilson lesion is found and the biopsy specimen does not have more than 50% global glomerulosclerosis it is classified as class III. Kimmelstiel–Wilson lesions appear in type 1 and type 2 diabetes (only one-third of microalbuminuric type 2 diabetic patients had them) as focal, lobular, round to oval mesangial lesions with an acellular, hyaline/matrix core, rounded peripherally by sparse, crescent-shaped mesangial nuclei. Compared to type 1 diabetes, type 2 diabetes

Paul Kimmelstiel and Clifford Wilson first described nodular lesions in glomeruli from eight maturity-onset diabetes patients in 1936. According to Cameron, they barely noted the association with diabetes, and it was Arthur Allen who clarified the association in 105 patients with diabetes in 1941. Nodular sclerotic lesions may also occur in the absence of DN that are clinically related to hypertension, smoking, hypercholesterolemia, and extrarenal vascular disease.

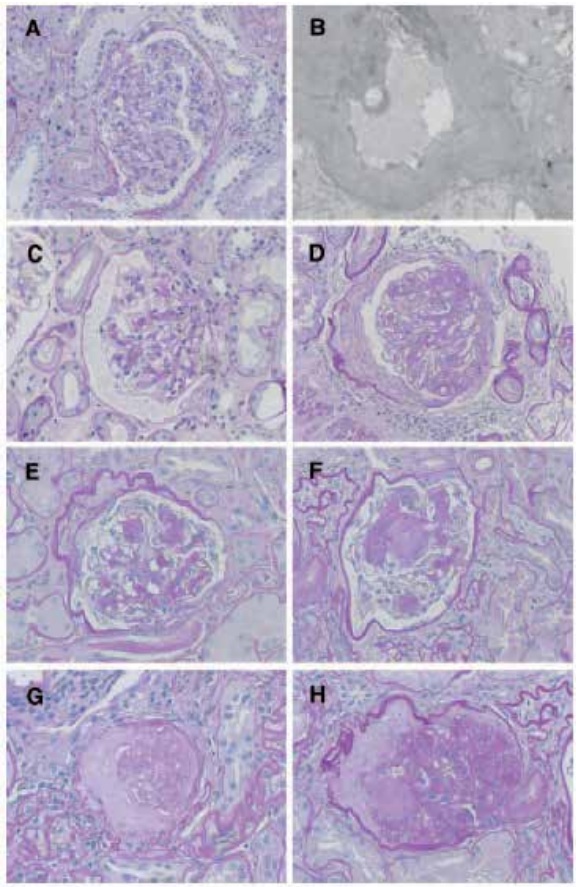


Fig. 10. Representative examples of the morphologic lesions in DN:
(A) Glomerulus showing only mild ischemic changes, with splitting of Bowman's capsule. No clear mesangial alteration.
(B) EM of this glomerulus: the mean width of the GBM was 671 nm (mean taken over 55 random measurements). EM provides the evidence for classifying the biopsy with only mild light microscopic changes into class I.
(C, D) Class II glomeruli with mild and moderate mesangial expansion, respectively. In panel C, the mesangial expansion does not exceed the mean area of a capillary lumen (IIa), whereas in panel D it does (IIb).
(E, F) In panel F is a class III Kimmelstiel–Wilson lesion. The lesion in panel E is not a convincing Kimmelstiel–Wilson lesion, therefore (on the basis of the findings in this glomerulus) the finding is consistent with class IIb. For the purpose of the classification, at least one convincing Kimmelstiel–Wilson (as in panel F) needs to be present.
In panel H, signs of class IV DN consist of hyalinosis of the glomerular vascular pole and a remnant of a Kimmelstiel–Wilson lesion on the opposite site of the pole.
Panel G is an example of glomerulosclerosis that does not reveal its cause (glomerulus from the same biopsy as panel H).
For the purpose of the classification, signs of DN should be histopathologically or clinically present to classify a biopsy with global glomerulosclerosis in > 50% of glomeruli as class IV

It is claimed that in the initial stage of developing nodular sclerotic lesions in DN, two important processes take place: lytic changes in the mesangial area called mesangiolysis and detachment of endothelial cells from the GBM. Exactly how these two processes relate remains uncertain. Paueksakon et al. detected fragmented red blood cells in Kimmelstiel–Wilson lesions, which supports the theory that microvascular injury contributes to the pathogenesis of these lesions. Dissociation of endothelial cells may disrupt the connections between the mesangial area and the GBM. This process precedes expansion of the Kimmelstiel–Wilson lesion. These lesions consist of an accumulation of mesangial matrix with collagen fibrils, small lipid particles, and cellular debris.

A completely developed Kimmelstiel–Wilson lesion destroys the normal structure of glomerular tuft with a decrease in mesangial cells, especially in the central area. In 1992, a graphic method of analysis of the position of Kimmelstiel–Wilson lesions demonstrated the nodules were distributed in a horseshoe-shaped area corresponding to the peripheral or intralobular mesangium, excluding the possibility of hyperfiltration as being their main cause of development.

The presence of at least one Kimmelstiel–Wilson lesion associates with longer duration of diabetes and less favorable clinical parameters. In a study of 36 patients with type 2 diabetes, patients with Kimmelstiel–Wilson lesions had more severe overall retinopathy and higher serum creatinine concentrations than those with mesangial lesions alone. In a study of 124 Chinese patients with type 2 diabetes, patients with at least one Kimmelstiel–Wilson lesion had relatively long duration of diabetes mellitus, a poor prognosis, and frequent evidence of diabetic retinopathy. Kimmelstiel–Wilson lesions are often found in combination with mesangial expansion. The occurrence of Kimmelstiel– Wilson lesions is widely considered transitional from an early or moderately advanced stage to a progressively more advanced stage of disease.

7.1.4 Class IV: Advanced diabetic glomerulosclerosis

Class IV implies advanced DN with more than 50% global glomerulosclerosis in which there is clinical or pathologic evidence that the sclerosis is attributable to DN. Glomerulosclerosis in DN is the end point of multifactorial mechanisms that lead to excessive accumulation of extracellular matrix proteins such as collagen types I, III, and IV and fibronectin in the mesangial space, which through stages of mesangial expansion and development of Kimmelstiel–Wilson lesions finally result in glomerulosclerosis. The clustering of sclerotic lesions in columns perpendicular to the kidney surface suggests that vascular factors relating to the interlobular arteries also contribute.

8. Tubulointerstitial lesions, vascular lesions and nondiabetic glomerular lesions

8.1 Tubular lesions

Concomitant tubular basement membrane thickening of nonatrophic tubules is apparent from the development of class II glomerular diabetic lesions and becomes more conspicuous in class III and IV, which is best seen in PAS or silver stains. Interstitial fibrosis and tubular atrophy (IFTA) follow glomerular changes in type 1 DN that ultimately lead to ESRD. A score of 0 is assigned when the biopsy specimen shows no IFTA, a score of 1 is assigned when less than 25% IFTA is present, a score of 2 is assigned when at least 25% but less than

50% of the biopsy has IFTA, and finally, a score of 3 is assigned when at least 50% IFTA is present, which is similar to the scoring in the recently published classification of IgA nephropathy.
Presence of mononuclear cells in the interstitium is a widely recognized finding in DN. Inflammatory interstitial infiltrates comprise T lymphocytes and macrophages. A score of 0 is assigned if interstitial infiltrates are absent, 1 if they only occur around atrophic tubules, and 2 if the inflammatory infiltrate is also in other areas than around atrophic tubules.

8.2 Vascular lesions

According to Stout et al., hyalinosis of the efferent arteriole is relatively specific for DN, but hyalinosis of the afferent arteriole occurs in numerous other settings. Chronic cyclosporine nephropathy is a typical example in which arteriolar hyalinosis occurs outside DN. Tracy et al. also report the presence of arteriolar hyalinosis in kidneys of young patients with coronary heart disease. Efferent arteriolar hyalinosis is an important lesion by which DN is distinguished from hypertensive nephropathy. However, most studies relate arteriolar hyalinosis to clinical parameters, not distinguishing between efferent and afferent arterioles, showing clear correlations with UAE and disease progression.
In addition to characteristic arteriolar hyalinosis, relatively nonspecific arteriosclerosis may be present in the biopsy specimen. Bohle and colleagues found increases in vascular disease associate with more severe glomerular disease. Osterby et al. use a so-called "matrix to media ratio" to investigate the role of arteriosclerosis and find this ratio is increased in patients with microalbuminuria, suggesting that arteriolar matrix accumulation occurs early in the course of DN. By assessing the most severely affected artery in the biopsy and assign a score of 0 if no intimal thickening is present, 1 if intimal thickening is less than the thickness of the media, and 2 if intimal thickening is more than the thickness of the media. Isolated or significant medial thickness may be associated with concurrent hypertension.

9. Other glomerular lesions

In 1994, Stout et al. defined "insudative lesions" as consisting of intramural accumulations of presumably imbibed plasma proteins and lipids within renal arterioles, glomerular capillaries, Bowman's capsule, or proximal convoluted tubules. Insudative lesions in Bowman's capsule are called capsular drop lesions, and in afferent and efferent arterioles they are called hyalinized afferent and efferent arterioles. In glomerular capillaries they are called fibrin cap lesions, although this term is considered obsolete and moreover is amisnomer because the lesion does not contain fibrin; we prefer the term hyalinosis for these lesions.
Capsular drops are mainly located between the parietal epithelium and Bowman's capsule of the glomerulus. Capsular drops are prevalent in advanced DN and associate with disease progression. The common belief, reviewed by Alsaad et al., is that capsular drops are specific but not entirely pathognomonic of DN. Stout et al. report a prevalence of capsular drops in 5.3% of biopsies without diabetes. However, finding a capsular drop in a biopsy can help distinguish DN from other causes of glomerulosclerosis.
By light microscopy, glomerular hyalinosis describes the same staining characteristics as the capsular drop lesion but it occupies the capillary lumen instead of being attached to Bowman's capsule. This lesion is not a specific finding in DN, because similar lesions are recognized in focal glomerulosclerosis, arterionephrosclerosis, and lupus nephritis.

Lesion	Criteria	Score
Interstitial lesions		
IFTA	No IFTA	0
	<25%	1
	25% to 50%	2
	>50%	3
interstitial	Absent	0
inflammation	Infiltration only in relation to IFTA	1
	Infiltration in areas without IFTA	2
Vascular lesions		
arteriolar hyalinosis	Absent	0
	At least one area of arteriolar hyalinosis	1
	More than one area of arteriolar hyalinosis	2
presence of large vessels	–	Yes/no
arteriosclerosis (score worst artery)	No intimal thickening	0
	Intimal thickening less than thickness of media	1
	Intimal thickening greater than thickness of media	2

Table 2. Interstitial and vascular lesions of DN

Finally, there is increasing recognition of abnormalities in the glomerulotubular junctions with focal adhesions called "tip lesions" and atrophic tubules with no observable glomerular opening (so-called "atubular glomeruli"). These lesions are typically found in more advanced stages of nephropathy associated with overt proteinuria.

One of the important questions that need to be validated is whether the new classification system, which makes no distinction between patients with type 1 and type 2 diabetes, is helpful for clinicians. Type 2 diabetes has more heterogenous clinical course with more heterogenous lesions. Type 2 diabetes also has different response towards treatments, and different relationship between diabetic nephropathy and retinopathy. In a study that published by Osterby R, Gall MA, Schmitz A, et al in 1993, virtually all patients with type 1 diabetes with overt nephropathy have retinopathy, whereas less than 50% of patients with type 2 diabetes and diabetic nephropathy have diabetic retinopathy. These figures may have been altered somewhat in the course of time. In 2010, Pedro et al. studied the prevalence and relationship between diabetic nephropathy and retinopathy in a population-based transversal study in northeastern Spain including 8187 type 2 and 488 type 1 diabetes patients. They distinguished between patients with microalbuminuria and those with overt nephropathy. The relationship between microalbuminuria and diabetic retinopathy was different between the types of diabetes, but the relationship between overt nephropathy and diabetic retinopathy was similar in both types. Overt nephropathy, in this study, was a risk factor for diabetic retinopathy in both types.

10. Biomarker studies

A number of studies reported on biomarkers in either plasma or urine for diabetic nephropathy, particularly in relation to type 2 diabetes. A group from Denmark reported on transthyretin, apolipoprotein A1, apolipoprotein C1, and cystatin C as promising biomarkers for diabetic nephropathy in the plasma of patients with type 1 diabetes (Overgaard AJ 2010). Proteomic analysis of plasma from a cross-sectional cohort of 123 type 1 diabetic patients previously diagnosed as normoalbuminuric microalbuminuric, or

macroalbuminuric, gave rise to 290 peaks clusters. Independent component analysis identified 16 candidate peaks that contributed significantly in their respective components with high stability and ability to separate the groups. Four of the peaks were identified as transthyretin, apolipoprotein A1, apolipoprotein C1, and cystatin.

Soluble CD40 ligand derived from platelets and mediating atherothrombosis, was shown to be elevated in type 1 diabetes patients with diabetic nephropathy in comparison with controls. The study was a prospective, observational follow-up study of 443 type 1 diabetes patients with diabetic nephropathy and a control group of 421 patients with longstanding type 1 diabetes and persistent normoalbuminuria. High levels of sCD40L did not predict development of end-stage renal disease (P = 0.85) nor rate of decline in glomerular filtration rate (GFR) (Lajer M 2010).

There were several studies on biomarkers in type 2 diabetes giving rise to a number of new markers. A group from China reported an independent association between plasma levels of osteopontin and the presence and severity of diabetic nephropathy in type 2 diabetes (Yan X 2010). In another study on type 2 diabetes, plasma levels of methylglyoxal, a side-product of many metabolic pathways, was found to be higher in patients with diabetic nephropathy than in those without and, furthermore, were shown to correlate with the urinary albumin: creatinine ratio (Lu J 2011). Fibroblast growth factor 23 (FGF-23), previously reported as a marker for outcome in chronic kidney disease in general, was found to be predictive of renal outcome in type 2 diabetes patients with macroalbuminuric nephropathy (Alkhalaf A, 2010). At baseline, serum FGF- 23 showed a significant association with serum creatinine and proteinuria. FGF-23 was an independent predictor of the primary outcome in this study, defined as death, doubling of serum creatinine, and/or dialysis need. The moderate consistency observed between biomarkers reported in different studies is puzzling, which is probably due to different technologies used and the lack of statistical power in some studies resulting in the reporting of artifacts. Also the distinction between those patients with and without diabetic nephropathy in most of these studies is not proven by biopsy.

It is evident that a huge amount of effort is being put into how to identify diabetic nephropathy in patients with diabetes without being invasive, that is, without taking a renal biopsy, both in clinical practice and clinical research studies. A drawback in most of these biomarker studies pursuing this aim is that no renal biopsy was taken to determine the presence and severity of diabetic nephropathy in the diabetic patients in the first place. Virtually all studies rely on clinical parameters, mostly expressed as the amount of albuminuria, for the diagnosis of diabetic nephropathy. How reliable is this? Chronic renal insufficiency and/or proteinuria especially in type 2 diabetes may stem from chronic renal disease other than classic diabetic nephropathy. In a recent retrospective study of 69 patients with type 2 diabetes with renal biopsies, 52% had non diabetes-related nephropathy (Mou S, 2010). Selection bias most likely plays a role in these data, as renal biopsies are typically performed in these patients if co-morbidity is suspected. It remains, however, most likely that some of the patients included in the biomarker studies were unrightfully given the diagnosis of diabetic nephropathy, and it is uncertain how important this contamination of the data is for the study outcomes. Only one marker study in 2010 was found that did incorporate renal biopsy findings and interestingly, in this study, a classification model was able to reliably differentiate diabetic nephropathy from nondiabetic chronic kidney disease (Papale M 2010). Among the best predictors of this classification model were ubiquitin and b 2-microglobulin.

There are probably also a considerable proportion of patients without albuminuria who are at the beginning stages of diabetic nephropathy. These patients are unrightfully diagnosed as not having diabetic nephropathy. Interesting in this light was the 2010 study by Nielsen et al., which evaluated the new marker of tubulointerstitial damage kidney injury molecule 1 (KIM-1) in type 1 diabetes patients with either normoalbuminuria, microalbuminuria, or macroalbuminuria in comparison with normal controls. Urine KIM1 was elevated in all type 1 diabetes patients in comparison with the normal controls and irrespective of the presence of albuminuria. Thus, normoalbuminuric patients with type 1 diabetes also had elevated urine KIM1. It was therefore concluded that tubular damage may be present at an early stage in all patients, the so-called 'tubular phase' of diabetic nephropathy. Whether or not this means that in fact all patients with type 1 diabetes have a latent form of diabetic nephropathy remained undiscussed, but that would be an interesting hypothesis. Another study that investigated urinary changes in non-overt diabetic nephropathy in type 2 diabetes came from Japan (Araki S,2010). This study included 254 patients with type 2 diabetes of whom 185 were normoalbuminuric and 69 had microalbuminuria. At baseline, urinary type IV collagen levels. were higher in patients with microalbuminuria. During a follow-up study with a median duration of 8 years, the level of urinary type IV collagen inversely correlated with the annual decline in estimated GFR, whereas overt proteinuria did not appear in a majority of patients. Two studies from Malaysia gave similar results, with type IV collagen levels correlating with the amount of urinary albumin in 30 type 2 diabetes patients at baseline Sthaneshwar P, 2010), but also with subsequent GFR change (Katavetin P 2010). Also in New Zealand, urinary collagen IV was studied in diabetes: spot urine samples from 457 unselected patients attending a hospital diabetes clinic were analyzed for albumin, creatinine, and a number of biomarkers including collagen IV (Cawood TJ, 2010). The proportion of patients with abnormal collagen IV increased from 26, 58, and 65%, respectively, across the normoalbuminuria, microalbuminuria, and macroalbuminuria groups. The authors conclude that longitudinal studies are now required to assess whether these biomarkers can detect early renal disease with greater specificity and sensitivity than the albumin: creatinine ratio. Including histopathological findings in these types of studies would certainly make a great contribution to our better understanding of the mechanism leading to diabetic nephropathy.

11. Treatment

Interventions that have been found useful in preventing or retarding the progression of DN include

- Strict glycemic control
- Strict blood pressure control
- Cessation of smoking
- Control of hyperlipidemia and
- Restriction of protein intake.

12. Strict glycemic control

Hyperglycemia is an important risk factor for development of microalbuminuria, progression of established microalbuminuria to macroalbuminuria and impaired glomerular filtration rate (GFR). Additional risk factors for microalbuminuria include older age, male

sex, long duration of diabetes, smoking, obesity, elevated blood pressure, and genetic predisposition. (de Boer IH 2011)

Studies have shown that strict glycemic control delays the development of microalbuminuria, stabilizes or reduces protein excretion in patients with microalbuminuria and overt proteinuria, and slows the rate of progression to chronic renal failure. The Diabetes Control and Complications Trial (DCCT) compared conventional with intensive glycemic management in 1,441 type-I diabetic patients. This study proved that intensive treatment reduced the risks of retinopathy, nephropathy, and neuropathy by 35% to 90% compared with conventional treatment. The absolute risks of retinopathy and nephropathy were proportional to the mean glycosylated hemoglobin (HbA1c) level over the follow-up period preceding each event. Intensive treatment was most effective when begun early, before complications were detectable. These risk reductions were achieved at a median HbA1c level difference of 9.1% for conventional treatment versus 7.3% for intensive treatment. ((The DCCT/EDIC Research Group 2000, The DCCT/EDIC Research Group 2002)) In the combined cohorts, intensive treatment reduced the development of microalbuminuria and clinical albuminuria by 39% and 56%, respectively. The benefits of intensive therapy were also long-lasting and persisted beyond the period of shortest intervention. (The DCCT/EDIC Research Group 2000, The DCCT/EDIC Research Group 2002) Thus, intensive treatment should be started as soon as possible safely after the onset of type 1 diabetes mellitus and maintained thereafter, aiming for a practicable target HbA1c level of 7.0% or less. (Writing Team for the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group 2002). These findings suggest that hyperglycemia has long-term chronic effects on the underlying pathophysiology of microvascular complications. It takes time for improvements in control to negate the long lasting effects of prior prolonged hyperglycemia, and once the biological effects of prolonged improved control are manifest, the benefits are long lasting. However, using the current intensive treatment regimen led to a 3-fold increase in severe hypoglycemic events and to excess weight gain in the DCCT. (DCCT/EDIC research group 2002)

Efforts need to be made to eliminate preventable severe hypoglycemic episodes that result from unsafe patient behavior and decisions, and to avoid inordinate weight gain. Irregular food intake, failure to check blood glucose before planned or unplanned vigorous exercise or before operating a motor vehicle, and excess alcohol ingestion have been identified as risk factors for hypoglycemia and serious complications and must be scrupulously avoided. Mealtime bolus doses of rapid acting insulin must be based on the pre-injection blood glucose level, the anticipated amount of carbohydrate intake and upcoming exercise. Thorough diabetes education and its regular reinforcement should be provided by diabetes nurse and dietitian educators. These professionals can negotiate individualized care plans with patients, give them training in self-management, and provide stimulation, motivation, and positive reinforcement for good self-care behavior, such as frequent self blood glucose monitoring and regular eating habits. While these measures can interfere with patients' lifestyles, they are the current price that must be paid to delay or reduce the risk of microvascular complications. (DCCT/EDIC research group 2002)

13. Blood pressure control

Long term and aggressive antihypertensive treatment induces a progressive reduction in the rate of decline in kidney function. Thus, this modality of treatment can postpone renal

insufficiency in patients with DN. Both systolic and diastolic hypertension markedly accelerate the progression of DN, and aggressive antihypertensive management has been shown to decrease the rate of fall of glomerular filtration rate, increase the median life expectancy, and reduce the need for dialysis and transplantation.

The primary goal of therapy for non-pregnant patients with DM older than 18 years is to achieve a blood pressure less than 130/80 mmHg for patients with proteinuria <1 g/day, and less than 125/75 mmHg for patients with >1 g/day of proteinuria. Angiotensin converting enzyme (ACE) inhibitors and ARBs are recommended as first-line antihypertensive therapy for patients with type-I and type-2 DM. Other agents that can be used include Beta blockers, calcium channel blockers, and diuretics. (American Diabetes Association 2003, Ayodele OE 2004).

Persistent clinical proteinuria is closely associated with the presence of hypertension in IDDM patients. In NIDDM patients the risk of developing clinical proteinuria is increased more than twofold in patients with blood pressure > 165/95 mm Hg compared to those with lower blood pressure after adjusting for age, sex and duration of diabetes. Moreover, in both insulin-dependent and non-insulin-dependent diabetes, the age adjusted total mortality rate is greatly increased in those patients with proteinuria or hypertension (Earle K 1994). Prospective studies have shown that normoalbuminuric patients who progress to microalbuminuria have higher blood pressures (albeit within the normal range) than those who persistently remain normoalbuminuric. Parents of insulin-dependent diabetic patients with nephropathy have a higher prevalence of hypertension and cardiovascular disease compared to patients without nephropathy (Earle K 1994).

ACE Inhibition versus Angiotensin II (Ang II) receptor type 1 blockade in the Renin-Angiotensin System (RAS). RAS is one of the most important physiological regulators of renal function. ACE inhibitors selectively dilate efferent arterioles. This decreases the arterial pressure and in turn reduces glomerular capillary pressure. In addition, Ang II causes mesangial cell growth and matrix production. Numerous animal studies and clinical trials have shown that ACE inhibitors significantly reduce the loss of kidney function in DN. They prevent progression of microalbuminuria to overt proteinuria and several studies evaluated the effect of ACE inhibitor on development and progression of DN.

The landmark study by Lewis et al. [1993], examined the effect of captopril on the progression of DN in patients with type 1 DM (Lewis, EJ 1993). This was measured as the rate of decline in creatinine clearance and the combined end points of dialysis, transplantation, and death. Treatment with captopril was associated with 48% risk reduction for doubling the serum creatinine as compared to placebo. The results of this study were subsequently confirmed by North American Microalbuminuria Study Group and EUCLID study group (EUCLID Study Group 1997, Laffel LM 1995). They extended the observation by showing a protective effect of ACE inhibitors in patients with a variety of renal diseases, including glomerulopathies, interstitial nephritis, nephrosclerosis, and DN. The exception was polycystic kidney disease. Importantly, the protective effect of ACE inhibition was independent of the severity of renal insufficiency. (Lewis, EJ 1993, Brown N 1998)

The MICRO-HOPE (Heart Outcomes Prevention Evaluation) studied the benefit of ramipril in type 2 diabetics. The diabetes sub-study of the Heart Outcomes Prevention Evaluation study showed ramipril reduced the risk of overt nephropathy by 24%. Moreover, ramipril reduced urinary albumin excretion at 1 year and at the end of the study. Thus, ACE inhibitors have also been shown to be renoprotective in patients with type 2 DM. (Heart Outcomes Prevention Evaluation Study Investigators 2000)

Two studies, Irbesartan in Patients with Diabetes and Microalbuminuria (IRMA-2) and Diabetics Exposed to Telmisartan and Enalapril (DETAIL) study examined the effect of ARB's in diabetic patients with microalbuminuria, but without overt DN (Parving HH 2001, Barnett A 2006). It is well known that, in patients with type 2 DM presence of microalbuminuria increases the risk of developing DN 10 to 20 times. IRMA-2 study showed that irbesartan significantly reduced the rate of progression of microalbuminuria to overt DN in patients with type 2 diabetes. Furthermore, the study discovered that irbesartan was associated with significantly more common restoration of normoalbuminuria as compared to standard therapy. All these effects were achieved independent of the systemic blood pressure. The more recent DETAIL study compared the renoprotective effects of ACE inhibitor enalapril and ARB telmisartan. In this head-to-head comparison the authors showed that both treatments were equally effective in preventing the progression of renal dysfunction, measured as decline in the GFR. Reduction of Endpoints in NIDDM with Angiotensin II Antagonist Losartan (RENAAL) study (Brenner BM 2001) showed that treatment with losartan was associated with a 25% decrease in the risk for doubling serum creatinine level. The risk of developing ESRD was also reduced by 28%.

Other individual factors may better define the impact of ACE inhibitors on the progression of renal insufficiency. In particular, Rigat et al described an insertion (I) and deletion (D) polymorphism in the ACE gene that correlates with ACE activity. ACE levels are highest in patients who are homozygous for the ACE D allele and lowest in patients homozygous for the ACE I allele (Rigat B 1990). They are intermediate in those who are heterozygous. Yoshida et al (Yoshida H 1995) later reported a greater reduction in proteinuria in response to ACE inhibition in patients with IgA nephropathy who were homozygous for the D allele. In contrast to this, other investigators have suggested a worse response to therapy in patients who carry the D allele (Parving HH 1996). Obviously, large-scale studies are needed to define the impact of genetic factors on the renal protective effects of ACE inhibition.

There are studies suggesting that dual blockade of renin-angiotensin system using a combination of ACE inhibitor and an ARB in patients with nephropathy is superior to the use of either drug alone. (Ayodele OE 2004, Mogensen CE 2000, Rosner MH 2003). The antiproteinuric effects of inhibitors of the renin angiotensin system are increased by sodium restriction and by concomitant administration of diuretics or non-dihydropyridine calcium channel blockers. (American Diabetes Association 2002, Ayodele OE 2004, Arauz-Pacheco C 2002).

In the United Kingdom Prospective Diabetes Study, atenolol showed beneficial effects comparable to captopril on diabetes-related mortality and microvascular complications in patients with type-2 diabetes. (United Kingdom Prospective Diabetes Group 1998) Beta-blockers have been shown to reduce mortality following myocardial infarction, and the absolute benefit of a given relative reduction is greater in diabetics compared to nondiabetics due to higher mortality from myocardial infarction in patients with diabetes. (Ayodele OE 2004) The nondihydropyridine calcium channel blockers have been shown to lower protein excretion in patients with diabetes. (Bakris GL 1990 and 1997) Their antiproteinuric effect may be due to reduction in intraglomerular pressure, reduction in glomerular hypertrophy, and improved glomerular size. The dihydropyridine calcium channel blockers have a variable effect on protein excretion ranging from increased protein excretion to no effect to a fall in protein excretion in various studies. (Melbourne Diabetic Nephropathy Study Group 1991, Vellussi M 1996, Salako BL 2002, Rosssing P, 1997)

14. Protein restriction

The role of dietary protein restriction in CKD is best described as controversial. However, restriction of protein (0.6 g/kg body weight per day) and phosphorus (0.5 to 1 g per day) were shown to reduce the decline in GFR, lower blood pressure, and stabilize renal function compared with higher intakes. This was suggested by a randomized trial involving patients with type-I DM and overt DN. In addition, another study showed that restriction of protein intake to 0.8 g/kg body weight per day reduced the rate of progression to ESRD in patients with type-I diabetes. The National Kidney Foundation recommends that patients with GFR <29 mL/min per m2 should have a daily protein intake of 0.6 g/kg body weight. More recently, a 4-year randomized controlled trial in 82 patients with type 1 diabetes with progressive DN showed that a moderately low–protein diet (0.9 g · kg−1 · day−1) reduced the risk of ESRD or death by 76%, although no effect on GFR decline was observed (Hansen HP, 2002)

A prospective, randomized controlled trial in patients with type 1 diabetes suffering from progressive diabetic nephropathy demonstrated beneficial effect of moderate restriction in dietary protein on the development of ESRD or death. The beneficial effect of protein restriction appeared within the first year, and persisted with continued treatment, as also has been demonstrated in non-diabetic nephropathies suggesting that type 1 diabetic patients with progressive diabetic nephropathy are highly sensitive to dietary protein restriction (Hansen HP, 2002).

The mechanisms by which a low-protein diet may reduce progression of DN are still unknown, but might be related to improved lipid profile and/or glomerular hemodynamics. (Jorge L, 2005) Since diabetic patients have other restrictions to the diet, this may reduce compliance to an additional low-protein diet, although better compliance can be obtained by applying much more intensive dietary counseling (Hansen HP, 2002)

15. Cessation of smoking

Smoking has been shown in many previous studies to effect diabetic complications. Cessation of smoking alone may reduce the risk of progression by 30% in patients with type-2 diabetes. (Ritz E, 2000) Recent studies demonstrated that smokers have increased systolic blood pressures and proteinuria amongst diabetics with nephropathy (Sawicki, PT 1996) More recent work by Chihuran et al has shown that renal function declines faster in smokers than nonsmokers with type 2 DN undergoing treatment to improve blood pressure including ACE inhibitors. Also, loss of renal function is slower in those who stopped smoking. Cigarette smoking remains a risk factor for renal function decline in type 2 DN despite currently recommended therapy (Chihuran T, 2002)

16. Hyperlipidemia

There is suggestion that elevation in lipid levels may contribute to the development of glomerulosclerosis in chronic renal failure. (Ravid M, 1995, Krolewski AS, 1994) Studies have shown that lipid lowering may have a beneficial effect on renal function. (Lam KSL, 1995) A meta-analysis of 13 controlled trials involving a total of 362 subjects, 253 of whom had diabetes, showed that statins decreased proteinuria and preserved GFR in patients with chronic renal disease. (Fried LF, 2001) Adequately powered randomized controlled trials will be needed to determine the role of lipid lowering therapy in retarding the rate of decline in kidney function in patients with chronic renal disease secondary to diabetes mellitus.

17. Renal replacement therapy

The renal replacement modalities available for patients with ESRD from diabetes include peritoneal dialysis, hemodialysis, and renal transplantation. Various studies have shown similar survival in hemodialysis and peritoneal dialysis, though patients are more likely to persist with hemodialysis than with peritoneal dialysis. Both hemo- and peritoneal dialysis limit social life, leisure, and sexual activity.(Hostetter TH, 1981) Patients with diabetes may manifest uremic symptoms at a relatively less-advanced degree of renal insufficiency than their nondiabetic counterparts. (Hostetter TH, 1982)

18. Emerging therapies

Extensive research is currently underway in this field and some new pathogenic mediators for DN have been discovered. These include

- Renin
- Advanced Glycosylation end-products [AGE]
- Protein Kinase C [PKC]
- Transforming growth factor – Beta 1 [TGF-1]
- Nitric Oxide [NO]
- Vascular endothelial growth factor [VEGF]
- Oxidative stress

18.1 Direct renin inhibitor – Aliskiren

Blockade of RAS is a key therapeutic strategy in slowing progression of DN. Interruption of the RAS may also be accomplished by blocking the activity of renin. Aliskiren is a direct renin inhibitor and thus, decreases angiotensin II and aldosterone levels. Aliskiren is a potent antihypertensive and anti proteinuric.

18.2 Advanced Glycosylation end-products [AGEs]

The formation of AGEs and their cross-linked products is a phenomenon of normal aging; however, it is accelerated in the DM. AGE–cross-linked products accumulate in patients with DM and have been implicated in the pathologic process of diabetic complications. Several anti-AGE agents have been tested and shown to be renoprotective in experimental diabetic animal models. (Thomas MC, 2005)

Aminoguanidine is the prototype of an AGE formation inhibitor which acts by scavenging intermediates in the advanced glycation catalytic process.

ALT-946 is a more potent and selective AGE formation inhibitor than aminoguanidine. It has minimal effects on NO synthesis and appears to have fewer toxic effects, although it has not been studied in as much detail.

Pyridoxamine (PYR) - Pyridoxamine is one of the three natural forms of pyridoxine (vitamin B6). It scavenges pathogenic reactive carbonyl species and inhibits the formation of AGEs from Amadori compounds. (Voziyan PA, 2002, Chetyrkin SV, 2008)

18.3 Thiamine

Experimental studies have suggested that thiamine and benfothiamine (S-benzoylthiamine monophosphate), a vitamin B1 derivative, can also prevent or decrease kidney injury. These drugs decrease formation of AGE compounds and protein kinase C (PKC) activity in DN.

AGE Breakers - This group of compounds decreases AGE accumulation by breaking the glycation cross-links.
AGE Receptor Antagonists - AGEs mediate their effects both directly and indirectly through receptor-dependent mechanisms. They bind to the transmembrane receptor for AGE (RAGE) and prevent the development of diabetic microvascular complications. (Bierhaus A 2005, Wendt T, 2003) Thus, RAGE is a potential target to prevent AGE effects.

18.4 Pentoxifylline

Pentoxifylline (PTF) is a methylxanthine derivative with hemorheological properties that has favorable effects on microcirculatory blood flow. In vivo, it also functions as a phosphodiesterase inhibitor. (Navarro J.F., 1999)

18.5 Protein Kinase C inhibitors

Recent studies have identified that activation of PKC initiated by hyperglycemia is associated with many vascular abnormalities in retinal, renal, and cardiovascular tissues. The blocking of PKC-beta isoforms has been shown to decrease albuminuria, structural injury, and TGF-beta expression in animal models of DM. (Koya D, 2000) Ruboxistaurin is one such PKC beta inhibitor.

18.6 Glycosaminoglycans

Glycosaminoglycans are important determinants of GBM permeability. An emerging body of evidence supports the notion that glomerular capillary wall and mesangial alterations in DN involve pathobiochemical alterations of glycoproteins in these structures. Heparin and sulodexide are examples of this class of drugs.

18.7 Endothelin receptor antagonists

DN is associated with enhanced renal synthesis of endothelins. A number of preclinical reports suggested that endothelin might be an appropriate target to decrease DM-related albuminuria. (Turgut F, 2010) Avosentan (SPP301) is a new orally available endothelin 1 antagonist.

18.8 Antifibrotic agents and growth factor inhibitors

Characteristic morphologic lesions of DN include glomerular hypertrophy, thickening of the basement membrane, and mesangial expansion. This leads to glomerulosclerosis, tubulointerstitial fibrosis, and, eventually, loss of kidney parenchyma. Several growth factors which are normally expressed in the kidney have been implicated in the pathogenesis of DN.
TGF-β Inhibitors - Pirfenidone (PFD) is a low molecular weight synthetic molecule that exerts dramatic antifibrotic properties in cell culture and various animal models of fibrosis. SMP-534, another antifibrotic agent is also being studied. Several AGE inhibitors also decrease TGF-β levels. (Turgut F,2010, Sugaru E., 2006)
CTGF Inhibitors - Connective tissue growth factor (CTGF/CCN2) has been associated with fibrosis in various tissues including the kidney. It is up-regulated in most models of DN. Clinical trials evaluating anti-CTGF ab (FG3019) are underway.

18.9 Nitric Oxide (NO) modulation

Abnormalities of renal NO generation have been linked to pathogenesis of renal disease in diabetes. NO and / or NO Synthase are targets for drug development for treatment and/or prevention of DN.

18.10 Vascular endothelial growth factor (VEGF) inhibitors

VEGF is a main regulator of blood vessel growth and plays an important role in promoting endothelial survival and maintaining the microvasculature. Loss of capillaries is strongly associated with the progression of CKD to ESRD (Doi K, 2010).

19. Alternative and complementary therapies for diabetic nephropathy

19.1 Exercise and Yoga

The American Diabetes Association recommends a minimum of 30 minutes of moderate-intensity aerobic physical activity 5 days per week, or vigorous-intensity aerobic physical activity for 20 minutes 3 times per week is recommended for healthy adults aged 18 to 65. Currently, there is no clinical evidence to suggest that vigorous exercise increases the rate of progression of diabetic nephropathy. In fact, some studies have shown that aerobic exercise actually decreased urine protein excretion (Gordon LA, 2008). Additionally, it has been demonstrated that resistance training may have a beneficial effect on muscle mass, nutritional status, functional capacity, and glomerular filtration rate. Therefore, the American Diabetes Association feels that there is no need to restrict exercise in patients with diabetic nephropathy. A more recent study that examined the effects of Yoga and conventional exercise showed findings that suggest better glycemic and blood pressure control obtained in type 2 diabetic patients after Hatha yoga than conventional PT exercises (Gordon LA, 2008).

19.2 Life style modifications

Obesity is often associated with diabetes mellitus and also with nephropathy independent of diabetes (often focal sclerosis). However the impact of weight loss in diabetic subjects with nephropathy on renal function and proteinuria remains as a subject of intense investigation. Short term studies recently reported that weight reduction using dietary therapy for 4 weeks resulted in significant reduction in systolic pressure, proteinuria, and serum creatinine in obese patients with diabetic nephropathy. (A Saiki, 2005). Longer studies involving larger group of patients need to be evaluated to validate such conclusions.

19.3 Herbal and Food derivatives

19.3.1 Curcumin

Curcumin is the active component in Tumeric Rhizomes (Curcuma Long Linn). Curcumin has been shown to possess anti-inflammatory, anti-oxidant and antifibrotic properties in many tissues, in vivo and in vitro studies. Tikoo et al have shown that curcumin treatment prevented the development of DN by significantly lowering blood urea nitrogen and plasma creatinine/body weight ratio in diabetic animals. (Tikoo K, 2008) Various biological actions of curcumin are mediated by inhibiting cell proliferation (Sikora E, 1997), oxidative stress and inflammation (Sharma C, 2006). Several other investigators have also shown that the anti-inflammatory property of curcumin can significantly improve kidney function in animals with chronic renal failure.

19.3.2 Cinnamon

Cinnamon has been known for its antidiabetic effects for some time now. Mishra et al have investigated its effects on nephropathy in diabetes in rodent models of type I diabetes. Histological studies of the kidney proved the protective effect of cinnamon oil by reducing

the glomerular expansion, eradicating hyaline casts, and decreasing the tubular dilatations. (Mishra A, 2010). The authors concluded that the volatile oil from cinnamon contains more than 98 % cinnamaldehyde and that it confers dose-dependent, significant protection against alloxan-induced renal damage. While the mechanism of its action remains unclear, it is believed to be mostly due to its antidiabetic and antioxidant effects leading to reduced formation of AGEs.

20. Conclusions

During the last 3 decades, considerable progress has been made in delaying the progression of CKD even as the frequency of DN continues to increase. This is a truly a reflection of the advances made in understanding the pathogenesis. As reviewed in this chapter many pathogenic cytokines and growth factors have emerged in the recent years that either initiate or contribute to the progressive renal injury in diabetes. Current treatment options are still suboptimal. However with the rapid strides being made in the field, several new therapeutic targets are being recognized and effective treatment strategies being developed.

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Metabolic Syndrome Associated Kidney Damage

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1. Introduction

We evaluated the incidences of metabolic syndrome (MS) and its components in the urban residents of southern China, analyzed their relationship to insulin resistance (IR), meanwhile compared the different of MS diagnostic criteria between Chinese Diabetes Society (CDS) and International Diabetes Federation (IDF) in clinic practice in the southern urban residents of China. The total incidence of MS was 8.7% according to the diagnostic criteria of CDS, but up to 19.8% according to the diagnostic criteria of IDF. The total incidences of hypertension, abdominal obesity and diabetes were 22.1%, 39.2% and 6.7%, respectively. The incidence of IR was 5.0% according the value of HOMA-IR. By means of binary logistic regression analysis, impaired fasting glucose, diabetes, obesity, abdominal obesity, elevated triglyceride and high sensitivity C reactive protein were independent risks of insulin resistance, but gender, hypertension, elevated low density lipoprotein and total cholesterol were not independent risks of insulin resistance. It is suggested by the data of our present screening in the residents of southern China that the incidence of MS according to the diagnostic criteria of CDS was lower than that according to the diagnostic criteria of IDF. Some residents with MS main presentation of abdominal obesity would be missed diagnosis by the criteria base on BMI. In the components of MS, hypertension, abdominal obesity and lower high density lipoprotein were more common than others. IR was associated to most of the components of MS and may be one of the main pathogenic factors.

By means of cross-section epidemiological analysis, we investigated the relationship of glycometabolic disorder and IR with chronic kidney diseases (CKD). The prevalence of CKD was 12.6% in the community population, with 11.2% in the youth group, 19.4% in the middle age group and 17.7% in the elder group. And there were a significantly difference between the three age groups ($P<0.01$). The awareness rates and treatment rates of CKD were very low in all of the three groups. In the whole screened population there was a higher CKD prevalence in IR residents when compared to non-IR residents, 36.9% versus 12.6% ($P<0.01$). Among population with only impair FBG but not diabetes, CKD prevalence in those residents with IR was higher than those without IR, 33.3% versus 16.0% ($P<0.05$).

Even among population with normal FBG, CKD prevalence in those residents with IR was higher than whose without IR, 39.1% versus 12.0% (P<0.01). It was observed in different age group that the prevalence of albuminuria and the mean albuminuria level were higher in the residents with IR when compared to the residents without IR. No difference existed between either the prevalence of decreasing eGFR or the mean level of eGFR in residents with IR and without IR in the middle age group and the old group, but not in the youth group in which. The mean level of eGFR was significantly higher in the residents with IR when compared to the residents without IR (P<0.01). It is indicated in our data that CKD is common and the awareness rate and treatment rate are very low in this investigated community population. IR might be associated with the increasing prevalence of CKD, especially with the increased prevalence of microalbuminuria, even in the population with normal FBG. Furthermore IR might also be associated with elevated eGFR in population at the early stage of diabetes but without CKD, while associated with decreased eGFR in those with CKD. It is suggested that IR might be a risk factor of CKD and also a prevention and treatment target of CKD in community residents.

We also explored the incidences of hyperuricemia (HUA) in the urban residents and the related risk factors. The total incidence of HUA was 23.5% in the cohort residents, and was 28.4% in the males and 19.7% in the females (P<0.01). The serum uric acid level was positively related to body weight (or BMI), waist circumference and the age (for females) when controling with serum creatinine (P<0.01). Alcohol consumption and smoke influences significantly on the serum uric acid level, and highest uric acid levels were in the residents frequently drunk and smoked in the past. More common prevalence of HUA was in the patients with chronic kidney disease and hypertension, and the serum uric acid levels were similar in these patients. There was no significant difference of the incidences of HUA between the patients with diabetes and non diabetes. It is suggested by our present investigation that the incidence of HUA is increasing in the residents of inland city of China, and that the change of their lifestyle with lose weight, prevention of obesity, avoid smoke and restriction of alcohol would be the most effective measures to change the high prevalence of HUA.

2. Pathogenesis of metabolic syndrome associated kidney damage

2.1 Insulin resistance and pathogenesis of metabolic syndrome-associated kidney damage

Insulin resistance and metabolic renal damage is closely related. So the metabolic syndrome were screened for kidney damage, assessment is very necessary. Regarding to the pathogenesis of insulin resistance and to improve the treatment based on will also be a metabolic control of renal damage in a new direction for the future.

2.1.1 Insulin resistance leads to kidney damage

The metabolic syndrome (MS) was defined as the presence of 3 or more of the following risk factors: elevated blood pressure, insulin resistance, low high-density lipoprotein cholesterol level, high triglyceride level, elevated glucose level, and abdominal obesity. MS disease risk factors in order to gather more focus is characterized by heart, kidney blood vessels and other target organs. The impact is clear. Now the increasing number of researches show that compared with simple hypertension, metabolic syndrome is more easily lead to kidney damage. Some studies further confirmed that the large vessels and kidney of metabolic syndrome patients damaged obvious. MS can cause kidney damage, and the kidney disease affects MS as well. This shows the relationship between MS and kidney disease is very close.

So people consensus that MS is started for obesity as the common factor. And insulin resistance is the central link of MS. This can be inferred that the relationship between insulin resistance and metabolic renal damage is close.

Insulin resistance refers to the uptake of insulin to promote peripheral tissue, the use of glucose output and inhibit the biological effects of glycogen decreased, with the changes in the compensatory ability of the body showed hyperinsulinemia and (or) high blood sugar status. The kidney damage mechanisms caused by high blood sugar is including that: ① polyol pathway activation; ② protein non-enzymatic glycation (advanced glycation end products formation); ③ activation of protein kinase C. The renal injury is caused by these lesions and GBM thickening. In addition, the recent study found, MS appears hyperinsulinemia is also often high viremia of amylin, amylin is a high-fiber protein, mainly deposited in the glomerular mesangial area widened, K-W nodules, blood vessel walls and renal interstitial, which become one of the causes of injury about glomerular and interstitial. In vitro experiments showed that amylin may be higher in mesangial cells through induction of apoptosis and increased permeability in endothelial cells kidney damage, but the exact mechanism is still not very clear.[1, 2]

Now that insulin resistance occurs most often in patients with metabolic syndrome, is the central link in the pathogenesis and pathogenic basis. It not only prompts a new-onset diabetes mellitus, cardiovascular events and all-cause mortality in high-risk, but also the renal damage and failure are independent risk factors.[3-6] The primary kidney disease before there is often severely impaired renal function also showed insulin resistance.[4] Therefore, insulin resistance and renal damage can reinforce each other, so to clear the relationship between insulin resistance and metabolic renal damage is useful that in the prevention and treatment of kidney disease is especially prominent role in the process. This article is the review on the causes of pathogenesis why insulin resistance leads to metabolic kidney damage.

2.1.2 Insulin resistance and pathogenesis of metabolic syndrome-associated kidney damage

Insulin resistance is the central link of metabolic syndrome. The U.S. NHANES Ⅲ data shows that the prevalence of metabolic syndrome has reached 23.7% for the adults who over 20 years 'old.[3] The study showed that In type 2 diabetes, more severe insulin resistance is independently associated with microalbuminuria.[5, 6] Animal experiments confirmed that clinical diabetes mellitus in the event of hyperinsulinemia stage before changes of structure and function in kidney have been.[7] Such early renal damage has its own characteristics and pathogenesis, which are different from diabetic nephropathy. Insulin resistance mechanisms lead to kidney damage mainly in the following areas.

Insulin-like growth factor (IGF) axis is involved in diabetic renal disease

Insulin-like growth factor I (IGF-I) is a potent mitogenic polypeptide under the regulation of growth hormone (GH). Evidence of significant involvement of the GH/IGF system in diabetic nephropathy and other nephropathies has been provided by several studies. Kidney tissue expresses receptors not only for IGF-I but also for GH, which suggests that although most of the biologic effects of GH are mediated by IGF-I, GH may also act independently of IGF-I. IGF-I may have pathogenic roles in diabetic nephropathy and other nephropathies. Serum IGF-I levels are reduced in hyperglycemic diabetic subjects, despite elevated GH levels. This phenomenon has been explained by inhibition of hepatic IGF-I synthesis, resulting from decreases in hepatic GHR expression and binding. The metabolic consequences of these

alterations produce a "vicious cycle," wherein the hyperglycemia/insulinopenia induce decreases in serum IGF-I levels, which in turn induce GH hypersecretion, making optimal metabolic control more difficult to achieve.[8] People speculate that the mechanism underlying the renal effects of this GHR antagonist involves renal GHR inhibition of renal IGF-I (and IGFBP-1) protein accumulation. They also speculate that the mechanism underlying the renal effects of this GHR antagonist involves renal GHR inhibition of renal IGF-I (and IGFBP-1) protein accumulation. This study demonstrates that the GH/IGF axis plays a central role in the pathogenesis of early diabetic renal changes, and it suggests specific GHR blockade as a new concept in the treatment of diabetic kidney disease.[9]

Insulin resistance increase renal damage through the rennin-angiotensin system

Angiotensin II (Ang II) and insulin are implicated in the mesangial cell hypertrophy and excessive accumulation of mesangial matrix seen in glomerulosclerosis. Therefore, the effects of Ang II with and without insulin on mRNA levels of several important extracellular matrix genes and transforming growth factor beta-1 (TGF-beta 1) were examined. The results of the studies suggest that insulin, itself, significantly increases TGF-beta 1 and extracellular matrix gene expression in rat mesangial cells. Ang II alone has modest effects, while Ang II and insulin have additive effects. To explain the mechanism of these additive effects, we investigated the action of Ang II on insulin signaling and the effect of insulin on Ang II AT1 receptor mRNA expression. Ang II did not enhance insulin-induced insulin receptor substrate-1 (IRS-1) phosphorylation or phosphatidylinositol 3 (PI-3) kinase activity, but did enhance insulin-induced mitogen activated protein (MAP) kinase activity.[10] Insulin increased message levels of AT1 receptor by twofold. These results suggest that enhancement of MAP kinase activity and AT1 receptor regulation by insulin may contribute to the additive effects of insulin and Ang II in mesangial cells.

The direct impact of insulin resistance on kidney

Insulin major role in the tubules, but the specific sites of action are not yet entirely clear.It has a strong role in preserving sodium and dose dependent, while the presence of insulin to counter, this is still Paul sodium. Therefore, insulin resistance and hyperinsulinemia that occurs when the sodium sensitivity of blood pressure increase in glomerular pressure increased, resulting in microalbuminuria. A study was performed by Vedovato M to measure the effect of Na+ intake on blood pressure and albuminuria, in relation with insulin sensitivity and kidney haemodynamics, in Type 2 diabetic patients with and without microalbuminuria. They found that high salt intake increases blood pressure and albuminuria in Type 2 diabetic patients with microalbuminuria. These responses are associated with insulin resistance and increased glomerular pressure. Insulin resistance could contribute to greater salt sensitivity, increased glomerular pressure and albuminuria.[11]

Insulin resistance increase the renal damage by the plasminogen activator inhibitor 1

The insulin resistance syndrome typically features glucose intolerance and elevated fasting insulin and triglyceride levels. Elevated levels of PAI-1 and tPA antigens associated with glucose intolerance, hyperinsulinemia, and hypertriglyceridemia support inclusion of impaired fibrinolysis as an additional feature of the insulin resistance syndrome. Elevated fibrinolytic factors are also correlated with elevated markers of inflammation and endothelial dysfunction, which has been hypothesized to cause insulin resistance and thereby be the common pathogenic mechanism underlying atherosclerosis, insulin resistance, and glucose intolerance.[12] Hagiwara H's study proved that renal PAI-1 gene

expression is up-regulated in both type 1 and type 2 diabetic rats, and changes in gene expressions of fibrinolytic factors may play important roles in the development and pathogenesis of diabetic nephropathy.[13] In addition, TGF-β, angiotensin Π and thrombin could stimulate the synthesis of PAI-1, the process by inhibiting fibrinolysis and plasmin-mediated matrix metalloproteinase activity, so that less matrix degradation, resulting in renal fibrosis.

Insulin resistance increases the rates of renal damage by endothelin (ET) -1

Endothelin-1, released from the vascular endothelium after cleavage from big endothelin-1, is a potent paracrine vasoconstrictor peptide. Small studies suggest that the circulating level of endothelin-1 is elevated in subjects with cardiovascular risk factors. High endothelin-1 level may better reflect endothelin-1 generation. It is indicated by studies that endothelin-1 level is not related to blood pressure, but higher in healthy young men with insulin resistance and obesity.[14] It was discovered in a diabetic mouse model treated with A-type ET receptor antagonist that glomerular TGF-β and collagen I, II, IV production were decreased.

Insulin resistance increase the renal damage through oxidative stress

Insulin-stimulated (or inhibited) pathways retain normal sensitivity to the hormone, hyperinsulinemia could, by its effects on antioxidative enzymes and on free radical generators, enhance oxidative stress. Other effects of insulin involve the inhibition of proteasome and the stimulation of polyunsaturated fatty acid (PUFA) synthesis and of nitric oxide (NO).[15] Prabhakar SS attempted to review the existing literature, discuss the controversies, and reach some general conclusions as to the role of NO production in the diabetic kidney. He found that genetic polymorphisms of the NOS enzyme also may play a role in the NO abnormalities that contribute to the development and progression of diabetic nephropathy.[16]

Insulin resistance increases renal damage through nitric oxide

The results of study performed by Steinberg HO argued that insulin effect on the endothelium is mediated by its own receptor and insulin signaling pathways, resulting in the increased release of nitric oxide. The vascular actions of insulin are impaired in insulin-resistant conditions such as obesity, Type II (non-insulin-dependent) diabetes mellitus and hypertension, which could contribute to the excessive rate of cardiovascular disease in these groups. Insulin-resistant state in obesity and Type II diabetes shows a multitude of metabolic abnormalities that could cause vascular dysfunction. Non-esterified fatty acid level increased long before hyperglycaemia becomes present.[17] Under the circumstance of insulin resistance, endothelial dysfunction leads to vascular complications in the central link, which results in microalbuminuria.

2.2 Hypertension and pathogenesis of metabolic syndrome-associated nephropathy

The abnormality of kidney structure and function caused by hypertension is called hypertensive renal damage. Arteriolar nephrosclerosis is the most characteristic pattern in hypertensive renal damage, including benign arteriolar nephrosclerosis and malignant arteriolar nephrosclerosis. The former caused by benign hypertension, the latter caused by malignant hypertension. Sustained hypertension can cause renal arteriolosclerosis for 5-10 years (tunica intimal thickening of arcuate artery and interlobular arteries, hyaline of afferent artery), wall thickening, lumens narrowing, and secondary ischemic renal

parenchyma ischemic lesions, including glomerular ischemic shrinkage, sclerosis, tubular atrophy, interstitial infiltration of inflammatory cells and fibrosis, which lead to benign arteriolar nephrosclerosis. Malignant arteriolar nephrosclerosis is an accelerated hypertension or malignant hypertension-induced renal damage.

2.2.1 Pathogenesis of benign arteriolar nephrosclerosis

Factor of hemodynamics

Due to normal autoregulatory mechanism of renal vessels, renal blood flow (RBF) can be kept relatively stable, which can protect the kidney from blood pressure fluctuation. Renal arteriolar constrictive response occurs in the presence of hypertension, which increase renal vascular resistance and decrease renal blood flow (RBF). The degree of contraction of efferent arteries is more significant than afferent arteries in early stage. Glomerular filtration rate (GFR) can still be maintained within the normal range. With the progress of hypertension, there is renal arteriosclerosis, compliance decreasing, arterial wall thickening, lumens stenosis, and RBF further decline, GFR falling, which lead to ischemic renal lesions. Impairment of kidney tubules secondary to ischemia is more sensitive than glomeruli. Furthermore, renal tubular load does not reduce for maintaining normal GFR, thus more likely to increase renal tubular injury secondary to glomerular hyperperfusion.[18] However, benign arteriolar nephrosclerosis has obvious individual differences. There is not observed renal arteriolar sclerosis in some glomerulosclerosis secondary to hypertension. Hypertensive renal injury is not completely ischemic lesions. In addition to ischemic hypoperfusion nephron, the recent view is that most hypertensive renal damage is characterized by hypertransfusion compensatory nephron. The existence of glomerular hyperperfusion, high pressure and high filtration promote renal parenchyma lesions, especially is the major pathogenesis of glomerulosclerosis.[19].

Under hypertension state, renal vascular sensitivity to Ang II was significantly enhanced. Renal vascular resistance increased in the patients with hypertension who are injected low dose Ang II, and did not significantly change in normal people. The mechanism of proteinuria occurence is currently considered that increased RAS activity lead to podocyte fracture membrane damage and basilar membrane permeability increasing. High intra-glomerular pressure and high shear stress-induced endothelial cell injury and dysfunction, activated local lesions, increased Ang II and aldosterone, induced renal vascular remodeling and renal arteriosclerosis.[20]

Non-hemodynamic factors

In hypertension state, vascular endothelium bear high pressure and shear stress, cause endothelial cell injury, injured endothelial cell can lease cytokines, such as transforming growth factor β (TGF-β), plasminogen activator inhibitor (PAI).[21] Hypertension can directly cause renin-angiotensin-aldosterone system activation and oxidative stress. These factors can cause together kidney damage, matrixfibrosis and tissue hardening.[22, 23]

Clinically nephroangiosclcrosis may occur before significant elevation of blood pressure, such as unilateral nephrectomy or early type 1 diabetes mellitus (TIDM). Glomerular capillary blood flow is still significantly increased. Therefore, the renal capillary pressure overload, it can lead to nephrosclerosis. high pressure and high shear in glomerular cause endothelial cell dysfunction, which could lead to increase in some active factors such as angiotensin II (Ang II), endothelin-1(ET-1), thromboxane A2 (TXA2), TGF-β2 and platelet-

derived growth factor (PDGF) factors, leading to vasoconstriction, mesangial cell proliferation and collagen deposition, promoting ECM synthesis and secretion. The high pressure in glomeruli can also lead to glomerular visceral epithelial cell injury, increasing permeability of the basement membrane, causing proteinuria, eventually leading to glomerulosclerosis, nephron loss. Therefore, the patients with essential hypertension have low perfusion of ischemic nephron and high perfusion nephron, the latter is more characteristic pattern.

In addition, reactive oxygen species, salt intake, lithium-sodium counter-transport abnormalities, racial and genetic backgrounds, metabolic disorders, age, gender, body mass index, smoking is also influencing factors.

In summary, hypertensive renal damage is secondary to vascular lesions caused by high arterial pressure. The main mechanism of hypertensive renal damage is hemodynamics abnormalities in glomerular, and cytokines, vasoactive substances and the ECM are involved in the disease process.

2.2.2 Pathogenesis of malignant arteriolar nephrosclerosis

The direct vascular injury of elevated blood pressure

When blood pressure is significant elevated, renal artery and glomerular capillary stress and shear stress can be changed, which induce endothelial cells to secrete varied adhesion molecules, promoting inflammatory cell adhesion to endothelial cells, leading to endothelial cell damage.[24] Vascular endothelial cell damage lead to increased permeability, plasma protein and fibrinogen deposition in the vessel wall, induce vascular fibrinoid necrosis and tunica intimal damage. Finally, it appears vascular lumen narrowed and renal ischemia. But some patients with severe and persistent hypertension, whose vascular injury can not become malignant state. It indicates that in addition to intravascular pressure, there are other factors involved in vascular damage.

Activation of renin-angiotensin-aldosterone system (RAAS) and endothelin

Malignant hypertension often accompanied with activation of RAAS, It should be noted that the activation of RAAS may be primary or secondary. Activation of RAAS can promote intermittent vasoconstriction, activate platelets, release thromboxane and platelet derived growth factor (PDGF), stimulate myointimal cell migration and proliferation, cause vascular lumen narrowed, increased level of blood pressure and renal ischemia. When the systolic pressure is over 180-190mmHg critical level, it can occur naturally natriuretic and diuretic phenomenon, the decline in blood volume can further activate the RAAS.[25] In addition, elevated angiotensin can promote inflammatory cells adhesion to endothelial cell, induce apoptosis of endothelial cells, damage the integrity of blood vessels, induce fibrinoid necrosis of arteriole by "vascular toxicity".[26] Endothelin is of powerful vasoconstrictor function, it can cause sustained elevation of blood pressure. Animal model of malignant hypertension has been confirmed to plasma endothelin and endothelin-mRNA expression in renal tissue increased.[27]

Microvascular coagulation and thrombosis

Hypertension damage vascular endothelial, which active directly coagulation system, lead to platelet aggregation and fibrin deposition in vascular lumen. When red blood cells pass through the damaged vascular lumen, it prone to result in damage and lead to microvascular coagulation. Meanwhile, both platelet aggregation and adhesion of

leukocytes on vessel wall lead to turbulence, promoting to form platelet micro-thrombus.[28]

Genetic factors

It is reported that HLAB15, DR3, BW35 and CW4 are significantly associated with the incidence of malignant hypertension.[29, 30]

2.3 Lipid metabolic disorders and pathogenesis of metabolic syndrome-associated nephropathy

Lipid metabolism and kidney disease are closely related. As early as 1982, Moorhead[31] proposed that lipid accumulation can lead to chronic renal injury, it is not only a lot of primary or secondary renal diseases with common clinical manifestations, but also the progress of the diseases.[32] Hyperlipidemia, in addition to proteinuria and hypertension, promotes CKD progression outside the third most important factor. In polycystic kidney disease, obesity, diabetes and hypertension in animal models, hypercholesterolemia were found to accelerate the progress of kidney diseases, and high-fat diet can induce kidney macrophages and foam cell formation leaching, resulting in glomerular sclerosis.[33] In obese Zucker rats, lowering serum triglycerides improves glomerular sclerosis.[34] These results suggest that lipid metabolism is closely related with renal dysfunction, and a series of clinical studies have also been to the same conclusion. Samuelsson et al[35] found triglyceride-rich lipoproteins containing Apo-B in patients with CKD are closely related to the progress of the disease; Muntner et al[36] found in 12 728 subjects with normal renal function that low HDL cholesterol and hypertriglyceridemia in individuals with impaired renal function appeared more dangerous.

2.3.1 Mesangial cell function

Regulation of glomerular filtration of mesangial cells to produce matrix components, involved in the development of many glomerular diseases. Mesangial cell surface LDL, oxidized HDL and very low density lipoprotein act through receptor pathway and the corresponding lipoproteins. LDL can bind to mesangial cells and mesangial cell function can offset.[37] LDL stimulates mesangial cells and had a "phase effect", ie, low concentration of LDL stimulates cell proliferation, and high concentrations inhibits cell proliferation as toxic cells effect. The effect of LDL promoting mesangial cell proliferation may be related to arachidonic acid metabolism. Mesangial cells cytochrome P450 monooxygenase system produces epoxide metabolic pathways, it can promote cell proliferation, LDL oxidation and the formation of more toxic OX-LDL in a dose dependent manner, which can further increase mesangial cell injury. The mesangial cells and macrophages form foam cells and release cytokines and growth factors, such as transforming growth factor β, tumor necrosis factor, platelet derived growth factor and interleukin-1 and so on. These cytokines can stimulate the LDL receptor gene transcription and expression of epithelial cells, mesangial cells and macrophages, and promote the deposition of lipid in kidney cells and induced renal injury.[38] Mesangial cells themselves can be oxidized by LDL. OX-LDL can induce apoptosis of mesangial cells.

2.3.2 Mesangial matrix

Glomerular mesangial matrix includes type IV collagen, fibronectin and laminin. LDL and LDL oxidation in vitro can stimulate the increase of extracellular matrix components. LDL

can be activated in mesangial cells by LDL protein kinase C, which promotes transforming growth factor-β synthesis in the cells, and TGF-β can stimulate the cells to produce tissue inhibitor of matrix metalloproteinases, inhibiting matrix degradation and leading to the increase of mesangial matrix.[39] It has been confirmed by in vitro experiments that lipids increased the expression of TGF-β mRNA in mesangial cells and epithelial cells.

2.3.3 Endothelial cells

Endothelial cells with LDL receptor, VLDL receptor and LDL receptor related protein. Lipoprotein receptors and related, or non-receptor pathway, causing cell proliferation, lipoprotein lipase and lipoprotein receptors can enhance and strengthen its effect in promoting cell proliferation. OX-LDL and Lp of endothelial dysfunction caused mainly by interfering with the vasodilators nitric oxide synthesis and direct inactivation; and increased thromboxane A2 and endothelin production, damage vascular endothelial NO dependent relaxation response, cause renal ball efferent arteries, increasing the pressure in the glomerular endothelial cells to further damage, the release of cytokines, to promote cell proliferation, glomerular sclerosis. In addition, the lipoprotein receptor pathway through the endothelial cells directly mediated endothelial cell injury, while activation of coagulation and fibrinolytic system activation and inhibition of platelet function, leading to fibrin deposition and thrombosis, increased glomerular injury.

2.3.4 Glomerular epithelial cells

Glomerular epithelial cell surface LDL, VLDL receptor and lipoprotein receptor related protein, which contains both apoB and apoE, respectively, with the lipoproteins, the annexation of the metabolism of cells. apoE with high affinity receptors, the degree of cellular cholesterol esterification increased, and inhibition of cholesterol synthesis, therefore, glomerular epithelial cellsith more and VLDL, thus not explain the clinical hypercholesterolemia, only lipoprotein formed an exception. Can also cause the deposition of lipids in the glomeruli, glomerulosclerosis occurring phenomenon.

2.3.5 Tubulo-interstitium

Tubular injury showed increased tubulointerstitial matrix proteins and fibrosis. Proximal tubular epithelial cells present LDL, very low density lipoprotein receptor, hyperlipidemia lipid deposition in the renal tubules, tubular epithelial cells by phagocytosis, the formation of foam cells, while LDL, OX-LDL for the expression and FN mRNA secretion, further stimulate tubulointerstitial synthesis, promoting fibrosis.

2.3.6 OX-LDL renal toxicity

OX-LDL can promote renal cell proliferation, apoptosis and phenotypic transformation, involved in the glomerulosclerosis process from cell to cell is too small too many states the state of the process of cell loss.[40] OX-LDL also has the monocyte chemotactic activity of macrophages can express clear the OX-LDL receptor, OX-LDL uptake by macrophages stimulated macrophages after the synthesis of growth factors, cytokines and other related matrix protein synthesis in the media. Extraordinary receptor exists in glomerular mesangial cells and epithelial cells. In the case of lipid metabolism, the oxidation of LDL can be modified first intake, OX-LDL accumulation in the kidney and stimulate the kidney cells to secrete TGF-β1 and other cytokines to promote renal fibrosis, leading to monocyte-

macrophage in the local infiltration. Moreover, the activation of macrophages and can promote LDL oxidation, secretion of TGF-β and PDGF-AD, caused by extracellular matrix and mesangial expansion, creating a vicious cycle.[41] Focal segmental glomerulosclerosis in animal models and human glomerular diseases, a number of chronic renal biopsy tissues were detected by OX-LDL deposition, and the extent of OX-LDL deposition and renal dysfunction and protein was positively correlated with urine.

2.4 Hyperuricemia and pathogenesis of metabolic syndrome-associated nephropathy

Hyperuricemia is highly prevalent in MS patients. A few studies showed that hyperuricemia was associated closely with progression of kidney disease.[42] About 20% to 60% of patients with gout have mild or moderate renal failure. The histological lesion named "gouty nephropathy" includes glomerulosclerosis, interstitial fibrosis, and renal arteriolosclerosis, often with focal interstitial urate crystal deposition.

The precipitation of uric acid in the renal medulla with formation of characteristic tophi was believed to activate an inflammatory response resulting in renal interstitial fibrosis, a loss of nephrons, and ultimately to irreversible chronic renal failure. When pH<5.5 in vivo or dehydration, urate can deposit in the renal tubules and interstitium which cause urate nephropathy. It also can form kidney stone in distal tubule and collecting duct and induce obstruction. Emmerson[43] found that some interstitial deposits of urate and uric acid in the kidney derived from intra-tubular deposits which react with the tubular epithelium and pass into the interstitium; loss of tubular integrity may not be a prerequisite for crystal migration. Toblli[44] confirmed that urate crystals deposit in renal tubular cells, evoke complement, platelet, inflammatory cell and macrophages by classical pathway or alternative pathway, induce the expression of cytokine and transforming growth factor beta increased; stimulate fibroblast to be fibrocyte, activate cross linkage of collagen and ultimately lead to renal fibrosis or renal failure.

However, it is difficult to ascribe the generalized renal damage in gout to the deposition of urate crystals, for they are often only focally present.

Recent studies have reported that mild hyperuricemia in normal rats induced by the uricase inhibitor, oxonic acid (OA), results in systemic hypertension, renal vasoconstriction, glomerular hypertension and hypertrophy, and tubulointerstitial injury independent of intra-renal crystal formation.[45-47] It has also been found that hyperuricemia can accelerate renal disease in the remnant kidney model and accelerate experimental cyclosporine nephropathy.[48,49] The main pathophysiological mechanism by which uric acid causes these conditions involves the inhibition of endothelial nitric oxide bioavailability and direct actions on endothelial cells and vascular smooth muscle cells.[50,51] The importance of these pathways is suggested by a recent prospective study in which lowering uric acid in individuals with hyperuricemia and renal dysfunction was associated with improved BP control and slower progression of renal disease.[52]

There are a lot of clinical evidences that hyperuricemia may induce endothelial dysfunction, as lowering uric acid with allopurinol can improve endothelial function as measured by brachial artery vasodilatation.[53] Interestingly, while both uric acid and nitric oxide (NO) exhibit circadian variation, serum uric acid peaks around 6 a.m. when the level of NO is lowest.[54] This relationship can be accounted for by the finding that uric acid also inhibits endothelial cell dependent vasodilatation of rat aortic rings[55] and NO production in endothelial cells.[56] Furthermore, uric acid blunts endothelial cell proliferation in response to serum. [56] The mechanism by which uric acid inhibits NO levels is complex. It may involve scavenging by

oxidants, which can be induced by NADPH oxidase under hyperuricemia.[57] A reduced NO bioavailability could also be due in part to inhibition secondary to CRP production.[56]
In addition, the activation of RAS plays an important role in the exacerbation of renal injury caused by uric acid, which has been shown to be an important mediator of progression of renal disease, not only by its hemodynamic effects to increase systemic and glomerular pressure, but also by its direct fibrogenic effect in kidney and vessels. The increase of renin expression is observed in hyperuricemic rats. The relationship between serum uric acid and plasma renin activity has been described in humans.[58] Blocking the renin angiotensin system can ameliorate hypertension and renal injury in hyperuricemic rats.[45] Furthermore, studies in humans suggest that uric acid acts on blood pressure and renal injury in part via the renin angiotensin system.[59] All of above discoveries suggest that the roles of uric acid may also be mediated by the activation of the renin angiotensin system.
Hyperuricemia also alters glomerular hemodynamics.[47] Hyperuricemia induces cortical renal vasoconstriction in rats as evidenced by a significant increase of afferent and efferent arteriolar resistances. A decrease in glomerular plasma flow and the ultrafiltration coefficient resulted in a 35% decrease in single nephron GFR whereas glomerular pressure was increased. These changes were restored by allopurinol treatment. Aberrant renal autoregulation appears to be responsible for the glomerular hypertension observed with experimental hyperuricemia. Under normal conditions, an increase in mean systemic arterial pressure causes a reflex vasoconstriction of the afferent arteriole, thus preventing the transmission of the increased pressure to the glomerular circulation. However, in the event that the afferent arteriolar vasoconstriction is insufficient, the transmission of increased pressure to the glomeruli results in glomerular hypertension.[47] While renal vasoconstriction occurs in experimental hyperuricemia, it may be insufficient for the degree of systemic hypertension, therefore glomerular pressures are increased. This may be due to the disease of the afferent arteriole that occurs in the hyperuricemic rats, as evidenced by an increase in the media to lumen ratio. Again, the observation that allopurinol was able to prevent arteriolar hypertrophy leading to a normal renal autoregulatory response indicates a potential role of uric acid on this process.[47]

2.5 Obesity and pathogenesis of metabolic syndrome-associated nephropathy

The metabolic syndrome is a cluster of the most dangerous heart attack risk factors: diabetes and prediabetes, abdominal obesity, high cholesterol and high blood pressure.[60] Abdominal obesity is the form of obesity most strongly associated with metabolic syndrome. Obesity and metabolic syndrome has been found to be independent risk factors for CKD.[3, 61, 62] Treating obesity might stabilize renal function or reverse early hemodynamic abnormalities and glomerular dysfunction.[63, 64] Since the first description of an association between massive obesity and nephrotic proteinuria in 1974, a specific histopathologic pattern characterized by glomerulomegaly, in many cases accompanied by focal segmental glomerulosclerosis, has been described repeatedly in obese patients without any other defined primary or secondary glomerular diseases (including diabetic nephropathy, hypertensive nephrosclerosis, and secondary focal segmental glomerulosclerosis) and now is referred to as "obesity-related glomerulopathy".[65, 66] Overweight, obesity, and the metabolic syndrome have recently emerged as strong independent risk factors for chronic kidney disease (CKD) and ESRD. The multivariate analysis made by Chen et al[3] showed that the risk for being affected by CKD was more than twice as high in patients with an increased waist circumference than in those without, suggesting that obesity may be an independent risk factor for CKD.

Obesity with the features of the metabolic syndrome causes renal dysfunction,[67] increase glomerular filtration rate (GFR), renal blood flow (RBF), and filtration fraction (FF) in experimental and clinical observations.[64, 68, 69] Obesity also increases the risk factor for diabetes and hypertension. Iseki[70] indicate that obesity, including metabolic syndrome, is a potential treatable cause of CKD and ESRD. In a large cohort of >320,000 patients who were followed at Kaiser Permanente, Hsu et al[62, 71] found that a higher BMI was a strong independent risk factor for ESRD even after adjustment for other major risk factors that are associated with ESRD, including smoking, baseline hypertension, and diabetes.

Hyperlipidemia, hyperinsulinemia, hyperleptinemia, hyperuricemia and hypercoagulability, together in obese patients may directly or indirectly affect renal structure and function, caused kidney damage.

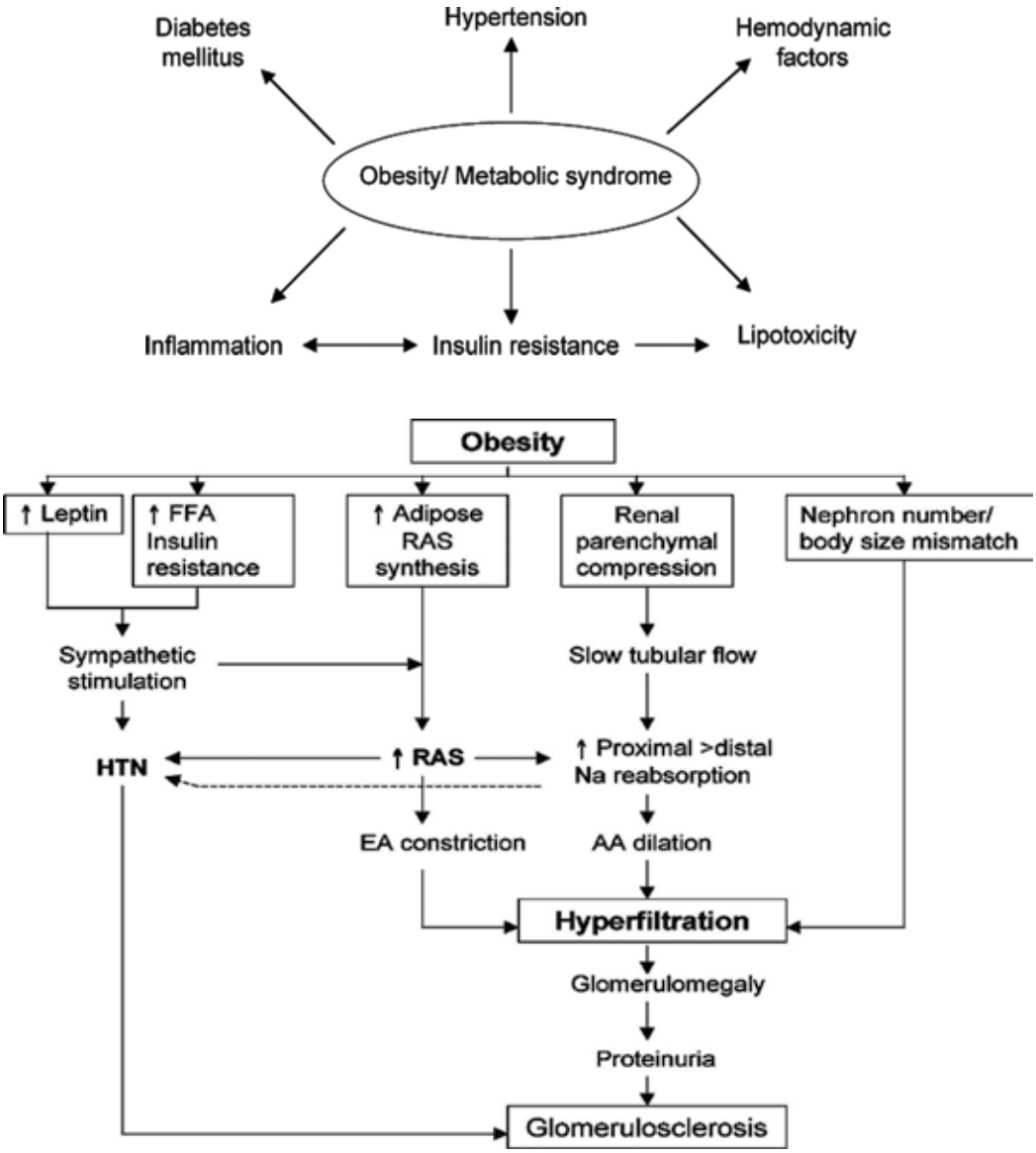
The pathogenesis of ORG may be implicated:

1. Renal hemodynamic alterations: Studies in animals and in humans have shown that obesity is associated with elevated GFR and increased renal blood flow.[68, 71] This likely occurs because of afferent arteriolar dilation as a result of proximal salt reabsorption, coupled with efferent renal arteriolar vasoconstriction as a result of elevated Ang II. In addition, Ang II may have a role in the regulation of adipokine production in adipose tissue and may increase insulin resistance in the setting of obesity.[72, 73]
2. Sympathetic stimulation: Obesity-related there is widespread increased sympathetic nerve activity, its causes and obese patients with baroreceptor dysfunction and vasomotor centers less on the incoming inhibitory signals.[74]
3. Leptin: Wolf[75] studies have shown that Leptin levels in obese patients was significantly higher, it was upregulated in glomerular endothelial cells and mesangial cells. Increased TGF-β1 and its receptor mRNA expression promote glomerular endothelial cells and mesangial cell proliferation.
4. Inflammation: The results of several studies have suggested that adipose tissue, especially visceral adipose tissue, is a major source of cytokine secretion in the metabolic syndrome, and that inflammatory cells, especially mature bone marrow–derived macrophages, invade adipose tissue early in obesity.[76, 77] Inflammation was linked to obesity and the metabolic syndrome in patients with CKD. Ramkumar et al[78] found a strong association between inflammation as defined by a CRP level >3 mg/dl and a high BMI in patients with CKD.
5. Changes in fatty acid composition in kidney, causing kidney reduced the release of vasoactive substances, increased the pressure of glomerular capillary. Hall et al[79] show that the mechanisms responsible for increased sodium reabsorption and altered pressure natriuresis in obesity include activation of the renin-angiotension and sympathetic nervous systems, and physical compression of the kidneys due to accumulation of intra-renal fat and extracellular matrix.

2.6 Diabetes and pathogenesis of metabolic syndrome-associated nephropathy

Diabetic nephropathy is the most important long-term complication of diabetes mellitus and a major cause of end-stage renal disease. The condition is associated with excess cardiovascular morbidity and mortality, as well as other diabetic microvascular complications.[80] The etiopathogenesis of diabetic nephropathy appears to involve both genetic and environmental factors leading to disease in a subgroup of patients. Improved knowledge of the natural history and pathophysiology of the condition have enabled

therapeutic strategies to be employed that have improved the outlook for patients with nephropathy.[81]



2.6.1 Historical background

DN has the characteristic of obvious family accumulation, but the incidence of the disease in different races is very different. Diabetic nephropathy is a serious problem resulted from microvascular complications of diabetes mellitus. Thus, genetic factors determining susceptibility plays an important role in DN, which was shown by studies about angiotensinogen (AGT) gene, angiotensin converting enzyme (ACE) gene, aloes reeducates (AR) gene, Glut21 gene, endothelial cells Nitric oxide synthase (eNOS) gene, cell receptor β-

chain of fixed area (TCRβ) genes. Correlation between gene polymorphism and the occurrence and development of DN has also been discovered.[82]

2.6.2 Chronic hyperglycemia

Hyperglycemia can activate intracellular key catalytic glucose into sorbitol AR. With high glucose increased AR activity, polyol metabolism in kidney is active, so that excessive accumulation of sorbitol and fructose. Sorbitol polarity is strong, thus can not freely through the membrane, while fructose has little further metabolism. It results in fructose accumulation and the increase of intracellular osmotic pressure and therefore cell edema. While the level of intracellular inositol is decreased, glutathione, NADH / NAD + ratio increased, Na2K2ATP activity decreased, which results in tissue hypoxia and endothelial cell damage, contributing to the occurrence and development of DN.[83] Some studies suggest that AR gene polymorphism is related to AR mRNA levels in peripheral blood mononuclear cells and diabetic micro-vascular complications. High glucose can lead to non-enzymatic glycosylation reaction, formation of advanced glycation end products (AGEs) in many organs. AGEs bind the AGE receptor (RAGE) of vascular endothelial cells, macrophages, vascular smooth muscle cells, mesangial cells and other cells. RAGE, as a signal transduction receptor, activates mitogen-activated protein kinase pathway (MAPK) and nuclear factor (NF) -κB signaling pathway (cell proliferation and inflammatory response), Ras pathway (stress and apoptosis), Rac/Cdc-42 pathway (cell growth and movement), Jak / Stat pathway (regulation of gene expression), raising the expression of a variety of growth factors, such as platelet derived growth factor and transforming growth factor, basic fibroblast growth factor, and adhesion molecules, such as intercellular adhesion molecules-1 and vascular cell adhesion molecule.[84]

Reactive oxygen species (ROS), including superoxide radicals, hydrogen peroxide, hydroxyl radicals, and lipid peroxidation, are of in vivo biological activity of inducing aerobic metabolism. Studies demonstrated that increased oxidative stress existed in diabetes. High glucose increased the generation of ROS by inhibiting the activity of the three glycerol phosphate dehydrogenase, leading to the development of microvascular complications mediated by diabetes-related signaling pathways, such as PKC pathway, polyol pathway, hexosamine pathway and AGEs formation. High glucose can also increase the the gene transcription of adhesion molecules and inflammatory NF-κB. Increased levels of ROS can also increase peroxynitrite synthesis and nitrotyrosine formation, leading to DNA damage, prompting the development of diabetic microvascular complications.[85]

PKC activation in diabetes is a common pathway of vascular injury. PKC family, has more than ten isozymes and plays, mainly PKC-β, a role in vascular injury in diabetes. PKC can be activated through a variety of ways. Hyperglycemia within the tissue cells can increase PKC activation, increase NADH / NAD + ratio, increase oxidative stress.[86] PKC inhibited endothelial nitric oxide synthase (eNOS) activity, decreasing NO level and NO-mediated inhibition of cyclic guanosine monophosphate (cGMP) generation, leading to vasomotor dysfunction.[87] PKC stimulated platelet aggregation and increased the content and activity of PAI-1, promoting the hypercoagulability of patients with diabetes. PKC promoted vascular endothelial growth factor (VEGF) expression, promoting angiogenesis, increasing vascular permeability.[88] PKC regulated the expression of transforming growth factor (TGF)-β and increased the expression of fibronectin and collagen type IV, leading to extracellular matrix expansion. Researches had shown that TGF-β promoted local extracellular matrix deposition.

2.6.3 Microvascular disorders

Homodynamic changes in early diabetic micro-vascular disorders characterized by increased pressure, is generally reversible. High glucose increased the plasma osmolality, increasing blood volume and renal blood flow. Diabetes changed the ratio between the resistance of glomerular arterioles, resulting in glomerular capillary hyperperfusion and high pressure, the mesangial matrix expansion and basement membrane thickening, and therefore leading to focal glomerular sclerosis.[89] Meanwhile, the capillary endothelial cell was damaged, the normal filtration barrier was damaged, protein filtration was increased, which result in the loss of glomerular function.

Microvascular disorders in DN is the pathological basis of clinical manifestations, the most prominent is the basement membrane thickening and the damage of glomerular filtration barrier function. The pathogenesis includes continuing glomerular hyperperfusion and hyperfiltration, increased collagen synthesis. Sustained high glucose leads to the non-enzymatic glycation of basement membrane protein components. Another major pathological feature of DN is increased mesangial matrix. Mesangial expansion is mainly resulted from the following factors: glomerular hemodynamic abnormalities, increased capillary pressure. High filtration can stimulate the increase of mesangial matrix, the damage of glomerular filtration barrier. The leakage and accumulation of macromolecules in the membrane system stimulate mesangial cell proliferation and promote matrix production. High blood glucose activates protein kinase C in mesangial cell and the increase in mesangial matrix protein synthesis. Cellular growth factors are the important factors playing roles in increasing mesangial matrix. The most important of which is TGF-β. High blood glucose, glomerular capillary pressure and angiotensin (Ang) - II can promote the synthesis of extracellular matrix of mesangial and tubular epithelial cell. In addition endothelin (ET) also stimulate the proliferation of mesangial cells and the secretion of matrix. NO had inhibitory effect on mesangial cells. NO level is increased in early stage of diabetic nephropathy, which results in glomerular hyperperfusion and hyperfiltration. And it is reduced in the late stage due to endothelial cell damage, which leds to the increasing of mesangial matrix, playing a role in accelerating glomerular injury.[90]

In diabetes, blood flow slows down and micro-thrombosis is easy to form, mainly due to abnormal endothelial cell and platelet function. The increase in serum of von Willebrand factor (vWF) is considered a sign of vascular endothelial cell injury. vWF is synthesized by endothelial cells, mediates platelet adhesion to endothelium, promote thrombosis. Overseas studies suggest that vWF is an independent risk factor for microvascular disease in diabetes.[91] In addition endothelial dysfunction is also reflected by the decreased activity of tissue plasminogen activator (t-PA) or the incresed activity of plasminogen activator inhibitor (PAI) which result in decreased fibrinolytic activity. Decreased prostacyclin-2 (PGI-2) synthesis reduced platelet inhibition. Increased release of ET can promote platelet aggregation. Plasma β platelet globulin (β-TG) and platelet factor 4 (PF4) levels reflect platelet activation. Increased thromboxane (TXA) -2 synthesis promote platelet aggregation and thrombosis. Many studies shown that increased platelet aggregation plays a very important role in the development of microangiopathy in type-2 diabetes mellitus, in addition to long-term hyperglycaemia.

2.7 Metabolic syndrome in pathogenesis of allograft renal damage

The metabolic syndrome (MS) is a cluster of interrelated common clinical entities, which include obesity, insulin resistance, glucose intolerance, hypertension and dyslipidaemia. Insulin resistance is their common pathophysiological basis. Metabolic syndrome

significantly increases the risk for cardiovascular disease and chronic kidney disease.[92] Recently it has been found that MS is also common in renal transplant recipients. How is kidney transplantation complicated with metabolic syndrome?

2.7.1 Immunosuppressive therapies

Immunosuppressive therapies induce post-transplantation diabetes

Post-transplantation diabetes is believed to be multi-factorial, probably involving β-cell toxicity and increased insulin resistance. In addition to other risk factors, studies suggest that immunosuppressive regimens may account for a large degree of the increased risk for the development of post-transplantation diabetes.

Corticosteroids and Calmodulin inhibitors (cyclosporine and tacrolimus) are widely used in kidney transplant recipients. They have long been recognized to potently affect glucose tolerance by a prevalent increase of peripheral insulin resistance.

Increasing daily prednisolone dose was independently associated with insulin resistance as glucocorticoids promote gluconeogenesis in the liver, inhibit glucose uptake, diminish glycogen synthesis in skeletal muscle cells and also may attenuate insulin secretion from pancreatic beta-cells. Several mechanisms displayed in vitro studies on murine β-cell or human cell lines incubated with dexamethasone, have been proposed: insulin secretion inhibition by increased expression of a2-adrenergic receptors, decreased cAMP levels, decreased Glut2 protein at the β-cell plasma membrane, down regulation of glucokinase mRNA, increased voltage-gated K+channel activity, β-cell apoptosis through the activation of the calcineurin phosphatase and the corticosteroid receptor.[93-96]

Calcineurin inhibitors, tacrolimus and cyclosporine cause reversible toxicity to islet cells and may directly affect the transcriptional regulation of insulin expression. Furthermore, both calcineurin inhibitors impair insulin gene transcription regulation through the inhibition of calcineurin signalisation. Other mechanisms have been proposed: closing of the ATP-sensitive potassium channel, interference with mitochondrial function of pancreatic β-cell, impairment of glucose-stimulated insulin secretion downstream of the rise in intracellular Ca2+ at insulin exocytosis, reduced ATP production and glycolysis derived from reduced glucokinase activity, decreased islet cell viability by a down regulation of anti-apoptotic factors and accumulation of pro-apoptotic mediators in cultures of freshly isolated human islets. Tacrolimus is more diabetogenic than cyclosporine.[97-99] At the first, it can be due to the steroid mimetic effect of tacrolimus. Tacrolimus binds to FK506-binding protein (FKBP), predominantly FKBP-12. Another immunophilin, FKBP-52 is associated with the cytoplasmic glucocorticoid (GC) receptor complex. When cells are exposed to glucocorticoids, the steroid binds to the GC receptor and liberates it from the complex. By binding to FKBP-52 in the GC receptor complex, tacrolimus may alter the affinity of interactions and either cause a release of the GC receptor at lower steroid concentrations, a steroid-sparing effect, or it may free the GC receptor I absence of steroids . Second, tacrolimus increases the bioavailability of steroids.

Immunosuppressive therapies induce obesity

Prednisone can induce overweight or obesity in transplant recipients, espically abdominal obesity, the abdominal fat which is sensitive to catecholamine can induce insulin resistance through elevating the level of low density lipoprotein and very low density lipoprotein, inhibiting the activity of phosphofructokinase, blocking glycolysis and glucose uptake.

2.7.2 Virus infection

Cytomegalovirus is one of the most important pathogenic microorganism after renal transplantation, Cytomegalovirus infection can infect insulin secretion, induce insulin resistance and impair the function of pancreatic β-cell, and it is independent infector of post-transplant diabetes mellitus（PTDM).[100] Cytomegalovirus infection increases the activity of tumor necrosis factor(TNF), TNF can affect the function of islet B cell and decrease the organism's sensitivity to insulin, that will promote insulin resistance in renal transplant recipients.

Patients with HCV infection were found to have a 8.3-fold higher risk of appearing PTDM compared with HCV(-) patient.[101] Patients with HCV disease have increased peripheral insulin resistance and are hyperinsulinemic, similar to those with type 2 DM. It also is postulated that patients with HCV have decreased β-cell responsiveness, possibly caused by direct viral effects. Other explanations include an autoimmune pathogenesis because HCV has been associated with several autoimmune diseases, including cryoglobulinemia, Hashimoto's thyroiditis, and Sjogren's syndrome. This would suggest antibody-mediated destruction of pancreatic β-cells. A potential role of viruses in the cause of type 1 DM has been suggested, as well as a role for enteroviruses. A greater prevalence of PTDM in patients with HCV therefore likely is caused by a combination of increased peripheral insulin resistance and either a direct viral- or immune-mediated effect of HCV on pancreatic β-cells that results in relative insulin deficiency.

2.7.3 Polycystic kidney disease

Insulin resistance with compensatory hyperinsulinaemia has been reported in adult polycystic kidney disease patients. It is reported that post-transplant diabetes mellitus (PTDM) occured in 10 adult polycystic kidney disease (APKD) patients and four controls (34.6% vs 15.3%; $P<0.005$).[102] It has been shown that increased membrane fluidity and abnormal erythrocyte Na/Li counter-transport, both abnormalities associated with insulin resistance, are present in APKD patient.[103, 104]

3. Diagnosis

Till now there is no formal denomination of metabolic syndrome associated kidney diseases which are often diagnosed as obesity-associated glomerulonephropathy, diabetic nephropathy, hypertension-associated kidney damage, lipid disorder associated kidney damage and hyperuricemia-associated kidney damage.

The diagnosis of metabolic syndrome associated renal disease is generally two levels criterion (Table 3-1). The first level is for large sample epidemiological screening, which is base on the diagnosis of MS and CKD, and often including MS complicated with CKD or CKD complicated with MS. The second is for clinical evaluation and treatment, which base on the diagnosis of MS and the clinical presentation and renal pathology of obesity-associated glomerulonephropathy, diabetic nephropathy, hypertension related renal damage lipid disorder associated kidney damage and hyperuricemia-associated kidney damage. Renal biopsy might be necessary for the investigation and clinical diagnosis of metabolic syndrome associated kidney diseases under some circumstances, and the pathological evaluation should be made with not only light microscope, but also immunofluorescence and electron microscope. The definite diagnosis of metabolic syndrome associated kidney diseases should exclude other primary or secondary kidney diseases. In clinical practice, metabolic syndrome

associated kidney diseases are often accompanied with other primary or secondary kidney diseases. Post-transplant metabolic syndrome associated kidney diseases are often complicated with acute or chronic rejection in renal allograft.

Level 1 Metabolic syndrome complicated with chronic kidney disease

1. Consistent with the diagnosis criterion of metabolic syndrome (necessary).
2. Consistent with the chronic kidney disease (necessary).

Level 2 Metabolic syndrome associated kidney disease

1. Consistent with the diagnosis criterion of metabolic syndrome (necessary).
2. One of the following diagnosis criterions.
    i. The obesity patient with the renal pathological character of obesity- associated glomerulonephropathy.
    ii. The diabetes patient with the renal pathological character of diabetic nephropathy.
    iii. The hypertension patient with the renal pathological character of hypertension related renal damage.
    iv. The patients with hyperuricemian and the renal pathological character of uric acid nephropathy

Table 3.1. The diagnosis of metabolic syndrome associated renal disease: two levels of criterion

3.1 Diagnosis of MS

A diagnosis of metabolic syndrome associated kidney diseases must be based on the diagnosis of metabolic syndrome which is not only composed of central obesity, diabetes or prediabetes, hypertension, lipid metabolic disorder, etc., but also insulin resistance as its critical pathophysiological basis. the American Heart Association (AHA) along with the National Heart, Lung and Blood Institute issued an up-to-date version on the diagnosis of the metabolic syndrome.[105] The International Diabetes Federation (IDF) also provided a working definition for the syndrome (Table 3-2).[106]

Clinical diagnosis criteria	**Categorical cut points**
Insulin resistance	None
Body weight	Increased WC (population specific) plus any 2 of the following
Lipid	TG ≥150 mg/dL (1.7 mmol/L)or on TG Rx HDL-C <40 mg/dL (1.03 mmol/L)in men or <50 mg/dL (1.3 mmol/L) in women or on HDL-C Rx
Blood pressure	≥130 mm Hg systolic or ≥85 mm Hg diastolic or on hypertension Rx
Glucose	≥100 mg/dL(5.6 mmol/L), includes diabetes

Table 3.2. Criteria for Clinical Diagnosis of Metabolic Syndrome (IDF, 2005)

The present AHA/NHLBI statement, in contrast to IDF, maintains the ATP III criteria except for minor modifications (Table 3-3).

Measure (any 3 of 5 constitute diagnosis of metabolic syndrome)	**Categorical cut points**
Elevated waist circumference	≥102 cm (≥40 inches) in men, ≥88 cm (≥35 inches) in women
Elevated triglycerides	≥150 mg/dL (1.7 mmol/L) Or On drug treatment for elevated triglycerides
Reduced HDL-C	<40 mg/dL (1.03 mmol/L) in men<50 mg/dL (1.3 mmol/L) in women Or On drug treatment for reduced HDL-C
Elevated blood pressure	≥130 mm Hg systolic blood pressure, Or ≥85 mm Hg diastolic blood pressure Or On antihypertensive drug treatment in a patient with a history of hypertension
Elevated fasting glucose	≥100 mg/dL (5.6 mmol/L) or On drug treatment for elevated glucose

Table 3.3. Criteria for Clinical Diagnosis of Metabolic Syndrome (AHA/NHLBI)

It is suggested by the data of our recent community-based screening that the incidence of MS according to the diagnostic criteria of CDS was lower than that according to the diagnostic criteria of IDF. Some residents with MS mainly presentation of abdominal obesity would be missed diagnosis by the criteria base on BMI. In the components of MS, hypertension, abdominal obesity and lower high density lipoprotein were more common than others.

3.2 Diagnosis of CKD

The early evidences of metabolic syndrome associated kidney diseases might exist before the occurrence of clinical diabetes or hypertension, and it should include elevated or decreased GFR, microalbuminuria or even smaller proteins occurring in the urine before microalbuminuria, the presentation of renal pathological disorders existing before the clinical manifestation.

According the K/DOQI definition and classification, CKD is defined as kidney damage or glomerular filtration rate (GFR) <60 mL/min/1.73 m^2 for 3 months or more, irrespective of cause. Kidney damage in many kidney diseases can be ascertained by the presence of albuminuria, defined as albumin-to-creatinine ratio >30 mg/g in two of three spot urine specimens. GFR can be estimated from calibrated serum creatinine and estimating equations.[107]

There are many methods to determine GFR have been used, such as measuring GFR using exogenous markers, or endogenous markers, using exogenous marker is extremely inconvenience and is not used in clinical and epidemic practice. The often used endogenous markers are serum creatinine (SCr), usually calculated as creatinine clearance (CCr), and serum cystatin C. Most studies have shown that serum cystatin C levels correlate better with GFR than does serum creatinine alone, especially at higher levels of GFR. In practice, combining use of multiple indexes, such as SCr, age gender and race to evaluate GFR will be more accurate. So there many equations have been used to estimate GFR. The two most commonly used equations to estimate GFR are serum creatinine based: Cockcroft-Gault (CG) and the Modification of Diet in Renal Disease (MDRD) equations. A recent study involving a pooled analysis of individuals with chronic kidney disease proposed an

estimation equation that included serum cystatin C in addition to serum creatinine, age, sex, and race. Studies concluded this equation provided the most accurate estimates.
The CG equation is as follows:

$$CCr(ml/min) = \{[140 - Age(yr)] \times Weight(kg)\}/SCr(mg/dl) \times 72 \times (0.85, \text{if female})$$

The six variable MDRD equation is as follows:

$$GFR = 170 \times (SCr)^{-0.999} \times (Age)^{-0.176} \times 0.762(\text{if female}) \times 1.18(\text{if black}) \times (BUN)^{-0.170} \times (Alb)^{0.318}$$

where BUN is blood urea nitrogen and Alb is albumin.
The abbreviated version or four variable version of the MDRD equation (ml/min per 1.73 m2) is as follows:

$$GFR = 186 \times (SCr)^{-1.154} \times (Age)^{-0.203} \times 0.742(\text{if female}) \times 1.212(\text{if black})$$

The equation base on SCr and serum CysC is as follow:

$$eGFR = 177.6 \times (SCr)^{-0.65} \times (CysC)^{-0.57} \times (Age)^{-0.20} \times 0.82(\text{if female}) \times 1.11 (\text{if black})$$

3.3 Renal pathological changes in metabolic syndrome

There is a small sample retrospective design.[108] The histopathologic presentation of patients with metabolic syndrome compared with controls had a greater prevalence of tubular atrophy, interstitial fibrosis, and arterial sclerosis, suggesting microvascular disease. Patients with metabolic syndrome had greater global and segmental glomerulosclerosis. Glomerular volume and cross-sectional surface area were not different. The combined end point of tubular atrophy greater than 5%, interstitial fibrosis greater than 5%, and presence of arterial sclerosis was more prevalent in patients with metabolic syndrome than controls.

4. Prevention and treatment

Aggressive multitargeted management of the metabolic syndrome can also improve cardio vascular and renal outcomes and is highly recommended by the American Heart Association. Although no study has evaluated whether multiple interventions can reduce the incidence or progression of CKD in patients with the metabolic syndrome.[109]

4.1 Lifestyle changes

Lifestyle interventions are the first line therapies recommended for treatment of the metabolic syndrome. The essential and important measurements include weight reduction, regular exercise, and a low-calorie, low-fat diet. Yet few data are available to indicate that such lifestyle interventions can prevent or reverse renal damage.[109]

4.2 Medication treatment

If lifestyle change is not sufficient, then drug therapies for abnormalities in the individual risk factors may be indicated. Such as medicine for treatment of diabetes, hypertension, lipid disorder and hyperuricemia.

4.2.1 Treatment of elevated blood glucose

In metabolic syndrome patients with IFG (or IGT if assessed), weight reduction, increased physical activity, or both will delay (or prevent) the onset of type 2 diabetes mellitus.

Intensive glucose control to lower the glycated hemoglobin value to 6.5% yielded a 10% relative reduction in the combined outcome of major macrovascular and microvascular events, primarily as a consequence of a 21% relative reduction in nephropathy.

Oral Antihyperglycemic Agents:[110] Metformin, thiazolidinediones and acarbose will lower risk for type 2 diabetes mellitus in people with IFG or IGT. The nonsulfonylurea insulin secretagogues repaglinide and nateglinide can be used in renal failure without dose adjustments. Metformin is contraindicated in renal failure because of the associated risk for lactic acidosis. It can be used at low dosages up to a creatinine clearance of 30 to 60 ml/min and should be avoided with clearances<30 ml/min. Although the metabolism of thiazolidinediones is unaffected by renal failure, they must be used with caution in this context because of their volume retaining effect with a risk for heart failure. The sulfonylureas (glyburide, gliclazide, glipizide, glibenclamide, tolbutamide, and chlorpropamide) have increased potency to prolong sulfonylurea induced hypoglycemia as the renal function decreases and are contraindicated in severe renal failure. α-Glucosidase inhibitors (acarbose and miglitol) are also contraindicated in renal failure.

Insulin therapy: Insulin therapy maybe benefits in glycaemic control, but not improves insulin resistance and metabolic syndrome. Insulin analogues, whose main objective is to stimulate physiologic insulin secretion, has opened new therapeutic possibilities in diabetic CRF patients.[111] Although only a few studies have evaluated the clinical efficacy and safety profile of insulin analogues in CRF patients, preliminary results appear hopeful. There is another concern of insulin injection for long term might be unfavourable, as ectogenous insulin may inhibit endogenous insulin secretion and results in the lack of the co-secretion of other beneficial substances such as C-peptide which is just now known to be potential in the treatment of diabetic nephropathy.[112]

4.2.2 Treatment of elevated blood pressure

In the presence of diabetes or chronic kidney disease, the blood pressure goal is <130/80 mm Hg. Mild elevations of blood pressure often can be effectively controlled with lifestyle therapies. Some investigators support angiotensin-converting enzyme (ACE) inhibitors as first-line therapy for hypertension in the metabolic syndrome, especially when either type 2 diabetes mellitus or chronic renal disease is present. ARBs may be used in those who cannot tolerate ACE inhibitors or as an alternative to ACE inhibitors.

4.2.3 Treatment of dyslipidemia

According to ATP III, as long as LDL-C remains above goal level, LDL-C is the primary target of therapy even in the metabolic syndrome. Other lipid risk factors are secondary. The LDL-C goals depend on estimates of absolute risk.

The main effects of Statin therapy are reduction of LDL-cholesterol, triglyceride and systemic inflammation, possible improvement of endothelial function and inhibition of renal endothelin 1-mediated proteinuria. The goal level of LDL-cholesterol: <1.80 mmol/l in very high-risk patients and <2.60 mmol/l in high-risk patients.[109]

Fibrates and nicotinic acid are the most commonly used drugs for elevated TG and reduced HDL-C. The main effects of fibrates therapy are decrease of triglycerides, increase of HDL, increase of insulin sensitivity, anti-inflammatory and antihypertensive action, and also reduction of mesangium-induced glomerular matrix deposition.[109]

4.3 New treatment

Islets transplantation or stem cell transplantation has shown very exciting future in more effectively controlling blood glucose and preventing and treating diabetes-associated organ damage. More early intervention and prevention targets have been found for metabolic syndrome associated kidney diseases.

4.3.1 Islets transplantation

Pancreas or Islets transplantation is indicated to treatment of type 1 diabetes which insulin is insufficient for normal glucose metabolism. After long term (more than five years) of normoglycemia, diabetic nephropathy will be reversed.[81] It is not clear that pancreas or Islets transplantation will be benefit to improve of type 2 diabetes. In fact, insulin resistance will be more serious after transplantation due to the use of immunosuppressive agent.[113] Our small sample of patients with type 2 diabetes and ESRD receive combined transplantation of islets and kidney shown reversion of the peripheral nerves and vascular diseases, and maybe a protection of renal graft from damage of hyperglycosemia.

Islet transplantation is an effective therapy for insulin-dependent diabetes mellitus, based on the research data which indicated that islet transplantation could not only retrieve the glycometabolism disorders but also prevent and reverse diabetes-associated microangiopathy. According to the registration data of the International Organ Transplantation Center, there had been up to 1300 patients received islet transplantation by the end of 2006. More than 40 institutes have developed islet transplantation for totally 550 cases of diabetic patients, since the Edmonton Islet Transplantation Protocol was available in 2000. The growth rate increased markedly compared to the era before 2000.

The islets isolated from donor's pancreatic tissue, by means of enzyme digestion and centrifugal purification, are injected into recipients' liver through portal vein. The transplanted islets locate in hepatic sinus, adjusting the synthesis of hepatic glycogen and reversing the disorders of glycometabolism through secreting insulin. Autogeneic islet transplantation is limited in patients received entire or partial resection of pancreas due to chronic pancreatitis and tumor. Allogeneic islet transplantation needs immunosuppressants and therefore is mainly suit for kidney transplant recipients with type I diabetes-associated renal failure. Along with the development of new immunosuppressants, the indications of islet transplantation are increasing. In recent years islet transplantation along has been applied in patients with "friable" type I diabetes with refractory severe hypoglycemia while without renal failure, and considered for patients with type II diabetes and complete lost of islets' function.

A four year cooperative study was carried out in 9 islet transplantation centers of USA, Canada and Europe and 36 cases of type I diabetes received islet transplantation according to coincident Edmondon Protocol. It was shown by the data that 16 cases were insulin-independent one year after transplantation (16/36, 44%) and, among them, 5 cases were still insulin-independent after 2 years follow-up (5/16, 31%). The study proved the efficacy of Edmondon Protocol in significantly increasing the success rate of islet transplantation. Besides, it is indicated by the success of Edmondon islet transplantation protocol that islet transplantation along is also fit to patients with type I diabetes while it is not always needed to be performed together with renal transplantation. In recent years some islet transplant recipients without renal failure were only administered short-term of immunosuppressive treatment (Daclizum administered on day 1 and day 3) and good efficacy achieved, which suggests that islet transplantation along may be do not need long-term immunosuppression therapy.

The surgical operation of islet transplantation is simple, but the isolation and the purification of islets belong to high-tech range. The skill and experience of operators are extremely important. Different operators have markedly different isolation results even they use the same pancreas from the same donor and apply the same isolation procedure. How to get enough functional islets from donor's pancreas is the key technique for islet transplantation. Islet transplantation is a program needs multi-department and multi-subject cooperation. A cooperation network should include endocrinologists, surgeons, transplantation center, net-serving staff, official administrators, transplant immunologists, transportation department. In addition, the qualities of a lot of procedures during transplantation can influence the result of islet transplantation, including patient selection, tissue matching, reservation and transportation, islet isolation and purification, islet implantation, blood glucose controlling during islet transplantation, immunosuppressant administration, and function evaluation after transplantation. The determination of islet cell auto-antibodies in recipients' blood after transplantation is important for the prognosis of long-term islet transplantation efficacy. We should further investigate the relationship between the production of islet cell auto-antibodies and the survival of transplanted islets, and make sure if the auto-antibodies is the main factor resulting in the damage of transplanted islets. Islet cell auto-antigens can be produced with gene recombination and immune absorption column can be prepare with the auto-antigens and used to in time eliminate the auto-antibodies in patients' blood, reducing islet damage induced by the antibodies and increase the long-term survival of transplanted islets.

4.3.2 Stem cell transplantation

Stem cell transplantation has shown effective in treatment of type 1 diabetes shown beta cell function increased,[114] and also in treatment of type 2 diabetes with significant increase of serum adiponectin and glucose tolerance.[115] Data from studies of NOD/SCID mice with diabetes shown that mesenchymal stem cells (MSCs) administration can prevent and treat diabetic nephropathy, prevent the pathological changes in the glomeruli and enhances their regeneration resulting in improved kidney function in diabetic animals.[116]

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Glomerulonephritis and the Cystic Fibrosis Patient

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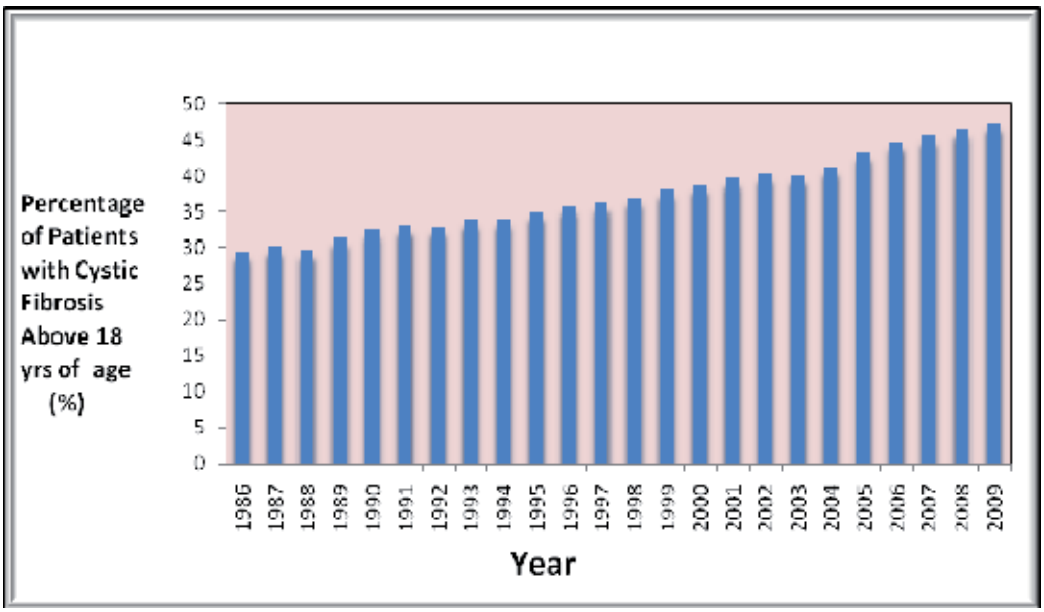
1. Introduction

Cystic Fibrosis (CF) is a disease with an evolving definition. Through earlier diagnosis and newborn screening programs, as well as a robust world-wide research program, we are able to treat individuals afflicted with this life-threatening malady more aggressively and with earlier interventions. Despite our progress in extending the life expectancy of the typical CF patient, the disease is still viewed by the general medical community as one of childhood.

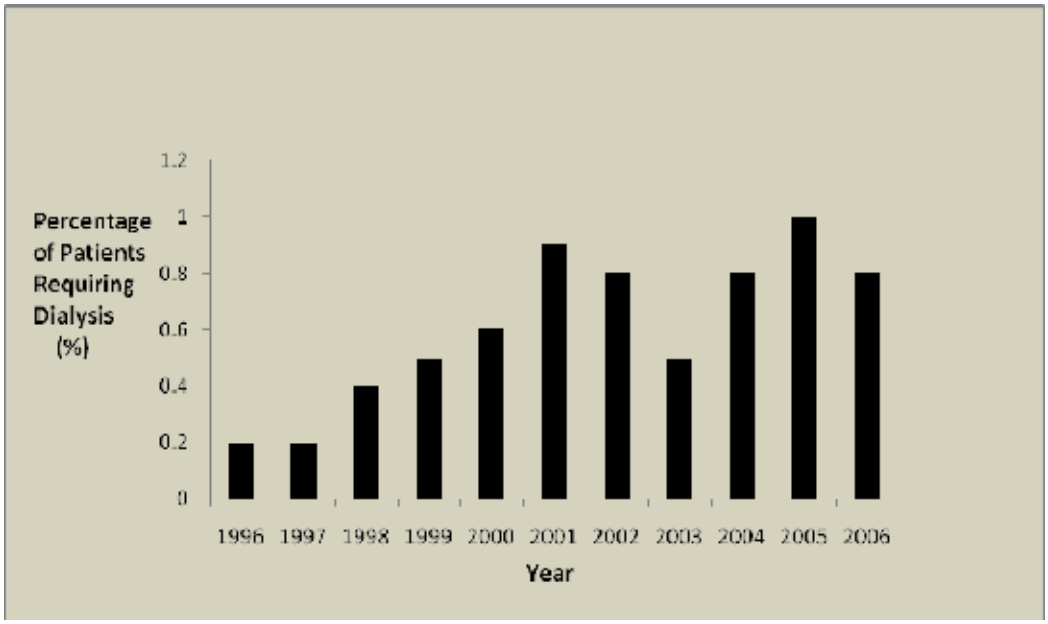


Source: Cystic Fibrosis Foundation Patient Registry Annual Data Reports, 1986-2009

Fig. 1. Prevalence of Cystic Fibrosis in North American Adults

This former "age constraint" on the natural history of CF is paralleled by other major developments, such as an expansion of the number of organ systems, which we now know are involved in this disease. We now know that this disease affects more than the pulmonary and gastrointestinal systems.

With the aging of the CF population, it has come to light that CF patients suffer from an increased risk of Diabetes Mellitus (Fischman & Nookala, 2008; Stecenko & Moran, 2010), Osteoporosis (Haworth, 2010), and malignancies (Hernandez-Jimenez et al., 2008). There is also a growing body of literature suggesting that as a result of treating other conditions associated with CF and as a result of the inflammatory and immunologic milieu associated with Cystic Fibrosis, these patients also suffer from renal disease (Stephens & Ridden, 2002; Katz et al., 1988) (Figure 2). In this chapter, we will discuss our current understanding of Cystic Fibrosis and review potential associations to renal disease with special attention to glomerulonephritis (GN).



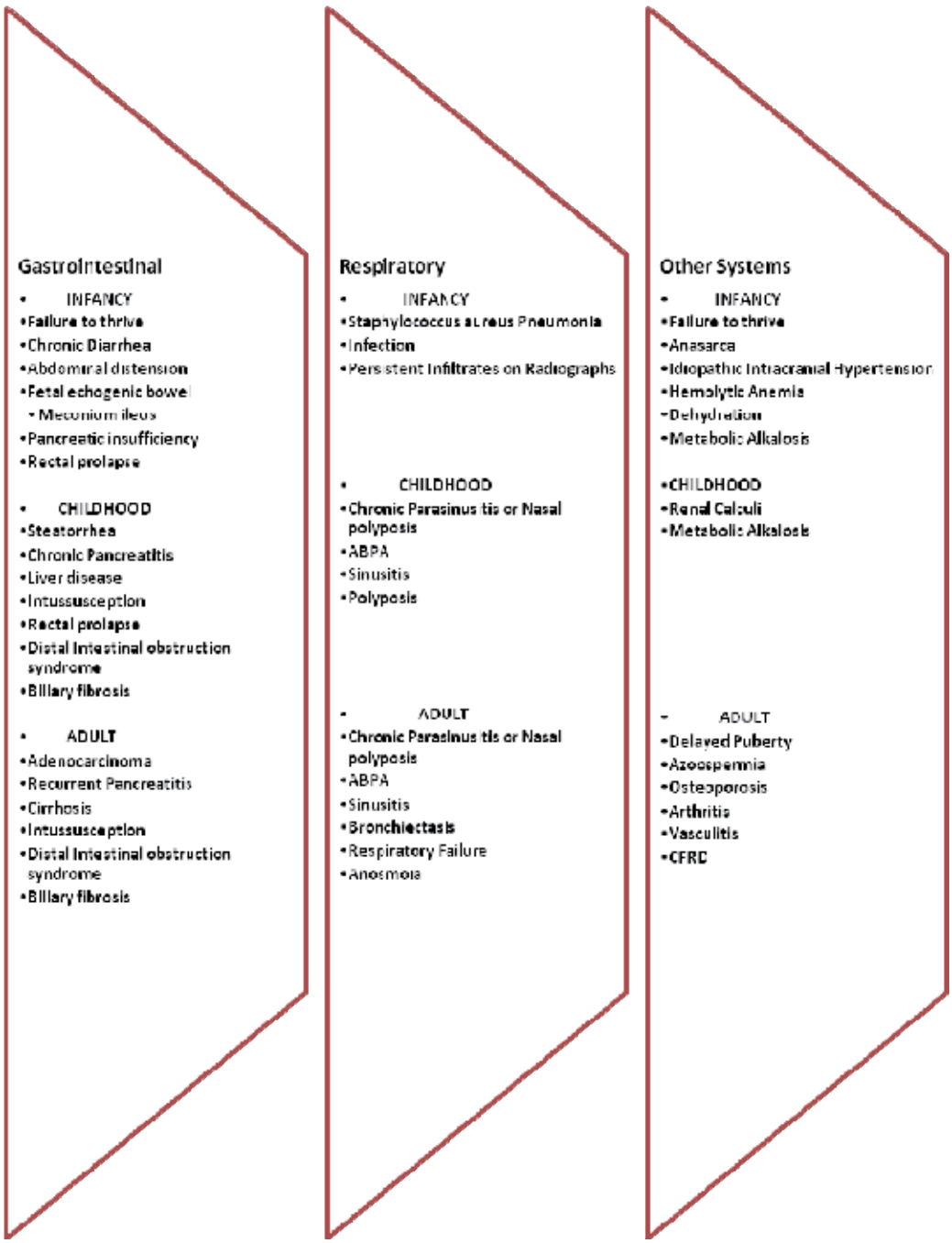
Source: Cystic Fibrosis Foundation Patient Registry Annual Data Reports, 1996-2006

Fig. 2. Prevalence of Cystic Fibrosis Patients Requiring Dialysis for Renal Failure

2. Background - Cystic Fibrosis

2.1 Genetics

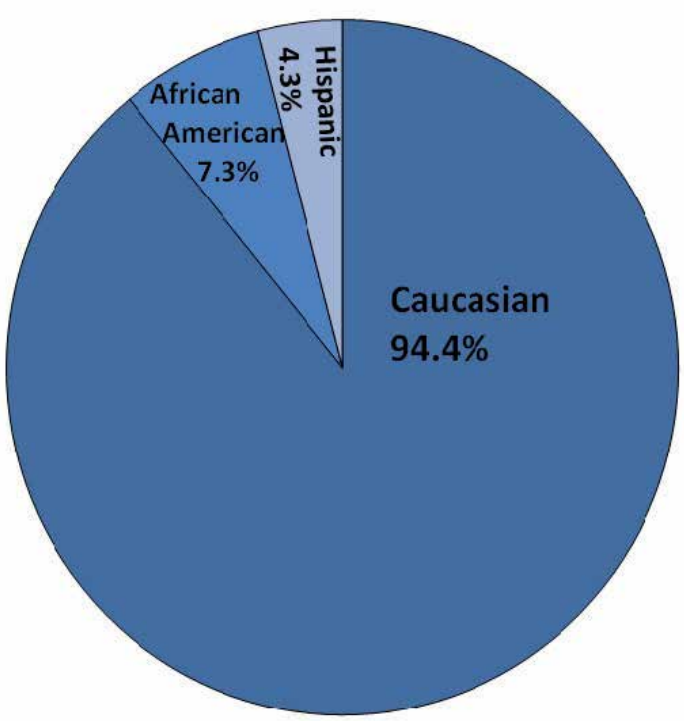
Cystic Fibrosis is the most common, lethal, autosomal recessive disorder seen among the Caucasian population. For this reason, its disease prevalence throughout much of the world is 1 in 2,000 to 1 in 3200 individuals. Among non-Caucasian populations, and those not living in North America or Western Europe, the prevalence is approximately 1 in 4,000 to



Legend: ABPA = Allergic Bronchopulmonary Aspergillosis,
CFRD = Cystic Fibrosis-Related Diabetes Mellitus

Fig. 3. Common Signs and Symptoms of Cystic Fibrosis, by Stages of Life

1 in 20,000 (Figure 4) (Sullivan & Freedman, 2009). Although we have known for decades of the association between salty sweat, obstructive lung disease, and pancreatic insufficiency, which comprise the hallmark symptoms of CF, and it was postulated as early as 1949 that a gene defect was the cause of CF, it was not until 1989 that the gene defect was localized to chromosome 7 (Rowe et. al., 2005). Since that time, more than fifteen hundred mutations have been identified that can lead to the cystic fibrosis phenotype (Boyle, 2007).



Source: Cystic Fibrosis Foundation Patient Registry Annual Data Report 2009

Fig. 4. Racial Demographics of Cystic Fibrosis Patients in North America

This specific gene encodes a protein called the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR), which is found on many different types of cells. CFTR plays numerous regulatory roles throughout the body. The role most commonly associated with CFTR is that of chloride channel. However, the CFTR protein also plays a role in the inhibition of sodium transport, the regulation of ATP channels, and the inhibition of calcium-activated chloride channels. We now know that there are multiple types of CFTR gene mutations. They range in effect from complete lack of protein production (Class I Mutation), and defective protein processing and trafficking to the cell surface (Class II), to reduced production of normal-functioning CFTR protein (Class V) (Sullivan & Freedman, 2009) (Table 1). The F508del mutation, which accounts for two-thirds of Cystic Fibrosis in Northern Europe and North America, is a class II mutation, resulting in production of a defective CFTR protein.

Mutation Class	*Effect on CFTR Protein*	*CFTR Ability to Function*	*Pancreatic Exocrine Dysfunction*
I	Protein not produced	No	Severe
II	Protein trafficking defect with CFTR degraded in ER/GolB; CFTR does not reach cell membrane	No	Severe
III	Defective protein regulation; CFTR reaches cell membrane but not activated by ATP or cyclic AMP	No	Severe
IV	Reduced Cl transport through apical membrane CFTR	Yes	Mild
V	Splicing defect with reduced production of functioning CFTR	Yes	Mild
VI	CFTR reaches cell membrane, but more rapid CFTR turnover	Yes	Presumably Mild

Legend: CFTR = Cystic Fibrosis Transmembrane Regulator Protein, ER = Endoplasmic Reticulum, GolB = Golgi Body, ATP = Adenosine Triphosphate, AMP = Adenosine Monophosphate, Cl = Chloride ion

Table 1. CFTR Mutation Classes

Since Cystic Fibrosis is an autosomal recessive genetic trait, full expression of this disease requires that a defective gene be present on each chromosome. However, having one abnormal gene typically conveys a milder level of morbidity. As the level of CFTR metabolic regulatory function decreases below fifty percent, the chance of the individual developing sino-pulmonary conditions such as sinusitis, nasal polyps, or asthma increases. Men with the cystic fibrosis trait may experience infertility due to Congenital Bilateral Absence of the Vas Deferens (CBAVD). Despite the fact that it takes the presence of only one abnormal CFTR gene and fifty percent CFTR function for sino-pulmonary and genitourinary symptoms to occur; it typically takes CFTR function at levels less than five-percent of normal, usually seen with two mutations, before a patient's sweat chloride excretion rises to levels diagnostic of CF. Furthermore, it takes CFTR protein function to be decreased by ninety-nine percent for pancreatic insufficiency to occur (Strasbaugh & Davis, 2007). Moreover, despite our efforts to characterize the association between genetic defect and phenotypic disease, neither sweat chloride level nor pulmonary function test values correlate with number or type of CFTR mutation. In two studies looking at this association, a mutation known to lead to Cystic Fibrosis could be found in only three out of five patients (Groman et. al., 2005; Groman et al., 2002). However, it is interesting that a 2009 study of urinary protein excretion with regard to Cystic Fibrosis did find an apparent association between level of renal protein excretion and genotype, suggesting that although we cannot directly correlate gastrointestinal or pulmonary phenotype with a patient's genotype, we may be able to correlate renal phenotype with CF genotype (Cemlyn-Jones & Gamboa, 2009).

2.2 Pathophysiology

The pathophysiology of Cystic Fibrosis results from abnormalities localized to the CFTR gene site resulting in an abnormality in CFTR protein production or processing. Either

through this absence of CFTR protein, or through the production of abnormally functioning protein, an electrolyte imbalance occurs on luminal surfaces in multiple organ systems. The common result is the production of thick, tenacious secretions, which have impaired immunologic function and result in dysregulation of the patient's inflammatory response, leading to an imbalance between pro-inflammatory and anti-inflammatory chemokines. In addition, there has been some evidence to suggest that the abnormal CFTR protein may facilitate protein binding of bacteria, leading to CF-related lung disease involving such bacterial pathogens as Pseudomonas aeruginosa and Staphylococcus aureus (Sullivan & Freedman, 2009; Rowe, 2005).

2.3 Diagnosis

The diagnosis of Cystic Fibrosis is typically made based on a combination of clinical symptoms consistent with the disease along with confirmatory testing. Since 1959, the diagnostic standard for Cystic Fibrosis has been the measurement of sweat chloride levels, as stimulated through a process using Pilocarpine Iontophoresis. In children younger than six months of age, a normal concentration of sweat chloride is considered to be less than 30 mmol/liter. In patients older than six months, the normal range would be less than 41 mmol/liter. Regardless of age, any repeatable sweat chloride level greater than 59 mmol/liter is consistent with the diagnosis of Cystic Fibrosis, particularly if accompanied by symptoms of sino-pulmonary disease, a gastrointestinal malady such as fat-soluble vitamin deficiency, malnutrition, or intestinal obstruction, metabolic alkalosis, dehydration, or acute salt depletion (Figure 2) (Farrell et al., 2008).

Consistent with the phenotypic pancreatic insufficiency seen in 90-95% of Cystic Fibrosis patients, pancreatic enzyme levels may be measured to help confirm the diagnosis. As of 2010, fourteen European countries and all states in the United States have a process in place for screening newborns for this disease. Most of these testing programs, at least to some degree, rely on measuring levels of Immunoreactive Trypsinogen, a pancreatic enzyme found to be elevated in the first six weeks of life in infants with CF (Barto & Flume, 2010).

In the situation where the sweat chloride analysis is indeterminate or the symptomatic phenotype is subtle, genetic testing is often employed. If the patient is found to have two mutations known to be consistent with the cystic fibrosis phenotype, then the diagnosis can be made. If one mutation is found, and it is unclear if the patient has a second, less well-characterized mutation, then the patient is considered to have a possible diagnosis of Cystic Fibrosis. As noted previously, genetic testing may still be performed in the circumstance of confirmed disease, since the patient's specific mutations may have prognostic significance. Furthermore, as we attain a greater understanding of the myriad of ways that defective CFTR genes may lead to inadequate or defective protein production and function, we will be able to tailor our therapeutic regimens more effectively to the patient's genetic circumstance, in order to modulate or restore CFTR function (Ashlock et al., 2009).

2.4 Clinical manifestations

To date, it has been impossible to draw a direct link between the degree of genotypic abnormality and a particular patient's morbidity. However, there are some common clinical patterns and associations seen in cystic fibrosis patients. Based on these clinical findings, an all-encompassing definition of Cystic Fibrosis would be "a chronic and progressive, multi-system disease leading to sino-pulmonary disease, with probable exocrine pancreatic insufficiency, malnutrition, and gastrointestinal obstructive symptoms". Patients with

Cystic Fibrosis typically have some degree of obstructive lung disease and present with a distinguishing factor of early colonization and subsequent infection from organisms not typically seen except in immunosuppressed or severely bronchiectatic individuals, such as Pseudomonas aeruginosa, Burkholderia cepacia, or Stenotrophomonas maltophilia.

As a consequence of pancreatic insufficiency and associated malnutrition, many Cystic Fibrosis patients are deficient in fat soluble vitamins and suffer from a chronic, negative protein balance. Mouse studies have shown that the presence of CFTR gene defects leads to a heightened risk of Osteoporosis and subsequent bone fracture (Haworth, 2010). Along with this fracture risk, CF patients have an increased risk of kidney stones. One autopsy study of thirty-eight CF patients found that thirty-five had evidence of nephrocalcinosis, including one still-born and two neonatal infants (Katz et al., 1988). This predilection seems to be multifactorial and results from the interplay of 1) impaired vitamin D absorption leading to impaired calcium absorption, 2) chronic disease characterized by increased metabolic tempo, 3) immobility, 4) increased osteoclast activity, 5) inadequate caloric intake due to increase work-of-breathing, 6) an imbalance between protein anabolism and catabolism, and 7) loss of oxalate-degrading bacteria due to frequent, and often chronic antibiotic use (Stephens & Rigden, 2002). Furthermore, Andrieux et al., in their 2010 study, found that 75% of children in their study population had hypocitraturia and 70% had hyperoxaluria, both of which are risk factors for nephrolithiasis (Andrieux et al., 2010).

One interesting aspect of the renal expression of CFTR is that cystic fibrosis patients often have greater antibiotic excretion than their non-CF counterparts. This necessitates the use of higher-than-typical antibiotic doses. The classic example of this occurs with the use of aminoglycoside antibiotics for treatment of pseudomonal infections, where Tobramycin is dosed at 10 mg/kg, sometimes in conjunction with inhaled Tobramycin, instead of the usual 5-7 mg/kg (Barto & Flume, 2010, Bergman et al., 2007). Indeed, in one study of renal failure in children with Cystic Fibrosis, twenty of twenty-four cases of acute renal failure were associated with recent or concomitant aminoglycoside administration (Bertenshaw et al., 2007). As a result of this uncertain pharmacokinetic and pharmacodynamic profile encountered with cystic fibrosis patients, monitoring drug levels is critical to insure that therapeutic doses are being achieved for efficacy.

3. Renal disease and the CF patient

3.1 A broad picture of renal disease in Cystic Fibrosis

Since Dorothy Anderson first generated the term, "Cystic Fibrosis," in 1938, we have known that kidney disease may be part of this malady (Abramowsky & Swinehart, 1982). However, the natural history of CF-related renal disease has been elucidated in few studies. In Abromowksy and Swinehart's 1982 study, they found that all thirty-four of the patients studied had some form of glomerulopathy, with nineteen having glomerulosclerosis. Twenty-five had what was described as a "Mesangiopathy,"and twenty-six had tubulointerstitial disease. In this study, the authors concluded that a number of factors had led to the myriad of renal lesions observed: 1) lung disease with resultant cyanosis, 2) liver disease, 3) Cystic Fibrosis-Related Diabetes Mellitus, 4) the effects of nephrotoxic medications, and 5) an altered immune response. Of note, the authors report that sixteen of thirty-four patients studied had complement-3 (C3) deposits in their kidneys while thirteen had evidence of immunoglobulin–M deposits (IgM) (Abramowsky & Swinehart, 1982).

A more contemporary, 2010 study investigating renal disease in Cystic Fibrosis followed 112 children, starting in the first year of life. This study revealed the presence of microalbuminuria in fifty-eight percent of patients. This finding was attributed to the presence of chronic inflammation as a result of the malfunctioning or absent CFTR protein, repeated infections, and the nephrotoxicity of many medications commonly used in the treatment of CF (Andrieux et al., 2010). Furthermore, a 2009 study following five hundred ten adults with CF, median age of thirty-one years, found that 13 developed renal disease severe enough to warrant renal biopsy, with eight different types of nephropathies found on histologic analysis. Twelve of these thirteen patients were found to have glomerular lesions. In this study, the main types of renal disease found were AA amyloidosis and diabetic nephropathy (Yahiaoui et al., 2009).

In literature reviewed for their 2002 article on renal disease in Cystic Fibrosis, Stephens and Rigden report that IgA Nephropathy appeared to be the most common form of glomerulonephritis described in CF patients, though the occurrence of this condition appeared rare (Stephens & Rigden, 2002). With respect to all renal disease in CF patients, the most common renal pathology found has been nephrocalcinosis. Drug-related nephrotoxicity also continues to be a common cause of renal morbidity among CF patients. Among the various agents responsible medication-induced renal disease, aminoglycosides continue to play a prominent role. As a result of the pharmacodynamic and pharmacokinetic eccentricities of the cystic fibrosis patient, Acute Tubular Necrosis (ATN) remains a constant concern when treating pulmonary exacerbations in this patient population. This risk of ATN is amplified by pulmonary biofilm formation and the need to use a combination of intravenous, oral, and inhaled antibiotics to effect a decrease in pathogen levels, and potentially facilitate bacterial eradication. It is for this reason that current Cystic Fibrosis Foundation guidelines recommend once-daily dosing of aminoglycosides to optimize treatment benefit, yet minimize risk of renal injury (Flume et al., 2009). Furthermore, a 2010 review of aminoglycoside toxicity in cystic fibrosis patients suggested that use of once-daily Tobramycin, particularly when dosed in the morning, may be superior to use of gentamicin in preventing aminoglycoside-induced renal injury (Prayle & Smyth, 2010).

3.2 Measurement of renal function in CF patients

Discussion of renal disease in CF patients is complicated by the fact that conventional methods of measuring renal function may not be accurate in this population (Prayle & Smyth, 2010). In Andrieux's 2008 study of renal disease in children with CF, his team observed that there was no correlation between a calculated Glomerular Filtration Rate (GFR), using the Schwartz Formula's manipulation of serum creatinine (SCr) values, and a urine creatinine-based (UCr) standard.

Schwartz Formula:

$$GFR(mL/min/1.73\ m^2) = ((k)(\text{Height in cm})/(\text{SCr in mg/dL})) \text{ where}$$

K = Constant as follows:

- 0.33 in premature infant
- 0.45 in term infants to 1 year-old
- 0.55 in children older than one, up to 13 years old
- 0.55 in adolescent females
- 0.65 in adolescent males

In one-third of children studied, the Schwartz Formula's serum-creatinine derived approach overestimated renal function (Andrieux et al., 2010). Furthermore, Yahiaoui's 2009 study investigating renal disease in thirteen adults with Cystic Fibrosis concluded that one of the reasons that renal disease is considered uncommon in CF patients is that measurements of SCr and calculations of GFR, using either the Cockcroft-Gault or MDRD equations, fail to adequately and reliably reflect a CF patient's true level of kidney function (Yahiaoui et al., 2009). To explain this apparent inadequacy in SCr-based evaluation of renal function observed in the CF population, it has been suggested that decreases in muscle mass seen among CF patients, and typically related to their underlying disease state, leads to decreased production of SCr. This would hinder the proportional rise in SCr levels in response to renal insufficiency, which has been observed in other studied populations. Recognizing this challenge of accurately measuring renal function in CF patients, methods that more directly measure kidney function have been proposed including methods using UCr collection, typically over twenty-four hours, or using plasma levels of an inert tracer which is only excreted via the kidneys (Prayle & Smyth, 2010).

In recognizing the fallacies of SCr-based formulae, the difficulties of collecting urine accurately over an extended period of time, and the invasiveness of nuclear tracer-based measurements of renal function, Beringer and colleagues studied levels of the biomarker Cystatin C (Cys C) as a potential method of estimating GFR. Their study showed that measurement of Cystatin C clearance was a suitable alternative to measurement of SCr or calculated GFR in following a CF patient's renal function. Furthermore, Cys C levels were not affected by age, gender, muscle mass, diet, or level of physical activity. Moreover, the authors make specific mention of using this method to follow CFRD patients for evidence of renal disease (Beringer et al., 2009). Unfortunately, at this time, Cys C levels have not become widely employed.

3.3 Cystic Fibrosis-Related Diabetes Mellitus and Renal Disease

Since Cystic Fibrosis-Related Diabetes Mellitus (CFRD) is becoming an increasingly common complication in the natural history of Cystic Fibrosis, and can lead to significant renal pathology, we discuss this type of renal disease as an independent section. By the time CF patients reach their thirtieth birthday, 45-50% will have developed CFRD, with an associated increase in morbidity and mortality. This complication of Cystic Fibrosis was formerly the most dreaded of sequelae, as it was associated with a six-fold increase in mortality, particularly among women (Fischman & Nookala, 2008). However, through aggressive efforts to increase screening for evidence of impaired glucose intolerance and overt diabetes, as well as through early use of oral diabetic medications and insulin, the mortality disparity associated with CFRD has all but disappeared (Stecenko & Moran, 2010) (Table 2).

As with other forms of Diabetes Mellitus, CFRD may lead to microvascular changes in such structures as the Eyes, Kidneys, Stomach, and Nerves. A 2007 study of the microvascular complications of CFRD suggested that CFRD-related nephropathy occurred less commonly than renal complications seen in association with other forms of Diabetes Mellitus (Schwarzenberg et al., 2007). The results found in Schwarzenberg's study appear to be consistent with our current understanding of CFRD, which suggests that the increased mortality risk that CFRD conveys is due to accelerated progression of the patient's underlying lung disease, not due to vascular complications (Stecenko & Moran, 2010). Moreover, the microalbuminuria that we typically associate with microvascular damage to the kidney was only seen among CF patients who had fasting hyperglycemia (Schwarzenberg et al., 2007) (Table 3).

Characteristic	CFRD	DM I	DM II
Age at Onset	18-21 years	< 20 years	>40 years
Prevalence	22% of CFFR population (2% of children, 19% of adolescents, 45-50% over age 30[a])	7% of US population	7% of US population (NB: > 15% of population > 50 years)
Body Habitus	Thin	Normal	Overweight/Obese
Insulin Secretion	Decreased, Release delayed	Absent	Decreased relative to need
Insulin Resistance	Increased or Unchanged	Increased Slightly	Increased Dramatically
Ketoacidosis	No	Yes	No
Microvascular Complications	Yes	Yes	Yes
Macrovascular Complications	Extremely Rare	Yes	Yes
Nutritional Support	**High calorie diet:** (120-150%) of RDA **Fat**: 40% of dietary intake (No restrictions on type) **Protein**: 10-20% of calories, not reduced for nephropathy **Sodium**: > 4 grams per day **Vitamins**: Routine supplementation	**Calories adjusted for goal:** growth, weight maintenance, or loss **Fat Restriction**: <30% of total calories, <10% from saturated fats **Protein**: 10-20% of total calories; reduced for nephropathy **Sodium**: <2.4 grams per day **Vitamin**: Supplementation for diagnosed deficiencies	**Calorie restriction for weight loss** **Fat Restriction**: <30% of total calories, <10% from saturated fats **Protein**: 10-20% of total calories; reduced for nephropathy **Sodium**: <2.4 grams per day **Vitamin**: Supplementation for diagnosed deficiencies
Pharmacologic Therapy	Insulin therapy(currently SOC); oral anti-diabetic agents(controversial)	Insulin replacement; SC synthetic Amylin analogues	Oral antidiabetic agents; Insulin therapy; SC Incretin Mimetic and synthetic Amylin analogues

Legend: CFFR = North American Cystic Fibrosis Foundation Registry; DM I = Diabetes Mellitus Type I; DM II = Diabetes Mellitus Type II ; RDA = recommended daily allowance; SOC = standard of care; SC = subcutaneously administered.

Table 2. A Comparison of Cystic Fibrosis-Related Diabetes Mellitus to Types 1 and 2 Diabetes Mellitus

Glucose Tolerance Catergory	Fasting Serum Glucose (mg/dl)	Two-Hour Oral Glucose Challenge: One-Hour Value (mg/dl)	Two-Hour Oral Glucose Challenge (mg/dl)
Normal Glucose Tolerance	<126	>/= 200	<140
Indeterminate	<126		<140
Impaired Glucose Tolerance	<126		140-199
Impaired Fasting Glucose	100-125		
CFRD *without* Fasting Hyperglcemia (CFRD FH-)	<126		>/= 200
CFRD *with* Fasting Hyperglycemia	>/= 126		>/= 200

Table 3. Cystic Fibrosis-Related Diabetes Mellitus Diagnostic Categories

It is interesting that a subsequent 2008 study comparing microvascular changes found in CF patients to a matched cohort of patients with Type I Diabetes Mellitus (DM1) found that while retinopathy occurred more frequently in DM1, renal disease as measured through microalbuminuria occurred with greater frequency in patients with CFRD. The authors of this study explained this discrepancy in microvascular findings by speculating that other factors, such as deficient CFTR function, chronic inflammation, repeated exposure to nephrotoxic agents, or genetic predispositions may be the cause (van den Berg et al., 2008). While the results of these two studies may appear to be conflicting, a closer analysis reveals that van den Berg's study methodology does not distinguish patients with fasting hyperglycemia from those without; thus, if van den Berg's CFRD population had a preponderance of patients with fasting hyperglycemia, then the results of these two studies may be consistent.

Within the last decade, case reports have emerged suggesting that Nodular Glomerulosclerosis (NGS) in cystic fibrosis patients may mimic the findings of Diabetic Nephropathy (DN) (Westall et al., 2004). Since the histopathology of these cases appears to be misleadingly reminiscent of that seen in Diabetic Nephropathy, we have chosen to discuss these cases in conjunction with our discussion of CFRD-related renal disease. In their case report, Westall's team reports that all three patients had pathologic findings thought consistent with DN, including Kimmelstiel-Wilson Nodules, without any evidence of impaired glucose tolerance. The authors speculate that the Focal and Nodular Glomerulosclerosis observed (e.g. the Kimmelstiel-Wilson nodules) may be an idiopathic occurrence, may be the result of CFTR deficiency or abnormal function, or may be the result of accumulation of toxic molecules. The authors explain that these toxic molecules are generated as the by-product of an inflammatory process or as a result of oxidative stress. Furthermore, they conjectured that these pathologic findings may be the consequence of undetected episodes of hyperglycemia, which would eventually become more persistent.

In an accompanying editorial to Westall's case series, Krous discusses the implications of Westall's work. In his commentary, Krous does raise the question of whether we should be

routinely screening cystic fibrosis patients for proteinuria, since renal pathology similar to DN has been described, even without evidence of hyperglycemia. Krous also calls for further investigation of the specific pathologic process that leads to the NGS seen in both CF and Diabetes Mellitus. In the end, the only answer that Krous leaves us with is that it may be prudent to screen CF patients for proteinuria (Krous HF, 2004).

3.4 Glomeulonephritis

3.4.1 An introduction to glomerular disease in Cystic Fibrosis

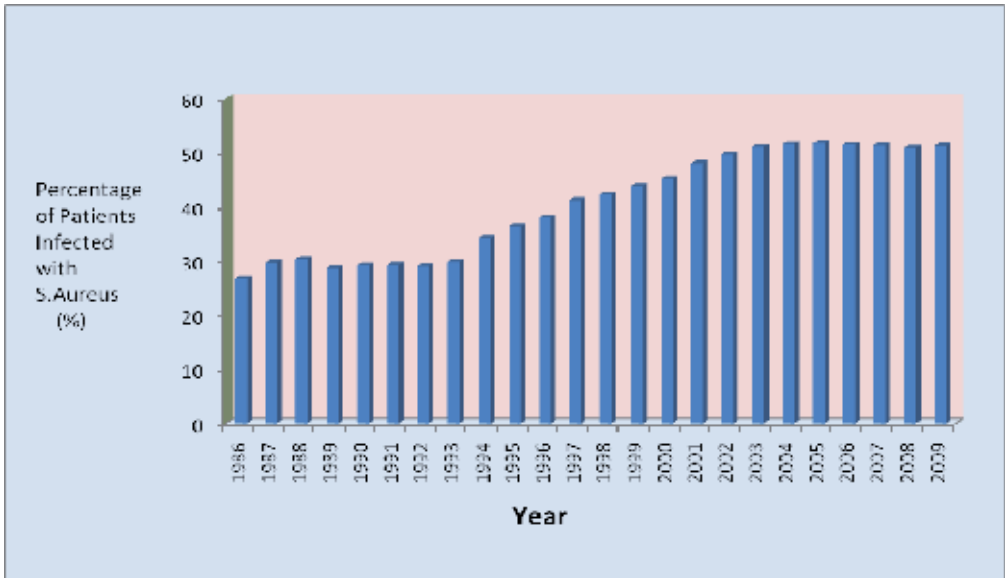
Despite the fact that renal pathology among cystic fibrosis patients was described from the time that CF's constellation of symptoms was first delineated, there still remains relatively little written on renal involvement in CF. There are many explanations as to why this may be the case: lack of a clear CF renal disease phenotype, difficulty measuring renal impairment due to inadequacy of SCr-based renal function tests, and, possibly, reluctance of cystic fibrosis center care teams to pursue the presence of renal pathology with potentially intrusive, and possibly invasive testing (Yahiaoui et al., 2009). However, with the aging of this patient population, an increasing number of case reports have suggested that renal disease is part of the CF phenotype. In 1972, Openheimer described the presence of glomerular pathology in autopsied CF patients (Openheimer, 1972). Abramowsky's 1982 study showed that a majority of patients studied not only had evidence of glomerular involvement, but also immunoflourescence findings of immunoglobulin and complement deposition in glomeruli, with localization to the mesangial regions and capillary loops (Abramowsky & Swineheart, 1982). A more contemporary study of thirteen adults with CF and renal disease, who underwent a renal biopsy, revealed that twelve had glomerular lesions (Yahiaoui et al., 2009). Thus, it is clear that, beyond a CF patient's very real risk of experiencing a renal injury as a result of a medication adverse drug reaction or toxicity, Cystic Fibrosis, itself, may be associated with renal pathology. In this section we will discuss the types of glomerular lesions that have been observed and current theories on the development of glomerular pathology in Cystic Fibrosis.

3.4.2 IgA Nephropathy

In Stephens and Rigden's review of renal disease in Cystic Fibrosis, they report that among the glomerulonephritides, IgA Nephropathy is the most frequently reported (Stephens & Rigden, 2002). In a 1999 case series reported by Stirati and colleagues, four out of the five CF patients who underwent a renal biopsy for proteinuria had findings consistent with IgA Nephropathy. It should be noted that the authors do admit that since IgA Nephropathy is a common type of GN in young adults, and the patients in their study ranged in age from twenty-two to thirty years, their findings may be a chance occurrence.

However, another plausible explanation for this association, put forth by the authors is that recurrent bacterial infections result in a robust immune response leading to increased levels of circulating immunoglobulins and immune complexes. These immune-mediating molecules may deposit in the kidney and lead to the histopathology and morbidity observed (Stirati et al., 1999). Interestingly, in Abromowsky and Swinehart's 1982 study, the explanation of chronic bacterial infection leading to a high level of immune activity resulting in glomerular pathology was also raised. Abramowsky and Swinehart conjectured that the proximate cause was chronic pseudomonas aeruginosa infection. However, contemporary immunologic investigations using pseudomonas antiserum did not reveal any antigens in the studied glomeruli.

If chronic pseudomonal infection is not the nidus for this robust immune response, then what is? The answer may actually lie in staphylococcal infection. Staphylococcus epidermitis bacteremia and staphylococcus aureus-asssociated endocarditis have long been known to cause glomerulonephritis. Furthermore, histopathology of staphylococcal-associated GN typically reveals glomerular immune complex deposits containing complement, particularly C3, and immunoglobulins, typically IgG and IgM. These findings are consistent with those described in CF-related Glomerulonephritis, and have even greater significance in light of the fact that staphylococcus remains a common colonizing pathogen (Figure 5), and a major cause of lung infection early in a CF patient's life, typically causing pneumonia before pseudomonas aeruginosa infections become common. Moreover, idiopathic IgA Nephropathy is known to occur within a few days of the patient experiencing an upper respiratory infection. Thus, there appears to be a viable association between staphylococcal colonization and IgA Nephropathy in cystic fibrosis patients (Satoskar et al., 2006).



Source: Cystic Fibrosis Foundation Patient Registry Annual Data Reports, 1986-2009

Fig. 5. Prevalence of Staphylococcus Aureus Infection/Colonization Among Cystic Fibrosis Patients

In a 2006 case series by Satoskar and colleagues, they report eight cases of staphylococcus infection-associated mesangial and/or intracapillary proliferative glomerulonephritis (GN) associated with IgA-laden immune complex deposition. In this study, seven of the eight patients described had infections other than endocarditis, and the eighth suffered from an epidural abscess which resulted in endocarditis (Satoskar et al., 2006). The reason why staphylococcal infections may lead to GN remains unclear. One theory proposed by Satoskar suggests that staphylococcus enterotoxins may behave as superantigens that bind Major Histocompatibility Complex Class II (MHC II) molecules on Antigen-Presenting Cells (APC). This complex of MHC II and enterotoxin then binds to T-cell receptors, resulting in widespread T-cell activation and a surge of cytokine release. These cytokines, in turn,

activate B-cells, which then produce IgA and IgG molecules. These immunoglobulins are then released, resulting in immune complex formation, and eventual deposition in the Kidney. Further elaborating on this mechanism of glomerular pathology, Koyoma's group described a specific staphylococcus aureus envelope antigen as a proximate cause for superantigen formation (Koyoma et al., 2004). Thus, research studying the association between staphylococcal skin and wound infections, as well as idiopathic IgA Nephropathy, may shed some light on the pathologic association between IgA Nephropathy and Cystic Fibrosis.

3.4.3 Membranoproliferative Glomerulonephritis

The occurrence of Membranoproliferative Glomerulonephritis (MPGN) in cystic fibrosis patients is not a new finding. Ambrowsky and Swinehart's 1982 autopsy study of thirty-four Cystic Fibrosis patients showed on eighteen patients who had evidence of immune complex deposition in the Kidney, and, of those, sixteen had evidence of mesangial proliferation with two also having evidence of membranoproliferative histopathology. Thus, this autopsy study would suggest that MPGN is a major cause of renal disease in the CF patient.

However, more contemporary reviews of the subject do not find nor discuss MPGN in the CF patient (Stephens & Rigden, 2002; Yahiaoui et al., 2009). Indeed, the only recent report of this association was published by Soriano and colleagues in 2008 (Soriano et al., 2008). In this paper, the authors discuss multiple, plausible explanations for the natural history of renal disease in CF. One mechanism proposed, the Factor H Deficiency Model, is based on the observation that genetic knockout mice who are deficient in alternate complement pathway Factor H not only develop MPGN, but also experience higher mortality with pseudomonas aeruginosa infections than factor H sufficient mice. Furthermore, factor H deficiency has been found to lead to aberrant activation of Complement Factor C3 and higher serum levels of various chemokines and cytokines (Soriano et al., 2008). This pathologic explanation is supported by the work of Wisnieski's group, who reported in 1985 that mortality among their cohort of one hundred thirty-nine patients was highly associated with decreased alternate complement pathway function and the presence of circulating immune complexes (Wisnieski et al., 1985).

Separate from the immunologic explanation proposed above, a plausible link between Cystic Fibrosis and Membranoproliferative Glomerulonephritis lies in the function of Toll-like Receptors (TLR), which are part of the Innate Immune System. This family of receptor proteins is responsible for recognizing recurring structures on pathogens, including single-stranded DNA, lipopolysaccharides, and RNA molecules. Upon recognition of a structure known to be associated with a pathogen, the TLR initiates inflammatory and immune responses whose end result is meant to be destruction of the pathogen. By modulating immune responses, including regulating helper T-cell immunologic responses, aberrant TLR function is conjectured to lead to kidney inflammation and glomerulonephritis (Smith & Alpers, 2005). A 2004 study by Muir and colleagues showed that TLR-2 expression was up-regulated in the lungs of CF patients (Muir et al., 2004). Moreover, in 2006, Shuto and colleagues suggested that this TLR-2 up-regulation and prolonged activation was critical to the pathogenesis of CF lung disease (Shuto et al., 2006). Separate from the Factor H Deficiency or the TLR models for the development of glomerulonephritis in cystic fibrosis patients, the presence of staphylococcal superantigens has been conjectured to lead to MPGN in CF patients (Soriano et al., 2008).

3.4.4 Treatment

To date, there has been very little written on the treatment of glomerulonephritis in CF patients. One available case series details the diagnosis and treatment of two adult patients. In this article, it is reported that one of the patients experienced an improvement in his renal disease to the point where he could safely forgo hemodialysis after undergoing a double-lung transplant and starting his anti-rejection regimen (Soriano et al., 2008). This would suggest that the conventional immunosuppressant therapy typically employed in the treatment of glomerulonephritis would be appropriate in this population as well. However, given the known pharmacokinetic and pharmacodynamic intricacies of the CF patient, further study is warranted.

4. Conclusion

Cystic Fibrosis is a disease in evolution. Through wide-spread newborn screening programs, patients are diagnosed earlier. Through an aggressive, world-wide therapy development network, new and revolutionary treatments for the manifestations of Cystic Fibrosis are shepherded from the lab bench to the patient's home. As we develop a better understanding of how genetic mutation translates into phenotypic dysfunction and symptoms, we will be able to regulate and modify protein function to ameliorate the symptoms of Cystic Fibrosis. However, with these revolutionary changes to how patients experience the morbidity of Cystic Fibrosis, and with the aging of the CF population, new manifestations of CF may emerge. Kidney disease, particularly glomerulonephritis, may be one of the more plausible morbidities to afflict CF patients with growing regularity in the future. Thus, we must stay ever vigilant, and not become complacent that we have a complete understanding of this disease process. Cystic Fibrosis is no longer a disease of children. We must, therefore, continue to broaden our understanding of what it means to be an adult with Cystic Fibrosis.

5. Acknowledgement

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22

Mild Forms of Alport Syndrome: Hereditary Nephropathy in the Absence of Extra-Renal Features

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1. Introduction

The type IV collagen nephropathies comprise a spectrum of abnormalities predominantly affecting the glomerular basement membrane (GBM) in the kidney, but also involving other organs such as the ear and eye. Type IV collagen nephropathies result from genetic mutations causing loss or deficiency of type IV collagen synthesis, and have been associated with Alport syndrome at one end of the spectrum, where individuals who are the most severely affected experience end-stage renal failure (ESRF) in their early teen-age years together with hearing loss and vision abnormalities. At the other end of the spectrum type IV collagen nephropathies are associated with mild defects, such as thin basement membrane nephropathy (TBMN) or benign familial hematuria where individuals may experience mild kidney abnormalities involving episodic hematuria but retain relatively normal renal function and show no extra-renal abnormalities. There are six different type IV collagen genes located on multiple chromosomes, and three of these genes (*COL4A3*, *COL4A4*, and *COL4A5*) are associated with X-linked, autosomal recessive or autosomal dominant inheritance patterns of Alport syndrome. In addition the type IV collagen genes are associated with the TBMN phenotype, involving heterozygous mutations of the *COL4A3* and *COL4A4* genes, with an autosomal dominant pattern of inheritance. The main focus of this chapter is the mild forms of Alport syndrome, and so in the following pages we review mild presentations of Alport syndrome, and illustrate this with a unique New Zealand family segregating mild X-linked Alport syndrome, some of whom display features of TBMN.

2. Synthesis and distribution of type IV collagen

The type IV collagen family is comprised of six homologous α-chains designated α1(IV)–α6(IV) encoded for by the *COL4A1-6* genes respectively, the corresponding genes of which are located pairwise on chromosomes 13q34, 2q36-37 and Xq22. Each α-chain has three domains composed of a short 7S domain at the amino terminus, a long collagenous domain of approximately 1400 residues of Gly-Xaa-Yaa repeats and a noncollagenous (NC1) domain of about 230 residues at the carboxyl terminus (Figure 1). Three α chains assemble into triple-helical molecules called protomers that then assemble into supramolecular networks by the association of four protomers at the N-terminus, forming a 7S tetramer, and the

dimerization of two protomers at the C-terminus, forming an NC1 hexamer (Timpl et al., 1981). Interruptions in the Gly-Xaa-Yaa amino acid sequence at multiple sites along the collagenous domain give rise to flexibility, allowing for looping and supercoiling of protomers into networks (Hudson 2004, Miner 2003, Zhou & Reeders 1996).

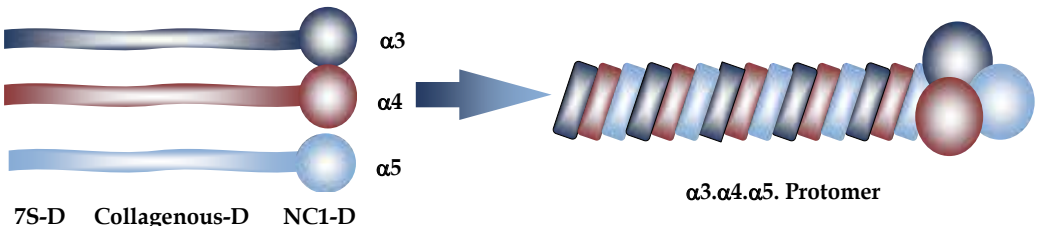


Fig. 1. A schematic drawing showing an example of α3-, α4-, and α5(IV)-chains and α3.α4.α5(IV) protomer formation. D: domain

To date, only three different types of collagen protomer have been identified; α1.α1.α2(IV), α3.α4.α5(IV) and α5.α5.α6(IV) (Hudson 2004). The protomer α1.α1.α2(IV) is ubiquitously present in most basement membranes (Hudson et al., 1993, Borza et al., 2001, Boutaud et al., 2000, Timpl et al., 1981). In contrast, α3.α4.α5(IV) and α5.α5.α6(IV) show restricted tissue distribution. In the kidney the α1.α1.α2(IV)-α1.α1.α2(IV) network predominates during early nephrogenesis in the GBM, the Bowman's capsular basement membrane, and tubular basement membrane. As the kidney becomes mature during the 2nd trimester of fetal development, the α3.α4.α5(IV)-α3.α4.α5(IV) network gradually becomes dominant and replaces the α1.α1.α2(IV)-α1.α1.α2(IV) network in the GBM and in tubular basement membranes while the α1.α1.α2(IV)-α5.α5.α6(IV) and the α1.α1.α2(IV)-α1.α1.α2(IV) networks are distributed in the Bowman's capsular basement membrane and in tubular basement membranes (Harvey et al., 1998, Milner 2003). The protomer α3.α4.α5(IV) is also expressed in the lung, testis, cochlea and eye while the α5.α5.α6(IV) network is present in skin, smooth muscle and esophagus (Cosgrove et al., 1998, Hudson et al., 2003, Kalluri et al., 1997). Alterations in any of the *COL4A3*, *COL4A4* and *COL4A5* genes may cause Alport syndrome.

3. Alport syndrome

Alport syndrome is a hereditary disorder with considerable genetic and clinical heterogeneity characterized by hematuria, proteinuria (1-2 gm of protein per day) and progressive renal failure and is frequently associated with diagnostic ocular abnormalities and high tone sensorineural deafness. Ocular abnormalities include lenticonus of the anterior lens capsule, retinopathy and cataracts. Other extra-renal manifestations include mental retardation or leiomyomatosis in rare cases (Alport 1927, Flinter et al., 1988, Hudson et al., 2003).

3.1 Genetics

In approximately 85% of patients with Alport syndrome there is X-linked inheritance of mutations in the *COL4A5* gene encoding the α5(IV) collagen chain on chromosome Xq22. *COL4A5* is a large gene comprising 51 exons. As many as 588 mutations have been described to date and are spread throughout the gene without any identified mutational hot spots. The types of mutations that involve *COL4A5* consist of missense, deletion, splice site, nonsense, insertion and duplication mutations (Hou et al., 2007, Mochizuki et al., 1994).

The remaining 15% of Alport syndrome patients show autosomal inheritance; of these 14% are recessive and 1% are dominant, which are caused by mutations either in the *COL4A3* or *COL4A4* genes on chromosome 2q36-37 encoding the α3(IV) or α4(IV) proteins. Heterozygous mutations of *COL4A3* or *COL4A4* could result in a less severe phenotype than that of homozygous or compound mutations in these genes (Jefferson et al., 1997). The authors also noted that heterogygous mutations of these genes could result in thin basement membrane nephropathy (TBMN) which typically does not result in renal failure. The authors postulated that mutations in the *COL4A3* or *COL4A4* gene can cause a spectrum of disease, ranging from TBMN/benign familial hematuria to autosomal dominant and recessive forms of Alport syndrome.

3.2 Pathogenesis

Ultrafiltration of plasma in the renal glomeruli is the major function of the kidney (Voskarides et al., 2008). The primary filtration barrier of the glomerular capillary consists of three layers: the fenestrated endothelial cells, the intervening GBM, and the epithelial podocyte foot processes. The foot processes are connected to each other by the slit diaphragm, and together these constitute an important component of the filtration barrier; the loss of podocyte foot processes results in massive proteinuria. The GBM is a special kind of acellular extracellular matrix, having properties of a viscous gel. The filtration barrier behaves as a selective sieve restricting the passage of macromolecules on the basis of their size, shape, and charge. Deterioration of the integrity of the GBM results in mild proteinuria. The major constituent of GBM is type IV collagen, which together with laminin, nidogen, and sulfated proteoglycans maintains the filtration barrier and provides the substrata and signals necessary for proper renal cell function (Hudson & Tryggvason 2003).

Mutations present in Alport syndrome that produce post-translational defects in α3(IV), α4(IV), or α5(IV) chains may result in incorrect folding or assembly of monomers. Such defective monomers are rapidly degraded. The mutations, therefore, arrest the normal developmental maturation during the fetal 2nd trimester period when the α1.α1.α2(IV) network is largely replaced by the α3.α4.α5(IV) network in the GBM. This maturation may be related to oxidative and physical stress in GBM (Kalluri et al., 2000) and perhaps also in the cochlea (Huang et al., 2000) and the lens capsule (Reddan et al., 1996). In the kidney, as plasma traverses glomerular capillaries, the protein content, including the levels of serum proteases, increases. The embryonic α1.α1.α2(IV) network is more susceptible to endoproteolysis than the more heavily cross-linked α3.α4.α5(IV) network (Kalluri et al 1997). It seems, then, that GBM that is more exposed to proteases or oxidants needs the protection of a resistant collagen IV network. Over time, patients with Alport syndrome probably become more sensitive to proteolysis, which may explain why their glomerular membranes thicken unevenly, split, and ultimately deteriorate (Kalluri et al., 1997, Kalluri et al., 2000). Immunohistochemical studies show that mutations in the *COL4A5* gene, which cause the X-linked form of Alport syndrome, frequently result in the loss of all three of the α3(IV), α4(IV) and α5(IV) chains in the GBM (Naito et al., 1996, Naito et al., 2003, Yoshioka et al., 1994). Thus, the absence of a functionally normal α5(IV) chain can disrupt assembly of the triple-helical protomer, and frequently leads to loss of the entire α3.α4.α5(IV) network in the GBM.

Mutations involving the NC1 domain of *COL4A5* result in no or severely reduced α3.α4.α5(IV) protomer formation within cells and/or in failure of secretion from cells

(Kobayashi T et al., 2008, Kobayashi & Uchiyama 2010). In the normal process of formation of the type IV collagen network, the NC1 domain plays an important role in forming protomers as three α chains specifically interact with each other. Additionally, in forming NC1 hexamers, two protomers dimerize at the C-terminus. Defective monomers or protomers of type IV collagen networks may be degraded rapidly.

4. Genotype-phenotype correlation in X-linked Alport syndrome

Approximately six hundred mutations in *COL4A5* have been reported, 588 of which are causally linked with X-linked Alport syndrome (Arup Laboratories 2011). Considerable allelic heterogeneity is observed by the high number of mutations and the associated phenotypic variability. Clinically the natural history of the nephropathy and other extra-renal lesions are quite variable. A number of researchers have attempted to link genotypes in Alport syndrome to phenotypes (Bekheirnia et al., 2010, Gross et al., 2002, Jais et al., 2000). Gross and colleagues have proposed a classification linking phenotype and genotype into three categories (Gross et al., 2002).

- Type S (severe); genotypic alterations in *COL4A5* include major gene rearrangements, premature stop codons, frameshift mutations, and donor splice site alterations. Also includes mutations involving the NC1 domain. The phenotype is characterised by early onset of ESRF at about 20 years of age and significant extra-renal manifestations including 80% with sensorineural deafness and 40% with ocular lesions.
- Type MS (moderately severe). The genotype in this group is characterised by non-glycine-XY missense alterations, in-frame deletions/insertions, acceptor splice site changes and glycine-XY substitutions involving exons 21-47. This type is associated with ESRF appearing in the mid-twenties with about 65% of individuals having hearing loss and 30% ocular defects.
- Type M (moderate). The genotype is glycine-XY substitutions involving exons 1-20. The phenotype appears to be milder with a later onset of ESRF at about 30 years of age, including a significant number of individuals with sensorineural deafness (70%) and ocular lesions (30%).

Bekheirnia and colleagues have confirmed previous reports (Gross et al., 2002, Jais et al., 2000) in that there is a strong genotype-phenotype correlation in X-linked Alport syndrome (Bekheirnia et al., 2010). The authors conclude that missense mutations are associated with the best prognosis with an average age at onset of ESRF of 37 yr, followed by splice site mutations at 28 yr, truncating mutations at 25 yr and small deletions at 22 yr. The authors also point out a strong relationship between mutation position and age onset of ESRF, with younger ages at onset of ESRF associated with the 5' end of the gene. Affected males with splice mutations or truncating mutations showed two-fold greater odds of developing eye problems and hearing loss than those with missense mutations. Mutations associated with hearing loss and ocular changes are located closer to the 5' end of the gene.

5. Mild forms of Alport syndrome

While many affected males of X-linked Alport syndrome show moderate to severe forms of nephropathy and extra-renal abnormalities between the second and third decades, it is also well known that there are occasional milder cases where ESRF may be delayed until

the fifth or sixth decade along with variable age occurrence of deafness (Bekheirnia et al., 2010, Kobayashi et al., 2008, Smeets et al., 1992). Of the six hundred or so *COL4A5* mutations that have been reported to date (Arup Laboratories 2011), 588 mutations were pathogenic for X-linked Alport syndrome, whereas 12/600 mutations were benign (silent). A total of 81/588 mutations (13.8%) were associated with a mild form of Alport syndrome where the age of onset of ESRF was over 30 yr old. These 81 mutations of a mild form are shown in Figure 2 and consist of 66 mutations within exons (red column) and 15 mutations within introns (blue column), widely distributed over the *COL4A5* gene. It appears that mutations involved in a mild form of Alport syndrome are widely distributed within 51 exons of the *COL4A5* gene with a tendency for more mutations between Exon 25 to 51.

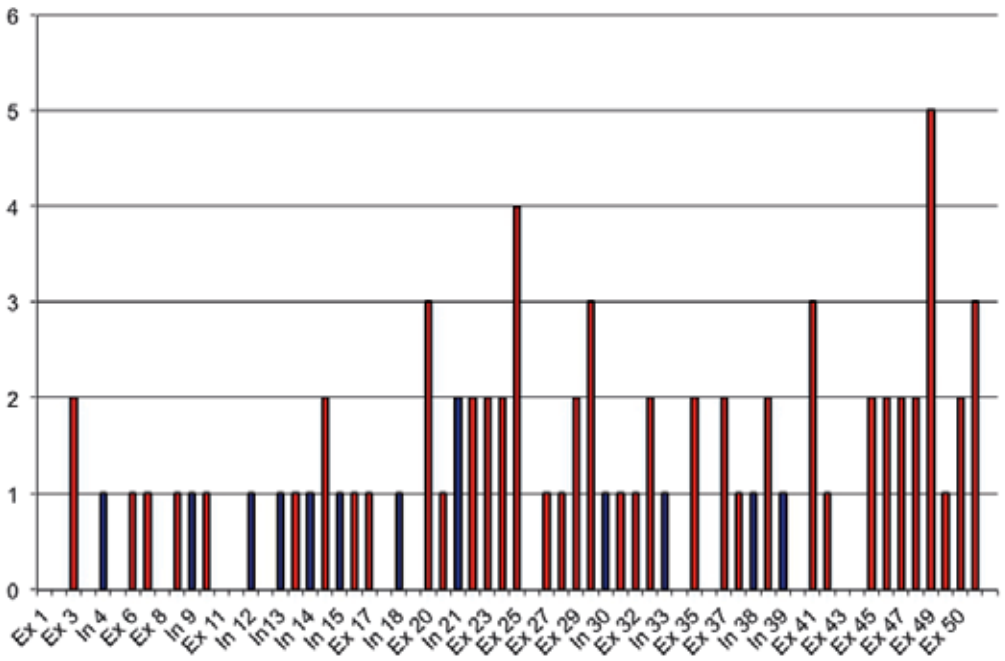


Fig. 2. Distribution and frequency of mutations in exons (red column) and introns (blue column) within the CO*L4A5* gene causally relating to X-linked Alport syndrome (Arup Laboratories 2011).

6. A unique mild form of Alport syndrome in New Zealand families

We previously described a novel Cys1638Tyr alteration in the NC1 domain of *COL4A5* identified in a large New Zealand family (Fig 3) with a hereditary nephropathy (Wilson et al., 2007). This family was identified when two sisters (IV26 and IV28) presented to the clinic to be considered as potential live kidney donors for their sons (V29 and V35, respectively) who had ESRF (see Tables 1 and 2). Both women were found to have significant proteinuria and hypertension and so it was decided to carry out renal biopsies. Following the results of the biopsies each family member was then evaluated for the presence of renal disease as indicated in Table 1, and only three male members of the extensive pedigree were found to

exhibit ESRF. Extra-renal manifestations such as sensorineural deafness or ocular changes were not observed in any family member. Further renal biopsies were carried out on additional family members, so that renal biopsies now totalled eight members of the family. The biopsies from a 39 year-old male with proteinuria of 1.1 g/24h and normal auditory and eye examination (V42) showed mild increase of mesangial matrix and mild periglomerular Bowman capsular fibrosis (Fig 4A). There were occasional areas showing focal interstitial accumulation of foam cells (Fig 4B), interstitial fibrosis (Fig 4C) and thick-wall hyalinized vessels surrounded by scattered aggregates of lymphocytes (Fig 4D).

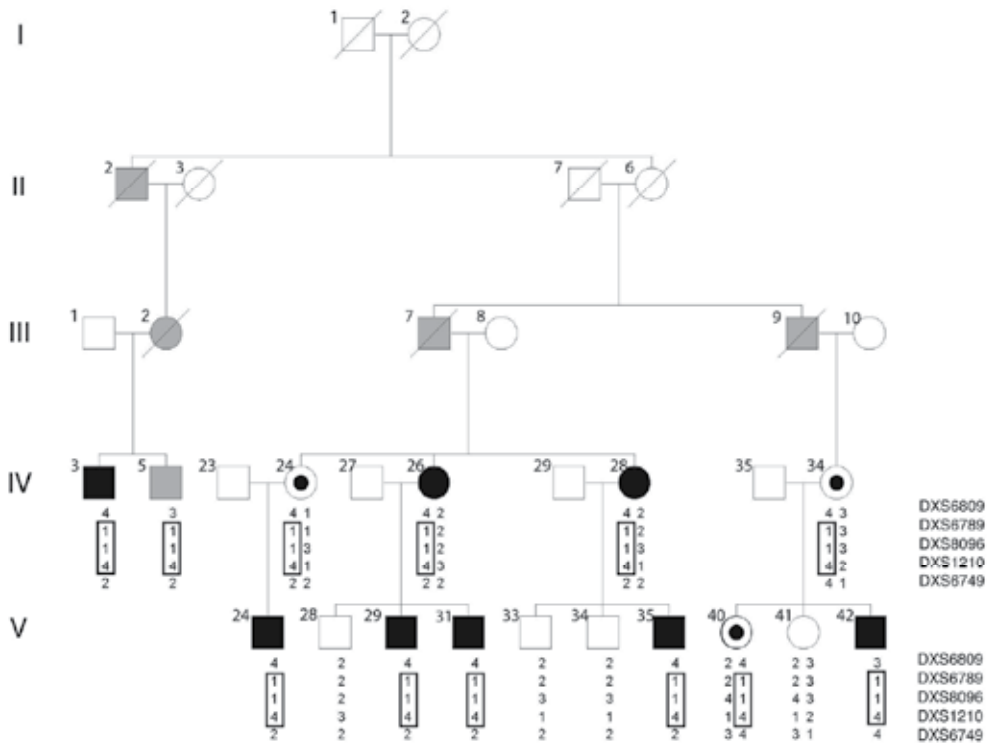


Fig. 3. Family pedigree. A simplified pedigree of the family showing males (squares) and females (circles) depicted by generation (I-V) is shown. While the disease appears severe in this pedigree, the extended pedigree was published previously (Wilson et al., 2007), and only 32/155 members of the extended pedigree are shown in this diagram. Some of the individuals indicated in Tables 1 and 2 are represented in this pedigree and are identified in each case by their corresponding number. Black symbols indicate individuals with biopsy-confirmed GN. Black dots inside the symbols indicate obligate carriers. Grey symbols indicate individuals who were not biopsied, with clinical manifestations of renal disease and therefore presumed GN. Open symbols indicate individuals without clinical signs of renal disease. X chromosome region markers (Xq21.33-Xq23) informative for linkage analysis are indicated on the right, and shown below the symbols are the genotypes for each individual that are associated with the relevant marker. The boxed region indicates a common haplotype inherited from the father or the mother, corresponding to genotypes of the 3 markers that segregate with the disease. The genomic region of chromosome X corresponding to this haplotype contained the *COL4A5* gene locus.

Identification number	Age Gender	Presentation	Renal Function and Blood Pressure	Biopsy *	Inheritance
III2	Female		Died on dialysis	Not done	Affected/ Carrier
IV3	57 yrs old male		ESRF at 40 yrs old. Dialysis. Renal transplant.	Not done.	Affected
IV5	46 yrs old male	Proteinuria 4.7g/24h Hypertension	Chronic kidney disease BP 200/120	Not done	Affected
V24	39 yrs old male	Proteinuria. Hematuria	BP 148/90	Mild mesangial matrix expansion	Affected
V29	41 yrs old male	Acute nephritic syndrome. Hypertension	ESRF at 28 yrs old. Dialysis. 2nd renal transplant	Chronic glomerulo-nephritis	Affected
V31	36 yrs old male	Proteinuria. Hypertension	Chronic kidney disease BP 136/86	Mesangial cell proliferation.	Affected
V35	32 yrs old male	Proteinuria. Hematuria	Chronic kidney disease. Progressed to ESRF, at 26 yrs old and renal transplant	Chronic glomerulo-nephritis	Affected
V42	39 yrs old male	Proteinuria 1.1g/24 hr	BP 126/80	Mesangial cell proliferation.	Affected

* Ig immunofluorescence negative

Table 1. Renal disease identified prior to mutation screening in the New Zealand family

Electron microscopy of a renal biopsy from (V42), of which histology is shown in Fig 4, demonstrated a classical basket weave pattern or splitting of the basement membrane characteristic of Alport syndrome (Fig 5A and 5B). However, a diagnosis of Alport syndrome was not necessarily an obvious diagnosis in this family, since the disease in all three males was relatively mild and there was a lack of extra-renal manifestations in any of the family members, raising some doubts as to whether this was Alport syndrome prior to carrying out genetic analysis.

To determine the genetic cause of the disease in this family, genomic DNA was isolated from whole blood of each of the family members, and used in linkage analysis with genetic markers spanning chromosome X carried out as described in Wilson et al (2007). Strong evidence for linkage to markers DXS6789, DXS8096, DXS1210, adjacent to the *COL4A5* (and *COL4A6*) genes located on chromosome X was obtained, indicating that this corresponded to a collagen nephropathy in the family, and that it was most likely due to a mutation in *COL4A5*.

Identification number	Age Gender	Presentation	Renal Function and Blood Pressure	Biopsy *	Inheritance
IV24	69 yrs old female	Trace microscopic hematuria	Normal renal function. BP 168/86	Not done	Carrier
IV26	64 yrs old female	Proteinuria 1.8g/24 hr Hypertension.	Normal renal function BP 152/76	Mesangial cell proliferation. Hypertensive arteriosclerosis	Affected/ Carrier
IV28	60 yrs old female	Proteinuria 1.4g/24 hr Hypertension	BP 160/98	Mesangial cell proliferation. Hypertensive arteriosclerosis	Affected/ Carrier
IV31	69 yrs old female	Hypertension Negative urine	Normal renal function	Not done	Carrier
IV34	65 yrs old female	Hypertension Negative urine	Normal renal function	Not done	Carrier
IV36	61 yrs old female	Microscopic hematuria	Normal renal function	Not done	Carrier
IV39	72 yrs old male	Proteinuria 1.6g/24 hr No hematuria Hypertension	Mild chronic kidney disease BP 144/76	Not done	Affected
IV47	54 yrs old female	Hematuria Hypertension	Normal renal function BP 148/70	Not done	Carrier
V44	36 yrs old female	Intermittent microscopic hematuria	Normal renal function BP 120/76	Not done	Carrier
V49	43 yrs old female	Negative urine	Normal renal function BP 120/70	Not done	Carrier
V37	39 yrs old female	Negative urine	Normal renal function	Not done	Carrier
V40	42 yrs old female	Hematuria	Normal renal function. BP 118/70.	Mild mesangial cell proliferation	Carrier

* Ig immunofluorescence negative

Table 2. Renal disease or carrier status identified after mutation screening in the NZ family

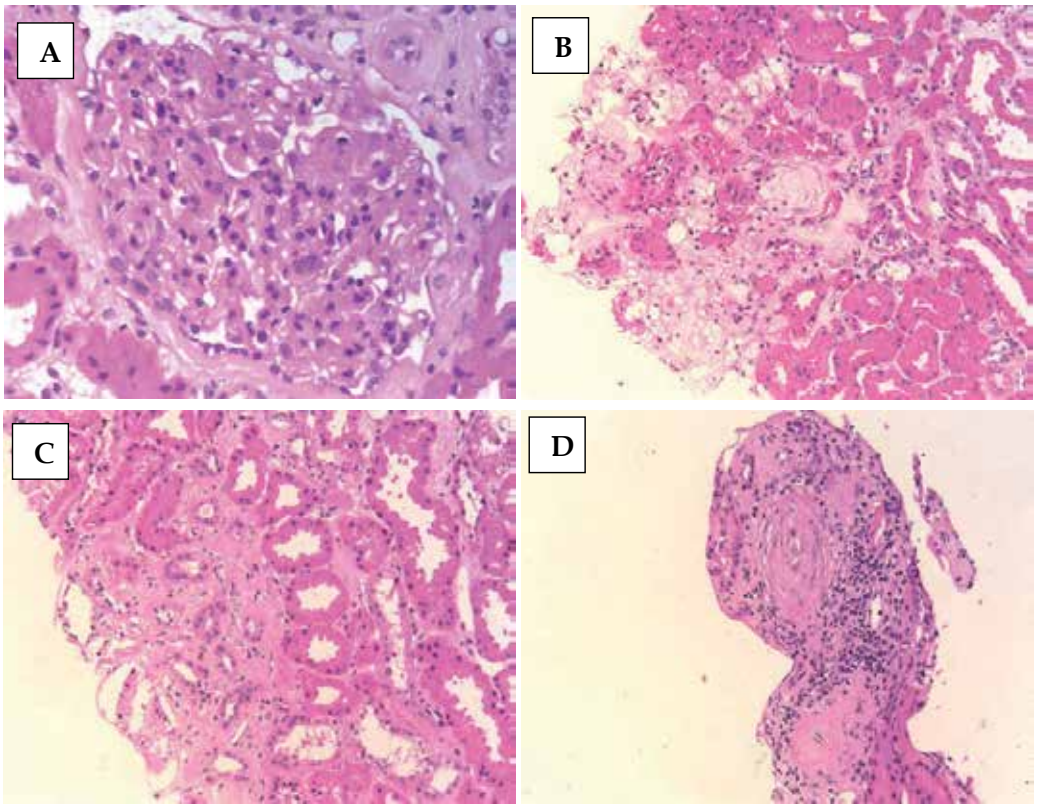


Fig. 4. Histological findings of the kidney of a patient, 39 yr old male with hematuria, proteinuria 1.1 g/24h and normal auditory and eye examination. (A) Glomerulus from kidney biopsy of this patient showing mild periglomerular fibrosis and mild mesangial matrix increase. Tubular atrophy is seen at the right upper corner. (B) Focal accumulations of foam cells in the interstitium. (C) Multiple focal areas of interstitial fibrosis associated with atrophic tubules. (D) Sclerosed vessels surrounded by lymphocytes.

Eventually the mutation, comprising a c.4913G>A nucleotide substitution in exon 50 of *COL4A5*, was identified by PCR amplification and sequenced following analysis using a series of primer pairs corresponding to each of the 51 exons making up the *COL4A5* transcript (Genbank accession number NM_000495) as well as the entire promoter region between *COL4A5* and *COL4A6*. Therefore, this analysis conclusively showed that the disease in this family was a mild form of Alport syndrome.

Since it is known that most cases of Alport syndrome result in loss of the synthesis or secretion of the collagen protein and/or protomer, which can be detected by the absence of the collagen staining by immunohistochemistry, in order to further understand whether the pathogenesis of this disease in the New Zealand family was due to the loss of synthesis of COL4A5, immunohistochemical studies of the α1 to α5 type IV collagens in kidney biopsies from affected and carrier individuals were carried out. This analysis showed that in affected men (V31, V35 and V42) and carriers (IV26 and IV28) the GBMs were positive for α1 *to* α5 type IV collagens, as exemplified in Fig 6 (α3, α4 and α5). These findings were considerably different from the previous reports of Alport syndrome, where the X-linked form of Alport

syndrome was generally found to result in the loss of all three of the α3(IV), α4(IV) and α5(IV) chains in the GBM.

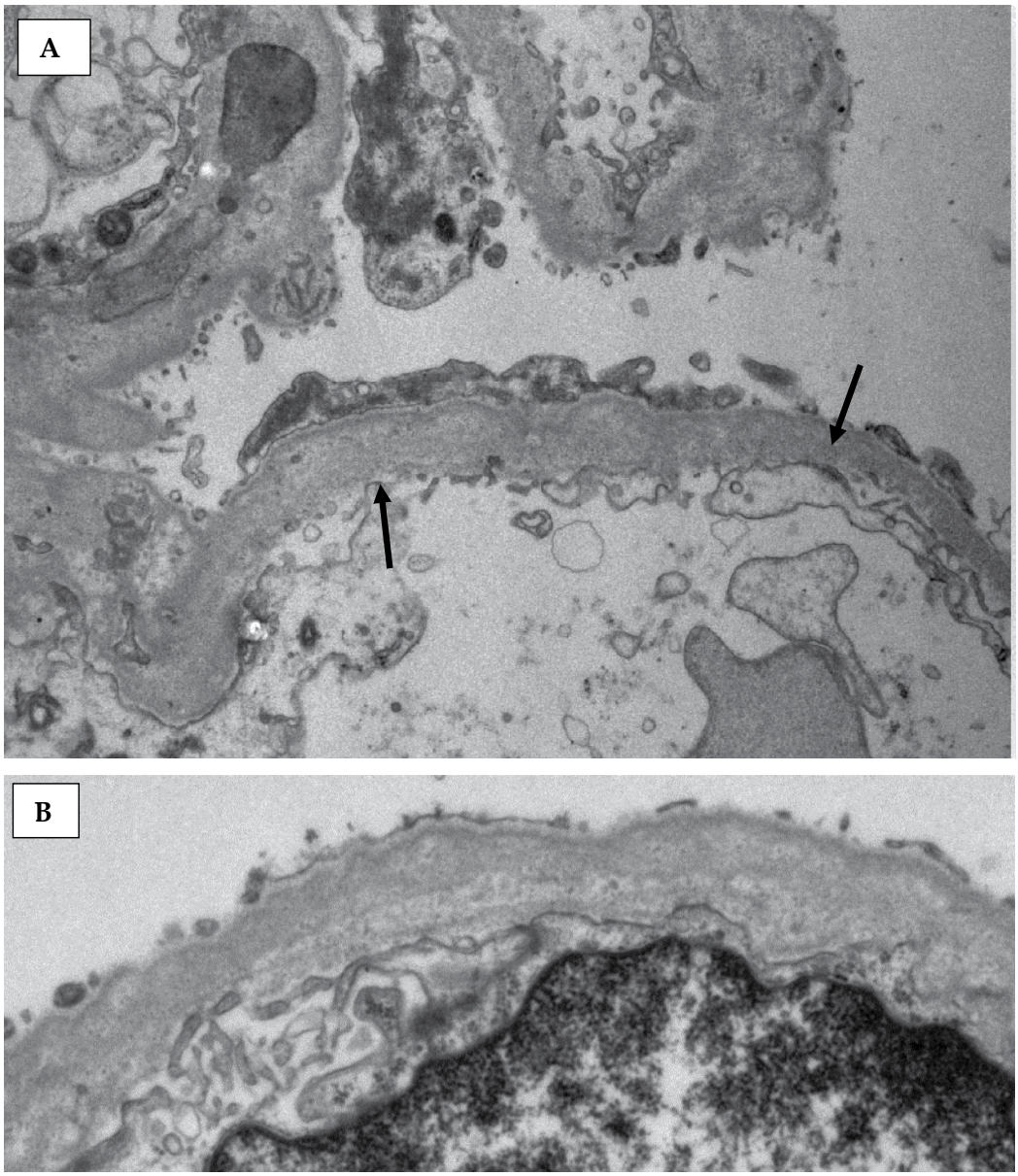


Fig. 5. Electron microscopy of glomerular basement membranes of the same patient (V42) of Figure 4. (A) Characteristic splitting or basket weave appearance of GBM (arrows) and abnormal podocyte foot processes. (original magnification x9,700). (B) Higher magnification of a basket weave appearance. (original magnification x13,500). Note, the ultra-structural changes in the glomerular basement membrane of patients with Alport syndrome were variably associated with areas of thick and thin basement membrane, and/or presence of a basket-weave pattern.

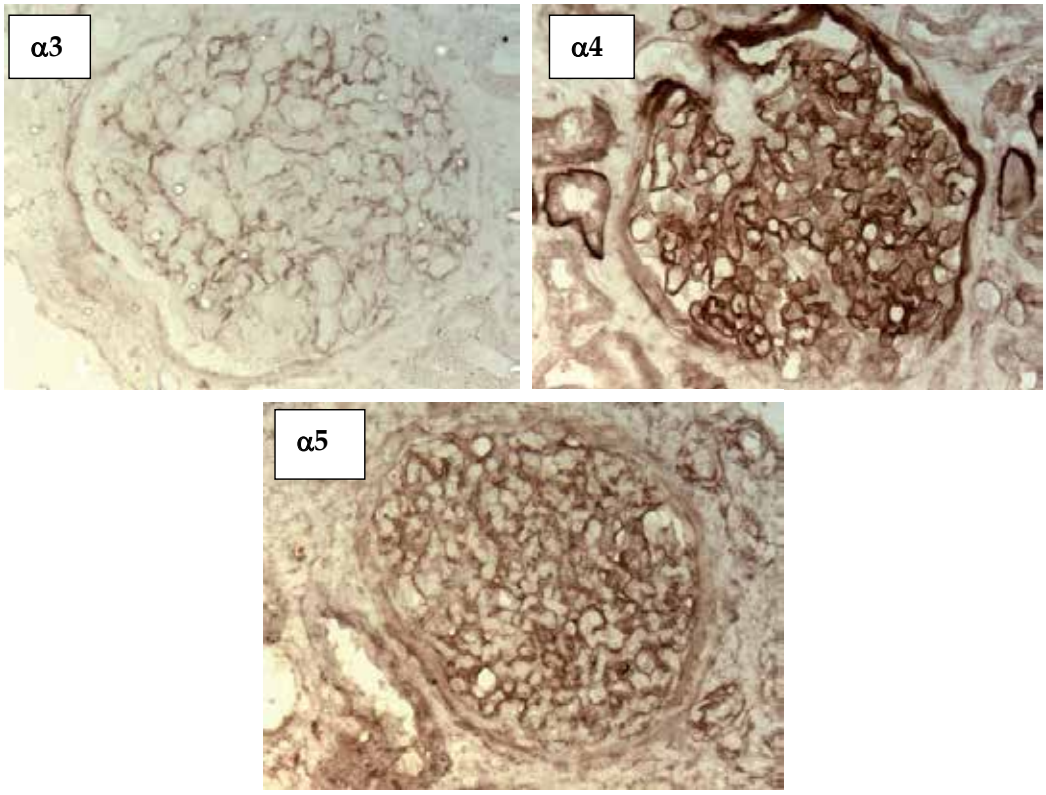


Fig. 6. Immunohistochemistry using monoclonal antibodies against αIV collagen in a kidney biopsy shows α3, α4 and α5 positivity in the GBM.

Unlike most other reports of Alport syndrome, and inconsistent with the disease severity and multi-organ involvement that is generally associated with NC1 mutations, the major manifestation of the renal abnormality in the New Zealand family was proteinuria, which occurred in six of the nine male members who carried the *COL4A5* mutation. Only three of the nine males in the family who inherited the mutation presented with glomerulonephritis and ESRF.

The NC1 domain plays an important role in the selection of α chains for assembly into heterotrimers. In general, substitution and missense mutations in the NC1 domain, as in other regions of *COL4A5*, lead to hematuria, proteinuria, ESRF and sensorineural hearing loss with an overwhelming predominance in males (Barker et al., 1996, Barker et al., 1997, Gross et al., 2002, Hertz et al., 2001, Inoue et al., 1999, Knebelmann et al., 1996, Nakanishi et al., 1994, Netzer et al., 1996, Zhou et al., 1991). A previous study classified X-linked Alport syndrome patients caused by mutations involving the NC1 domain into Type S (severe) phenotype (Gross et al., 2002). Mutations involving other cysteine residues in the NC1 domain have also been reported and include C1486S (Zhou et al., 1991), C1567R (Knebelmann et al., 1996) and C1586R (Hertz et al., 2001) and others (see Figure 7).

Using the proposed classification suggested by Gross and colleagues, the New Zealand family would be placed in 'type S' on the basis of the mutant genotype involving the NC1 domain. However, none of the family members had a phenotype as severe as reported previously in association with NC1 domain mutations. In all cases the presentation of renal

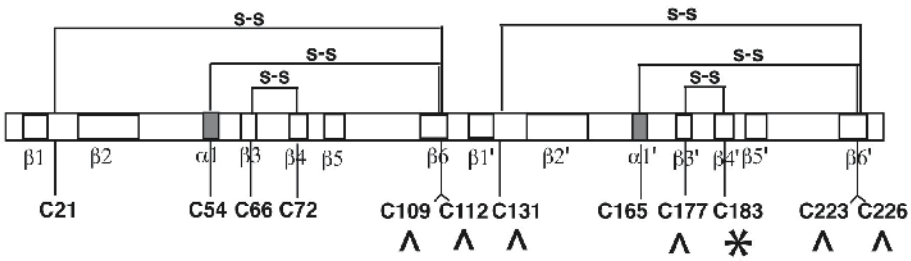
disease in the affected male members of the New Zealand family was relatively late. The lack of extra-renal manifestations in the males is also contrary to previous reports correlating NC1 mutations with 'type S' Alport syndrome.

Therefore, while NC1 domain mutations in *COL4A5* are thought to be associated with severe forms of Alport syndrome, the pattern of disease in this family was comparatively mild, and only 27% of the affected or presumed obligate mutant males in the family developed end-stage renal disease. Indeed, considerable variability and phenotypic heterogeneity in the extent of renal disease was observed in the affected males and carrier females. For example, one family member, a 72 year-old male (IV39) shown in Table 2, was later found to carry the sequence alteration, and was initially apparently phenotypically unaffected, but then further investigation revealed proteinuria (1.6g/24h) and hypertension. This man was not biopsied. Furthermore, the presentation of ESRF in one female carrier in this study (III2) leads to the conclusion that female carriers were also affected. Skewed inactivation of the X chromosome could account for this, although other genetic or environmental factors, such as hypertension, could also be contributing factors to the variability in disease progression. This amount of phenotypic variation between males and females is unusual in Alport syndrome, and even more unusual is the fact that none of the family members exhibited the full spectrum of renal, auditory and ocular abnormalities typifying Alport syndrome. The inheritance pattern was clearly consistent with an X-linked dominant mode, albeit with reduced penetrance, as the linkage analysis, together with the scan of the entire *COL4A5* gene for the mutation, clearly identified that the NC1 domain mutation identified in *COL4A5* was the causative mutation in this family.

The Cys1638Tyr alteration in the New Zealand family is predicted to affect the 10th conserved cysteine residue among 12 cysteine residues in the NC1 domain, thus disrupting the disulfide bond linking the C-terminal β3′-β4′ hairpin (Fig 7). In the kidney GBM the β3′ to β4′ disulfide-bridge could be involved in inter-molecular rather than intra-molecular interactions. For example, inter-protomer disulfide cross-links, or interactions with other molecules, such as integrins could involve formation of disulfide linkages to the cysteine residues at positions 66, 72, 177 or 183 in the N-terminal or C-terminal β3-β4 sheets in the type IV collagen NC1 domain.

During protomer assembly the NC1 domains of the α3(IV), α4(IV) and α5(IV) chains specifically interact to select chains for triple-helix formation. In Alport syndrome, NC1 domain cysteine substitutions (see Figure 7) are thought to affect the folding of the monomeric NC1 domain, preventing its participation in trimer assembly. The NC1 domain is also important for network assembly, whereby the NC1 trimers of two protomers specifically interact forming a NC1 hexamer. Variants that result in a loss of, or a defect in any of the α3(IV), α4(IV), or α5(IV) chains result in incorrect folding or assembly of the entire protomer leading to a complete absence of the α3.α4.α5(IV) network from the GBM. However in kidney biopsies from affected patients in this family the α3(IV), α4(IV) and α5(IV) collagens were still present in the GBM, implying that the p.Cys1638Tyr alteration must still allow for the correct assembly of the triple helical protomer. It is possible, however, that an organ-specific defect in protomer function rather than assembly could explain the lack of sensorineural hearing loss or ocular defects in this family, although it remains to be determined whether the p.Cys1638Tyr variant could indeed disrupt the dimerization of two protomers at the C-terminus, thus affecting network assembly.

Further to the New Zealand family, there have been 7 other mutations involving cysteine residues in the NC1 domain (Bekheirnia et al., 2010, Gross et al., 2002, Hertz et al., 2001, Inoue et al., 1999, Knebelman et al., 1996, Wang et al., 2005., Wilson et al., 1997, Zhou et al., 1991) affecting males and females (Figure 7). These mutations are shown together with information of age at the time of diagnosis. From refs 2, 3, 6, 7 and 8 patients were detected during the ages of 6–16 yrs while one was at 31 yrs old. From refs 3 and 2 two males showed ESRF at 14 and 16 yr old, respectively. When clinical information was available, all of the affected individuals appeared to show hearing loss, except for three individuals who had mutations involving C226 (Wang et al, 2005), C177 (Bekheirnia et al., 2010) and C183 (Wilson et al., 2007). The latter report is our own New Zealand family described here. Patients with other mutations of the NC1 domain either lacked a5(IV) in the GBM, or were clinically more severe than the patients with the C177 and C183 mutations, both of which, interestingly involved mutations in the same disulfide linkage.



Ref (no of patients)	1 (122)	2	3, 8	4 (5)	5 (19)	6, 7	8
Gender	M&F	ND	M, M	ND	M&F	M, F	M
Diagnostic age	ND	16	14, 10	ND	ND	31,15	6
Family history	+	-	+, +	ND	+	+, -	+
Hematuria	ND	ND	ND, +	+	+	ND,ND	+
Proteinuria	ND	ND	ND, +	+	+	ND, ++	+
Renal insufficiency	+	+	+, +	+	+	+, ND	-
Hearing loss	+	+	+, +	-	-	+, ND	-
Ocular lesions	ND	+	ND, ND	-	-	+, ND	-
Ultrastructural change in GBM	ND	+	ND, +	ND	+	ND, +	+
α5 (IV) in GBM	ND	ND	ND, -	ND	+	ND, +	-

Fig. 7. Depiction of NC1 domain showing locations of cysteine sequence alterations, and the clinical details of patients. Shown are the positions of the beta sheet domains (β1-β6, β1'-β6'), and alpha helix (α1, α1') and cysteine residues arranged linearly, and their disulfide linkages. Cysteine residue missense mutations of the NC1 domain that have been previously reported are shown (^), together with the cysteine mutation in the New Zealand family (* under cysteine 183; amino acid numbering in this figure is from the start of the NC1 domain, which is one amino acid longer than in our previous report (Wilson et al, 1997). ND, not determined. +, characteristic is present. -, characteristic is absent. M, male, F, female. Refs; 1 (Zhou et al., 1991); 2 (Knebelmann et al., 1996); 3 (Hertz et al., 2001); 4 (Bekheirnia et al., 2010); 5 (Wilson et al., 1997); 6 (Gross et al., 2002), 7 (Inoue et al., 1999); 8 (Wang et al., 2005).

Kobayashi and colleagues constructed a plasmid containing mutations corresponding to a variety of missense or deletion mutations of the NC1 domain of *COL4A5*, which were grown in a kidney cell line. The results showed that mutations render the collagen chain defective

in terms of heterotrimer formation between the α3, α4 and α5 collagen chains, and/or the secretion of the heterotrimer from cells (Kobayashi et al., 2008). After our publication, these researchers further constructed a plasmid containing the mutation corresponding to Cys1638Tyr into the α5(IV) chain. The results of this experiment showed that heterotrimer formation in the cells and secretion of the α5(IV) chain in the monomeric form from the cells were markedly decreased compared to cells containing the wild-type chain. However, the heterotrimer that was formed from the mutant chain was still secreted from the cells. They concluded that the residual ability of the mutant chain to form and be secreted may have led to the unique mild phenotype formed in the Alport syndrome family with the Cys1638Tyr mutation (Kobayashi & Uchiyama 2010).

7. Renal lesions in carrier women of X-linked Alport Syndrome and Thin Basement Membrane Nephropathy

Thin basement membrane nephropathy (TBMN) is the most common cause of inherited renal disease and its incidence has been reported to be as high as 1% of the world population (Kashtan 2005, Tazon et al., 2003, Wang & Savige 2005). It is defined as diffuse thinning of the GBM characterised by persistent glomerular hematuria, minimal proteinuria, and normal renal function. Genetic studies of TBMN have helped to establish that many patients with benign familial hematuria are actually the carriers of autosomal recessive Alport syndrome, carrying mutations only in the one allele of *COL4A3* or *COL4A4* (Voskarides et al., 2008). A novel missense mutation of *COL4A3* in a Chinese Han consanguineous family was identified and the underlying pathogenic role in the homozygous form was investigated in autosomal recessive Alport syndrome and in the hetrozygous form in TBMN within the identical family (Hou et al., 2007). These studies showed that while TBMN manifest as a dominant disorder in the family with the *COL4A3* mutation, Alport syndrome manifested as a recessive disease in the same family. In our experience, by light microscopy the kidney glomerular features of the carrier females (eg V40) of X-linked Alport syndrome in the New Zealand family were relatively unremarkable. However there was occasional periglomerular fibrosis and focal areas of protein casts in occasional tubules associated with epithelial cell atrophy (Fig 8A and 8B).

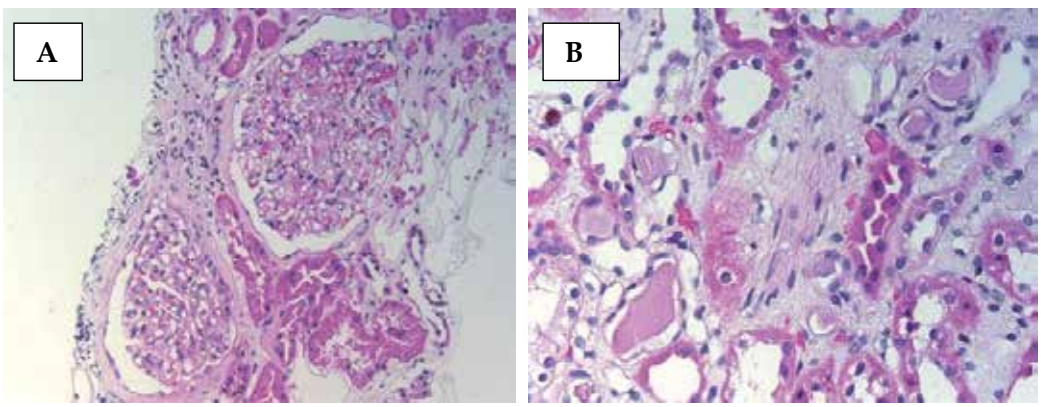


Fig. 8. Histology from a carrier mother (V40) in the New Zealand family with Alport syndrome. (A) Two glomeruli show periglomerular fibrosis. Glomerular tufts are relatively unremarkable. (B) Focal areas show protein casts in occasional tubules that show atrophic epithelial cells.

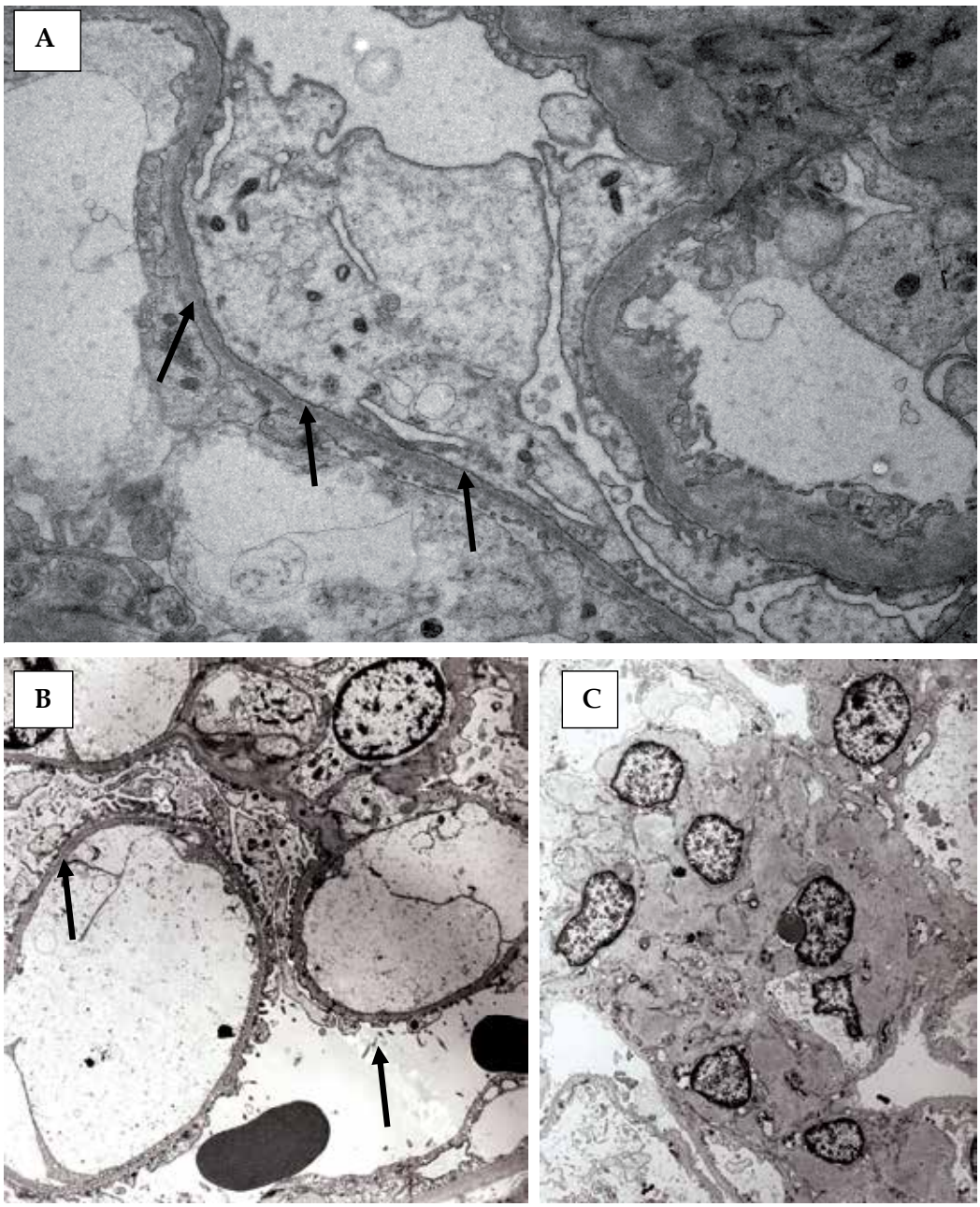


Fig. 9. Electron microscopy of carrier females (V40 and IV28). (A) The same carrier mother (V40) of the Figure 8 and (B) another carrier female (IV28), both showing focal areas of extremely thin GBM (arrows). (C) In addition there are focal areas of irregular GBM thickening. (A x7,400, B x3,000, C x2,100, original magnification respectively).

Electron microscopic findings on one of the carrier women of the New Zealand X-linked Alport syndrome family showed severe thinning of the GBM (Fig 9A) where the thickness

was approximately 150 nm, and much thinner than the normal GBM (300-400 nm). Another carrier woman also showed focal areas of severely thin GBM (Fig 9B). In addition there were occasional regional areas of thick segments of GBM (Fig 9C). It is notable that the typical basket weave appearance or splitting was not present in the females. One message to take from these studies is that electron microscopic examination of the kidneys in carrier women with clinical symptoms should be mandatory because the light microscopic observations on their own often provide unremarkable findings or only subtle changes and may not show a full range of pathology.

Although TBMN has been regarded as a benign condition with an excellent prognosis, as high as 38% of *COL4A3/COL4A4* heterozygous mutant carriers, of all ages, develop chronic renal failure and 19.5% progress to ESRF (Voskarides et al., 2008). These authors emphasize a strong association between TBMN and focal segmental glomerulosclerosis (FSGS). Several studies report that TBMN predisposes to premature glomerular obsolescence and that this may then lead to late onset renal insufficiency followed by ESRF (Nieuhof et al., 1997, Nogueira et al., 2000). Other studies suggest that there may be other factors that predispose transition from TBMN to FSGS (Sue et al., 2004). These factors could be due to involvement of modifier genes such as podocyte specific genes or environmental factors.

These findings concur with a previous report (Jais et al., 2003) in that hematuria was observed in 95% of 323 female carriers of X-linked Alport syndrome. Proteinuria, hearing loss, and ocular defects also developed in 75%, 28%, and 15%, respectively. Moreover, the probability of developing ESRF or deafness before the age of 40 yr was 12% and 10%, respectively, in females versus 90% and 80%, respectively in men. In their study ultrastructural change of the GBM were found in 26 of 28 carriers and consisted of typically thick and split or alternatively thick and thin GBM in 19 patients. When taken together, our results and those of Jais et al (2003) suggest that TBMN may frequently develop in carrier women with a heterozygous *COL4A5* mutation.

8. Conclusions

In conclusion, mild forms of Alport syndrome may occur in association with certain mutations in the collagenous domain of the collagen proteins. In addition, we showed a p.Cys1638Tyr mutation occurring within the NC1 domain of *COL4A5* in a New Zealand family was associated with a mild form of Alport syndrome. Mild forms of Alport syndrome also occur in females with *COL4A5* mutations, in whom there is considerable phenotypic variation. In particular, it appears that electron microscopy carried out in female carriers of *COL4A5* mutations reveals much more about the health of their kidneys than does routine light microscopy alone. The abnormalities present in the kidneys of female carriers suggest that with appropriate management of diet and hypertension this could prevent the onset of renal disease in these women. Additional investigations of the pathogenic role of *COL4A5* mutations in female members of Alport families, and of the role of the NC1 domain in the New Zealand family, will further help to better understand the role of collagens in the structure and function of the filtration barrier in the GBM.

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Part 5

Miscellaneous Topics

23

Nephrotic Syndrome in Children – Studies from South Africa

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1. Introduction

Worldwide research has shown that racial differences occur with regard to the histological subtypes, response to treatment and outcome of idiopathic nephrotic syndrome (INS) in children (Bhimma et al. 2006:1847; Bhimma, R. 2009:15; Ingulli &Tejani 2001:393). Several reasons have been suggested to explain these differences such as a higher prevalence of infections, lower socio-economic status and inequalities in access to health care resources, genetics, and environmental factors but none of these have been substantiated by data. Results from the International Study of Kidney Disease in Children (International Study of Kidney Disease in Children ISKDC 1978:159) showed that the majority of children with INS have minimal change nephrotic syndrome (MCNS) which responds to corticosteroid treatment and that a kidney biopsy is not indicated. Based on these findings empiric corticosteroid treatment was recommended, without performing a kidney biopsy. The study population consisted of predominantly white children from North America, Europe and Asia. These recommendations have been implemented worldwide as standard of care for the past 40 years despite the lack of prospective renal biopsy studies to substantiate this recommendation and which may not be applicable to other settings with a predominance of black patients. An increasing incidence of focal segmental glomerulosclerosis (FSGS) in children and adults with INS has been reported recently (Borges et al. 2007:1309; Filler et al. 2003 :1107; Srivastava et al. 1991:13). Studies reporting the outcome of INS associated with FSGS are variable, which is not surprising, as differences in the population mix, aetiology, pathogenesis and duration of disease are often not taken into consideration. This begs the question whether the recommendation of the International Study of Kidney Disease in Children for the management of INS should still be adhered to. Or should it be revised taking into consideration different racial groups? On the other hand, in the light of the rising incidence of FSGS, it may be prudent to withdraw the recommendation.

Paediatric nephrologists from developing countries, and specifically Africa, need to formulate guidelines specific to their patients with INS. To this end, relevant clinical characteristics such as the antenatal and family history, birth weight, feeding and nutrition, growth and onset of disease should be documented and analysed in their reports. Low birth weight (LBW) which has been shown to be associated with decreased glomerular endowment (Manalich et al. 2007:770, Vehaskari , VM. 2007:490) and subsequent increased risk for the development of chronic kidney disease (CKD), is a case in point, since it is more common in impoverished population groups living in Africa. However, most publications describing the influence of LBW on CKD come from developed countries. (Teeninga et al. 2008:1615)

Limited resources are often the stumbling block for clinicians in developing countries. For the standard care of a child with INS the minimum investigations necessary in the work-up include urine biochemistry, urine microscopy and investigations to exclude infectious and immune disorders. A kidney biopsy is recommended for all children and should include light microscopy, immuno-histochemistry or immunofluorescent studies and electron microscopy. These investigations are costly, but essential to make a definitive diagnosis. Without a specific diagnosis of the underlying pathology and the associated complications it will not be possible to make reliable recommendations for targeted treatment in a child with INS in a developing country.

2. Aim

The aim of the study is to describe the clinical characteristics, histological subtypes, response to treatment and outcome of children with INS treated at the paediatric renal unit of the Steve Biko Academic Hospital (SBAH). This is a level 3 South African hospital, affiliated to the University of Pretoria and the referral centre for the surrounding multiracial population of roughly 5 million children under the age of 14 years.

3. Methods

A retrospective audit was performed of consecutive children admitted with a clinical diagnosis of INS. The latter was defined as ≥2+ proteinuria on a urine dipstick test, hypoalbuminaemia of <25 g/L and oedema. Children who had macroscopic haematuria (red or brownish discolouration of urine) substantiated by >2+ blood on a urine dipstick test and hypertension in addition to the criteria for INS, were also included and were categorised as nephritic-nephrotic. Hypertension was diagnosed according to the 4th Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents. (The Fourth Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents. 2004:555).

The racial groups were documented as black, white, Indian, and mixed. The Indian and white racial groups were pooled and categorised as white. The black and mixed racial groups were similarly pooled and categorised as black. This grouping was done because of known similarities in clinical presentation, response to treatment and outcome in the respective groups. (Bhimma et al.1997:429).

The clinical characteristics analysed were age at presentation, gender, anthropometry (height/length for age and weight for age), and blood pressure.

The following investigations were analysed: urine dipstick tests, urine protein:creatinine ratio (mg/mg), s-albumin and s-cholesterol, s-creatinine and estimated glomerular filtration rate (eGFR) in ml/min/1.73m2. eGFR was calculated using a modified Schwartz formula (Schwartz. et al. 1987:571), i.e.: [40 x height (cm)/s-creatinine (μmol/L)]. Kidney function at presentation was categorised according to the National Kidney Foundation Kidney Disease Outcomes (K/DOQI clinical practice guidelines 2002:S1). Investigations to rule out secondary nephrotic syndrome include: the third and fourth components of complement (C3 and C4), immunologic tests for systemic lupus erythematosus (antinuclear antibodies and anti-double-stranded DNA antibodies), antistreptolysin O and anti DNase B titers, hepatitis B and C serology, cytomegalovirus (CMV) antibodies and HIV enzyme-linked immunosorbent assay (ELISA).

The indications for a kidney biopsy were: a family history of kidney disease, congenital and infantile nephrotic syndrome, children with clinical features suggestive of nephrotic syndrome (NS) other than MCNS, children who failed to respond to an 8-week course of corticosteroids, prior to the administration of cyclophosphamide, children with persisting elevated s-creatinine levels and all black children.

4. Treatment

The mainstay of treatment was corticosteroids with the aim to achieve and maintain remission rather than adhering to a standardised protocol. For this reason a higher dose and a longer course were used (daily dose versus alternate day treatment) compared to the ISKDC guidelines (International Study of Kidney Disease in Children 1978:159). The primary contraindications for corticosteroid treatment were children with CNS, secondary NS (e.g. hepatitis B associated nephropathy, Henoch-Schőnlein purpura), children with CKD Stage 3 or more associated with stunting and wasting, cardiomegaly, anaemia and bone mineral disease and presumably immune-compromised children.
Second line immunosuppressive therapy was initiated in a selective group of children, influenced by compliance and socio-economic factors, and only after parental consent had been obtained. Indications for second line immunosuppressive therapy included children with steroid dependent or frequently relapsing NS, those who developed secondary steroid resistance and a selective group of children with partial response. Exclusion criteria for cyclophosphamide treatment included children with underlying chronic infections, e.g. untreated *Mycobacterium tuberculosis* or HIV infection and those at increased risk of developing acute or chronic infections due to poor nutritional status.

4.1.1 Corticosteroid treatment

Prednisone was administered as a single daily dose at 2 mg/kg/day and tapered to 1 mg/kg/day after 4 weeks in children who responded and achieved remission. Further tapering was only initiated after another 4 weeks and treatment stopped after a total of 20 weeks. For those who had not responded to corticosteroid treatment at a dose of 2 mg/kg/day by the 4th week – which occurred commonly in the event of black children - this high dose was continued for a maximum of 8 weeks when the dose was tapered according to the response of the child.
Response to treatment was classified as: remission, partial response and steroid resistant.
Remission was defined as no or trace proteinuria on dipstick test for three consecutive days or urine protein:creatinine ratio of <0.2 mg/mg. Relapse was defined as proteinuria of ≥ 2+ on dipstick test for three consecutive days or urine protein:creatinine ratio of ≥ 2.0 mg/mg. Partial response was defined as ≤ 2+ proteinuria on urine dipstick or urine protein:creatinine ratio < 2.0 mg/mg after a maximum of 8 weeks of high dose steroid treatment (2 mg/kg/day). Steroid resistance was defined as persistent proteinuria ≥2+ and urine protein:creatinine ratio ≥ 2.0 mg/mg after a maximum of 8 weeks of high dose steroid treatment (2 mg/kg/day). Steroid dependence was defined as relapse when the dose of corticosteroid treatment was decreased or within two weeks after stopping it. Frequent relapse was defined as ≥2 relapses per 12-month period.

4.1.2 Cyclophosphamide treatment

The total course of oral cyclophosphamide for an individual child was calculated as 168 mg/kg over 8 or 12 weeks administered as 3 mg/kg/day for 8 weeks or 2 mg/kg/day

for 12 weeks (=168 mg/kg). Cyclophosphamide tablets are sugar coated and contain 50 mg cyclophosphamide/tablet. It cannot be crushed or divided. The dose to be taken per 7-day week was therefore calculated for each patient and was limited to a maximum dose of 3 mg/kg/day. Tablets were administered for fewer than seven days per week for smaller patients weighing less than 25 kg who required <50 mg/day (i.e. no treatment on weekend days).
A kidney biopsy was performed in all children before cyclophosphamide treatment was initiated. No child received a second course of cyclophosphamide.

4.1.3 Intravenous methylprednisolone pulse treatment

A course of intravenous methylprednisolone was administered in three scenarios, i.e. children with anasarca and who were resistant to their first course of oral corticosteroid treatment, children who became steroid dependent or developed frequent relapses after having had a course of cyclophosphamide treatment and children with partial response to oral corticosteroid and cyclophosphamide treatment. Selection of patients for this form of treatment was further influenced by their ability or willingness to frequently return to the hospital and by the absence of recurrent infections.
Intravenous corticosteroid treatment consisted of methylprednisolone 30 mg/kg/dose (maximum dose of 1000 mg) administered according to established guidelines (Mendoza et al 1990:303), but excluding cyclophosphamide treatment (Table 1).
It was discontinued when a patient experienced unacceptable adverse effects, e.g. progressive fluid retention or volume overload, with worsening hypertension or when proteinuria remained unchanged after 6 to 8 weeks.

Week	M-P[a]	N	Prednisone
1-2	30 mg/kg thrice weekly	6	None
3-10	30 mg/kg per week	8	2 mg/kg/day [b]
11-18	30 mg/kg per 2 weeks	4	1.5mg/kg/day tapered slowly
19-52	30 mg/kg per 4 weeks	8	1 mg/kg/day tapered slowly
53-78	30 mg/kg per 8 weeks	4	0.5mg/kg/day tapered slowly

a = Maximum dose 1000 mg
b = Maximum dose 60 mg

Table 1. Intravenous methylprednisolone (M-P) pulse regimen

4.1.4 Adjunctive treatment

Adjunctive treatment included diuretics for symptomatic management of oedema, antihypertensive drugs, multivitamin and folic acid supplementation, alpha-calcidol for children on high-dose corticosteroid treatment and with persisting nephrotic-range proteinuria. Treatment with an angiotensin converting enzyme inhibitor (ACE-inhibitor) was given for its antiproteinuric effect to alleviate persistent proteinuria and as an antihypertensive drug if needed. Other antihypertensive drugs used included a β-blocker (atenolol), calcium antagonist (amlodipine), α-blocker (prazosin) and a vasodilator (hydralazine). Angiotensin receptor blockers were not prescribed because of unavailability.

Diuretics of all classes, namely furosemide, hydrochlorothiazide and spironolactone were used in combinations if needed for children with diuretic-resistant NS. Hydroxy-methylglutharyl coenzyme A reductase inhibitors (statins) were selectively prescribed to children older than 5 years with persistent NS associated with an elevated total cholesterol level of >10 mmol/L. All children received a diet containing reduced salt and saturated fat. Protein intake was not restricted.

5. Ethical approval

The study was approved by the University of Pretoria Research Ethics Committee and permission was obtained from the chief executive officer of the hospital to access hospital files.

6. Statistical analysis

Categorical data was reported as proportions and quantitative data as means (standard deviations) and medians (range).
Frequencies of variables within groups were compared using the two-sided Fisher exact test and a p-value <0.05 was regarded as significant. Renal and patient survival in the two population groups were assessed using Kaplan Meier non parametric life table survival analysis.

7. Results

Over a period of 23 years (1986 – 2009) 358 children with a clinical diagnosis of NS were admitted and comprised the study group. Of these 278/358 (77.7%) were black and 80/358 (22.3%) white. The median age was 58 months (range 0.5 – 144 months) and the male: female ratio 1.3:1.
The age at presentation was categorised in 4 groups as depicted in table 2. Twenty seven children were ≤ 12 months of age at the time of presentation, of whom 15/27 (4%) presented within the first 3 months of life and were diagnosed with CNS. An additional ten children were also diagnosed with CNS but were referred later, when they were older than 3 months. Fifty percent (179/358) of the children were in the age group >12-72 months.

Age category (months)	Number	%
0-3	15	4.2
>3-12	12	3.3
>12 - 72	179	50.0
>72	152	42.5
Total	358	100%

Table 2. Age Categories of Children at Presentation

Stunting (height/length for age z-score > -2SD) was present in 88/358 (24.6%) children. Microscopic haematuria was present in 52/109 (47.7%) and macroscopic haematuria in 34/109(9.5%) children with MCNS.
A constellation of clinical and laboratory features including, age >12 months to 72 months, normal blood pressure, absence of haematuria, normal renal function and normal levels of

serum C_3 have been quoted as suggestive of MCNS with an expected good prognosis (International Study of Kidney Disease in Children 1978:159). In this study 67% of the children with MCNS fulfilled the criteria for the diagnosis, apart from not having a normal blood pressure. This finding has been described by others (Habib et al 1971) which questions the validity of the criteria for diagnosing MCNS.

Hypertension was present in 227/358 (63.4%) children at presentation of whom 171/227 (47.8%) required treatment with antihypertensive drugs for at least 6 weeks. Diuretics were used for symptomatic management of oedema and were often used as a first line antihypertensive drug in children considered to have volume overload. Of those with persistent hypertension 146/171 (85.4%) received an ACE-inhibitor as the preferred antihypertensive drug. Overall 213/358 (59.5%) received an ACE-inhibitor for its anti-proteinuric effect. Renal function and s-potassium were monitored in all children who were treated with an ACE-inhibitor. No child experienced an allergic response or developed significant coughing with this treatment. An acute increase in s-creatinine levels occurred in some patients, usually in association with volume contraction, which was reversible in all cases with fluid resuscitation.

The mean s-albumin level at the time of presentation was 13.2 ± 5.2 g/L. Some patients had below detectable s-albumin levels and for these the lowest value documented was an arbitrary level of 10g/L for the purpose of statistical analysis. This means that the true mean level was in fact lower. The mean s-cholesterol at the time of presentation was 11.4 ± 7.3 mmol/L. As already stated above, statins were only prescribed to a limited number of older children with persistent hypercholesterolaemia. The main reason why younger children with similar high cholesterol levels were not treated with statins is the lack of long-term safety information on the effects of these drugs on the developing brain, immune functions, hormones and energy metabolism.

Kidney function was monitored using change in eGFR over time. Despite its limited accuracy, especially in children with poor muscle bulk, it was the only feasible test which could be done at regular intervals at follow-up visits. The results of eGFR at the time of presentation and at last follow up for the two race groups are depicted in table 3.

CKD Stage	**eGFR[1] (ml/min/1.73m²)**	**eGFR at presentation n = 358**		**eGFR last follow up n = 358**	
		Black children n=278	**White children n=80**	**Black children n=278**	**White children n=80**
1	**≥90**	189 (68.0%)	55 (68.8%)	178 (64.0%)	66 (82.5%)
2	**60-89**	42 (15.1%)	20 (25.0%)	29 (10.4%)	10 (12.5)
3	**30-59**	27 (9.7%)	4 (5.0%)	18(6.5%)	1 (1.3%)
4 + 5	**<29**	20 (7.1%)	1(1.3%)	53(19.0%)*	3 (3.8%)

eGFR = Estimated glomerular filtration rate

*One black child with CKD stage 5 who had a successful kidney transplant is included in the number of black children with stage 4 and 5 CKD.

Table 3. eGFR at the Time of Presentation and During Follow Up in the Two Race Groups

Significantly more black compared to white children had stage 4 or 5 CKD on presentation, 7.1% vs. 1.3% respectively (p=0.03), or had developed stage 4 or 5 CKD at the time of last follow up, 19.0% vs. 3.8% respectively (p= 0.000).

A secondary cause for NS was identified in 26 (7.2%) children including Henoch-Schőnlein purpura, systemic lupus erythematosus, chronic hepatitis B infection, HIV infection and IgA glomerulonephritis. Ten children (2.8%) had chronic hepatitis B associated nephropathy. None of them were given any immunosuppressive treatment, interferon or other specific antiviral treatment. In eleven children a genetic cause of NS was suspected.

CNS was diagnosed in 25/358 (7%) children, none of whom had a syndromic form of CNS. Investigations for mutations of *NPHS1, NPHS2* and *WT1* were not done in any of the children with CNS or suspected familial NS because of unavailability.

Kidney biopsies were performed in 318/358 (89%) children. The main histological diagnoses are depicted in table IV. Eighteen children had inconclusive histology which was reported as "FSGS cannot be excluded." In these cases there was a chronic inflammatory cell infiltrate in the interstitium, interstitial fibrosis and tubulo-interstitial atrophy suggestive of FSGS but the biopsy sample did not have glomeruli with focal sclerosis. If this group of children is added to the group with definite FSGS, the frequency of FSGS increases to 98/318 (31%). Eleven children (10 black, 1 white) were diagnosed with immune complex glomerulonephritis (ICGN), based on the presence of immune deposits in the basement membrane on electron microscopy examination and another 26 (25 black, 1 white) had ICGN with secondary glomerular sclerosis.

Histological diagnosis	**White Number (%)**	**Black Number (%)**	**Total (%)**	**p-value**
MCNS	42(66)	67(26.4)	109 (34.2)	<0.001*
Focal segmental glomerulosclerosis (FSGS)	9 (14)	71(28)	80 (25)	0.01*
MCNS – FSGS **	7(11)	11 (4.3)	18 (5.7)	0.048*
Immune complex glomerulonephritis (ICGN)	1(1.6)	10 (4)	11(3.4)	0.31
ICGN and secondary FSGS	1 (1.6)	25(9.8)	26 (8.2)	0.02*
Membranous nephropathy (MN)	0 (0)	14 (5.5)	14 (4.4)	0.04*
Mesangiocapillary glomerulonephritis	1 (1.6)	12 (5)	13 (4)	0.22
Congenital nephrotic syndrome (CNS)	0 (0)	25 (9.8)	25 (8)	0.02*
Other	3 (4.7)	19 (7.5)	22 (7)	
Total ***	64(100)	254 (100)	318 (100)	

*Statistically significant

**MCNS-FSGS: Histology was reported as "FSGS cannot be excluded" and was therefore inconclusive but very suggestive of FSGS due to the presence of a chronic inflammatory cell infiltrate in the interstitium, interstitial fibrosis and tubular atrophy on the biopsy.

***Includes all children who had kidney biopsies, including the children with congenital nephrotic syndrome.

Table 4. Main Histological Subtypes of Nephrotic Syndrome (n = 318)

In table 5. the frequencies of all the histological subtypes excluding CNS are depicted, which is in line with the procedure followed by the ISKDC. CNS is considered a distinct form of NS with a unique etio-pathogenesis and a high frequency of underlying genetic mutations

and is therefore usually not analysed with other forms of INS. Kidney biopsies were done in 318 children (64 white and 254 black). After exclusion of the 25 children with CNS, all of whom were black, the frequencies of the histopathological subtypes of only the remaining 229 black children changed.

Histological diagnosis	White Number (%)	Black Number (%)	Total (%)	p-value
MCNS	42(65.6)	67(29.3)	109 (37.2)	<0.000*
Focal segmental glomerulosclerosis (FSGS)	9 (14.0)	71(31.0)	80 (27.3)	0.004*
MCNS – FSGS **	7(10.9)	11 (4.8)	18 (6.1)	0.07
Immune complex glomerulonephritis (ICGN)	1(1.6)	10 (4.4)	11(3.7)	0.26
ICGN and secondary FSGS	1 (1.6)	25(11.0)	26 (8.9)	0.01*
Membranous nephropathy	0 (0)	14 (6.1)	14 (4.8)	0.03*
Mesangiocapillary glomerulonephritis	1 (1.6)	12 (5.2)	13 (4.4)	0.18
Other	3 (4.7)	19 (8.3)	22 (7.5)	
***Total	64 (100)	229 (100)	293 (100)	

*Statistically significant
**MCNS-FSGS: Histology was reported as "FSGS cannot be excluded" and was therefore inconclusive but very suggestive of FSGS due to the presence of chronic inflammatory cell infiltrate in the interstitium, interstitial fibrosis and tubular atrophy on the biopsy.
***All children who had kidney biopsies, but excluding the children with congenital nephrotic syndrome.

Table 5. Histological Subtypes of Nephrotic Syndrome Excluding Children with Congenital Nephrotic Syndrome (n=293)

The incidences of the four major histological subtypes (MCNS, FSGS, membranous nephropathy, mesangiocapillary glomerulonephritis were significantly higher in the black children (Table 5).

7.1 Results of treatment

Remission with oral corticosteroid treatment was achieved in 33/41(81%) white vs 33/59(56%) black children (p=0.02) who had MCNS. The response rate of the black children is similar to the 60% response rate in black children reported previously from Kalafong Hospital (Prinsloo JG. 1986:375) but lower than the 78% response rate reported a decade later in children from the Chris-Hani Baragwanath Hospital (Johannesburg), both tertiary hospitals in South Africa (Thomson 1997:402). Oral corticosteroid treatment resulted in remission in 3/9 white children and 8/40 black children with FSGS (p=0.6). In the combined group of children with FSGS and MCNS-FSGS 9/13 (69.2%) white children vs 4/14 (28.5%) (p<0.05) black children went into remission with this treatment. Twenty five children who failed to respond to oral corticosteroid treatment were treated with a course of intravenous methylprednisolone of whom only 4 (one white and 3 black children) went into complete remission. This form of treatment was abandoned because of its poor efficacy, high toxicity and cost and the disruptive effect it had on school attendance. Hundred children were

treated with cyclophosphamide, 46 were white and 54 black. The response rate to this treatment was statistically significantly different in the white and black children. Sustained remission was achieved in 37/46 (80%) white and in 23/54 (43%) black children (P=0.002; 95% CI 2.2 – 13.7). The ISKDC reported no benefit of orally administered cyclophosphamide and prednisone compared to prednisone alone for the treatment of steroid resistant NS (Tarshish, P et al. 1996: 590). Their report and the poor response of children with steroid resistant NS in this study prompted discontinuation of cyclophosphamide treatment in children with steroid resistant NS since 2007. No patient experienced side effects of cyclophosphamide treatment, but they were all monitored at least every 10 to 14 days throughout the duration of the treatment. Several children who had been in contact with chicken pox were given human varicella-zoster immune globulin and/or acyclovir prophylactically, but none developed serious chicken pox.

7.2 Morbidity and mortality

Acute reversible renal failure occurred in 35/358 (9.8%) children and thrombotic complications, other than strokes in 9/358 (2.5%). Six children (1.7%) developed strokes, one of whom developed bilateral sequential middle cerebral artery thromboses a few months apart. She was one of a family of 3 children who all had steroid resistant NS. At the time that she developed the first stroke she was not dehydrated, but had a mild lower respiratory tract infection, iron deficiency anaemia and a thrombocytosis, which are known risk factors for thrombo-embolic complications in children with nephrotic syndrome.

Fourty eight percent of all children experienced acute invasive bacterial infections, including pneumonia, peritonitis and septicaemia. In those with steroid sensitive NS acute bacterial infections occurred during relapses. The frequency of infection was inversely related to age and was particularly high in children younger than 3 months. Of these children 87% developed serious infections compared to 39% of children older than 6 years. Streptococcus pneumoniae was the predominant causal organism, followed by Escherichia coli and other gram negative organisms. Twenty six children (7.3%) developed peritonitis of whom three demised due to pneumococcal septicaemic shock. Pneumococcal infections occurred in 7/80 (8.7%) white children vs 19/278 (6.8%) black children (p=0.6). Until recently pneumococcal polysaccharide vaccine was given to all children younger than 5 years at the time of their first presentation, despite its limited efficacy in children younger than 2 years. Since 2009 the pneumococcal conjugate vaccine is available in South Africa which is used for revaccination of this group of children. Long term prophylactic penicillin was not used.

Chronic hepatitis B infection (positive HBsAg and/or HBeAg) occurred in 10/358 (2.8%) children, all of whom were black. Hepatitis B vaccine was included in the routine immunization schedule of children in South Africa since 1991 and since that time no child was diagnosed with hepatitis B related NS. No child had hepatitis C related NS. Investigations to rule out CMV infection were only done in children with CNS and in those with atypical clinical features of NS, including anaemia, hepatosplenomegaly, skin rash or positive central nervous system signs. In most cases only CMV IgM and IgG were done, which were often both positive due to unexplained reasons at the time, because the test for CMV viral load was not available. Should a CMV infection be diagnosed it is not necessarily proof of the causality of the NS.

All children were screened for underlying Mycobacerium tuberculosis infection with a chest X-ray, gastric aspirates or induced sputum cultures and Mantoux test (skin prick test with intradermal injection of purified protein derivative of Mycobacterium tuberculosis). Because

children uncommonly have sputum positive tuberculosis, several of our patients received empiric anti-tuberculous treatment for 6 months when the diagnosis could not be unequivocally excluded. A high prevalence of tuberculosis associated with a deleterious effect on renal function was reported in black children with FSGS. (Kala et al.1993:392). It has been postulated that immune responses mediated by infections with Mycobacterium tuberculosis and HI-virus may contribute to glomerulosclerosis. Mycobacterium tuberculosis infection was present in too few patients in this study to draw any conclusion.
Investigations for HIV infection were only done in children who had clinical features suggestive of the disease and whose parents had given consent to testing. It is therefore not possible to report on the true incidence of NS associated with HIV infection. Patients with HIV infection had a variety of histological lesions, including, immune complex glomerulonephritis, immunotactoid and fibrillary glomerulonephritis and FSGS. No patient had HIV collapsing glomerulopathy which has been reported as one of the commonest histological lesions in black adult patients with HIV infection. Recently HIV- associated kidney disease has been reported to have become the most common form of kidney disease in children seen in the renal unit at one of the academic hospitals in South Africa. (Bhimma R, 2009:15)
Only one infant with CNS had congenital syphilis and treatment with penicillin did not result in cure of the disease. Chronic "quartan malarial nephropathy" or other parasitic related forms of NS did not occur.

7.3 Outcome

At presentation 21/358 children had CKD stage 4 or 5. Of the black children 20/278 (7.2%) had CKD stage 4 or 5 compared to 1/80(1.3%) white children (p=0.03). Over the period of follow up more black children (53/278) (19%) developed stage 4 or 5 CKD compared to white children (3/80)(3.8%)(p= 0.000). Kaplan-Meier estimation of renal survival depicting the difference in renal survival in black and white children is demonstrated in Fig1. One black child was successfully transplanted during the follow up period and had normal renal function at last follow up. Persistent nephrotic range proteinuria is associated with a rapid progression to end-stage kidney disease. Several children in this study had long standing suboptimal management of nephrotic range proteinuria when presenting to the SBAH which contributed to a more rapid progression to end stage kidney disease.
Forty three patients died during the follow up period. Three (3.7%) white children died of whom 2 succumbed to complications of renal failure (renal deaths) and one died due to pneumococcal septicaemia (non renal death). Forty black children died during the follow up period, mostly due to end stage renal failure. In the black children infectious related deaths occurred mostly in the children with congenital NS. Black children had a significantly higher mortality compared to white children (40/268 vs. 3/80) (p<0.001). Kaplan-Meier patient survival estimate depicting the difference in patient survival for black and white children is demonstrated in Fig 2.

8. Discussion

This study population differs in several aspects from those reported from developed countries. The majority of patients are black with an inherent risk of CKD due to a genetic predisposition aggravated by poor socio-economic circumstances and chronic infections. Poor prognostic indicators namely stunting, profound hypoalbuminaemia, long standing nephrotic-range proteinuria, hypertension and impaired kidney function are common at presentation against a background of tuberculosis and HIV infection.

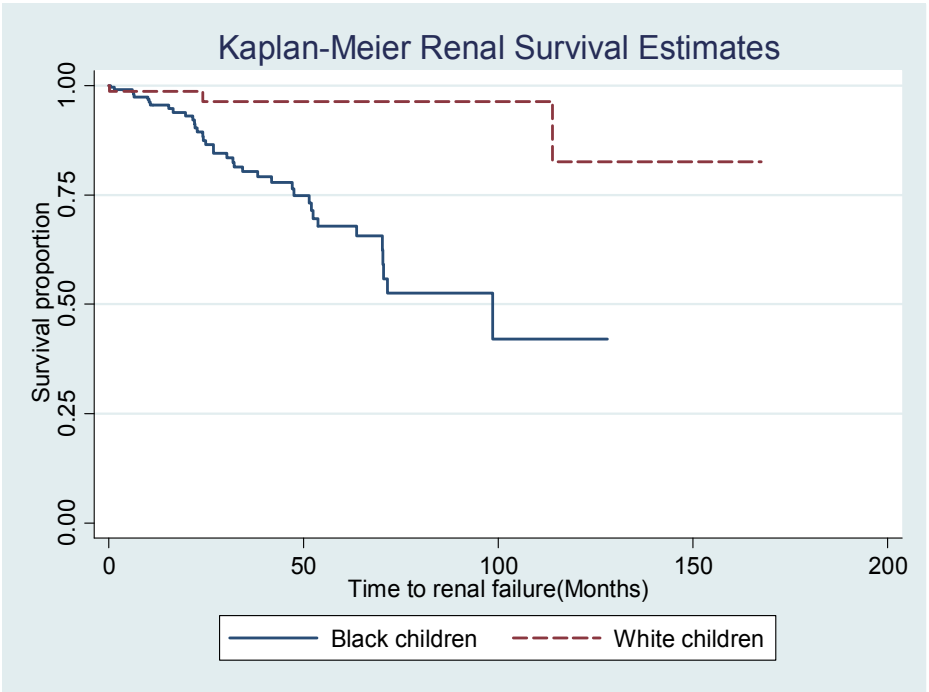

Fig. 1. Kaplan-Meier estimation of renal survival

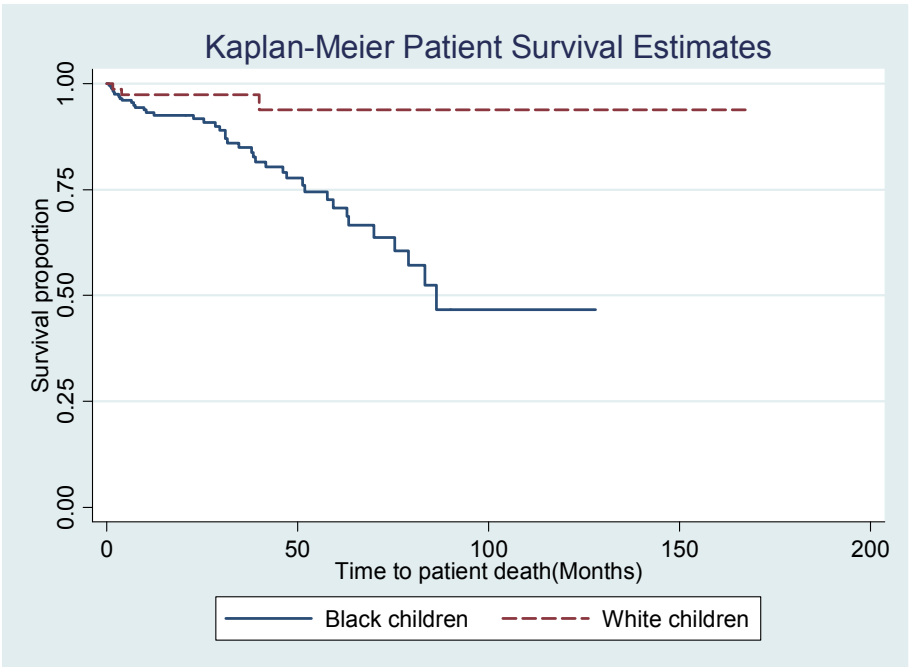

Fig. 2. Kaplan-Meier estimation of patient survival

The results of this audit confirm that significant racial differences exist in the clinical presentation, histological subtypes, response to treatment and outcome in South African children with NS. Similar findings have been reported by other study groups in South Africa (Bhimma et al. 2006: 1847; Bhimma R, 1997: 429), from other countries in Africa (Yao Doe, J. et al. 2006: 672; Olowu et al. 2010:200) and also from elsewhere in the world (Ingulli, E. & Tejani, A. 2001:393). In their study on idiopathic FSGS in children Ingulli et al. (Ingulli, E. & Tejani, A. 2001:393) reported that the rate of FSGS was higher (32.2% vs. 20%), and progress to end stage kidney disease was more common (78% vs. 33%) in black and Hispanic compared to white children. Olowu et al. (Olowu et al. 2010:200) reported an incidence of 18.5 % MCNS and 25.9% FSGS in their study of black Nigerian children with INS. Ethnic differences in the incidences of FSGS have now been well established. Over the past 10 years there have been several reports of an increasing incidence of FSGS in children (Borges et al. 2007:1309; Filler et al. 2003: 1107)

In this study 77% of the children were black and 23% were white. There was a significant difference in the incidences of the main histological subtypes (MCNS, FSGS, immune complex glomerulonephritis, secondary FSGS and membranous nephropathy) between black and white children. The incidence of MCNS was 65.6% and 29.3% ($p<0.000$) and of FSGS, 14% and 31% ($p=0.004$) in white and black children respectively.

The first course of corticosteroid treatment was often longer or more intense than that recommended by the ISKDC (International Study of Kidney Disease in Children: 1978:13) because of delayed achievement of remission or only partial response after the first four weeks. Corticosteroid treatment was also often given daily rather than on alternate days because parents failed to understand alternate day dosing schemes. White children with MCNS had a better response to oral corticosteroid treatment compared to black children with MCNS (80% vs 56%; $p=0.02$). Remission with oral corticosteroid treatment was achieved in more white children with FSGS compared to black children with FSGS, although this difference did not reach statistical significance (33% vs 20 %; $p=0.6$). Response rates in both race groups in this study were much lower compared to the 93.1% response rate reported by the ISKDC (International Study of Kidney Disease in Children: 1978:13).

After 2000 we stopped using high dose intravenous methylprednisolone for the treatment of steroid resistant NS in our patients for several reasons. It is too costly, it places a heavy burden on the family and child, and only 4 of 25 children (16%) went into remission with this treatment. This very poor response rate compared to that of Mendoza et al (Mendoza, S.A. et al. 1990:303), who reported complete remission in 52% of their patients, can possibly be explained by the fact that we omitted an alkylating agent in our treatment regimen. Adhikari et al (Adhikari et al. 1997:423) reported a dismal outcome in 12 South African children with FSGS who were treated with a combination of intravenous methylprednisolone and an alkylating agent. Although they considered this treatment as "promising," their patients developed serious side effects including alopecia, cataracts, leukopaenia, systemic candidiasis, gram negative septicaemia, and one child demised of a serious infection which was undoubtedly caused by the severe immune suppression associated with the treatment.

Infections remain a serious risk to all children during a relapse of NS, which is practically always for those with steroid resistant NS. This risk is intensified in those living in poor socio-economic circumstances. Forty eight percent of our patients experienced acute invasive bacterial infections and three succumbed to documented pneumococcal

septicaemia. Management of the children with CNS was particularly challenging, due to their serious immune compromised state and high frequency of recurring infections. All children with CNS were black and screening for a possible infectious cause was not very fruitful. Genetic studies were not undertaken in any of our patients as we do not have access to genetic laboratory services. Kidney biopsy revealed idiopathic FSGS or secondary glomerular sclerosis in 15/25 children with CNS. A very dense chronic inflammatory cell infiltrate was present in all cases. Children with CNS were not treated with corticosteroids or other immunosuppressive drugs. Most of them remained in hospital for long periods or required frequent admissions for treatment of bacterial infections or gastroenteritis. An ACE inhibitor was not prescribed to any child less than 3 months old and was usually only given to children older than 12 months.

Because of the lower incidence of MCNS in black children in South Africa first reported in 1979, (Bhimma et al. 1997:429; Lewin, et al. 1979: 88) it has been our practice to biopsy all black children at the time of their first presentation. For the same reason paediatric nephrologists elsewhere in Africa have also advocated pre-treatment renal biopsies in their patients. Olowu et al. (Olowu et al. 2010:200] reported that only 18.5% of the black children with INS in their study of Nigerian patients had MCNS.

Most centres in developed countries are still following the ISKDC recommendation regarding biopsies despite worldwide reports of an increasing incidence of FSGS in both children and adults. Filler et al (Filler et al. 2003 :1107) reported a declining incidence of MCNS from 81.1% to 64% and an increasing incidence of FSGS from 10.8 to 24.7% of FSGS in their childhood population in Ontario, over two time periods 1985-1993 and 1993-2002. The incidence of MCNS in the first period did not differ significantly from that reported by the ISKDC (International Study of Kidney Disease in Children 1978: 13) and although not specifically reported, the inference is made that the majority of their patients responded to corticosteroid therapy. The incidence of FSGS in their patients has more than doubled over 17 years while the population under study remained stable. Race is not mentioned in their study. Despite an alarming increase in the incidence of FSGS in their patients, they argue that empirical steroid treatment with a cut-off point at 28 days is still justifiable. During the second period of their study the incidence of MCNS is similar to the incidence of MCNS in white children in our study, 64.7% vs. 65.6 % and the incidence of FSGS slightly less than that of FSGS in black patients in our study 24.7% vs. 31%.

Already in 1997 Thomson et al. (Thomson, P.D. 1997:508), performing pre-treatment biopsies in all their black patients, reported an incidence of FSGS in 31.3% which is identical to the 31% incidence of FSGS in black patients in this study. It appears that there has not been an increase in the incidence of FSGS in black children in Gauteng Province in South Africa over this period.

It can be expected that the HIV epidemic has contributed to the incidence of CKD in South Africa in general, but its possible role in the development of FSGS, or its contribution to an increase in the incidence of FSGS, is uncertain. Local multi-centre prospective research studies in patients with HIV-associated nephropathy will be necessary to explore this question.

Primary FSGS is a spectrum of podocytopathies caused by a variety of contributing etiologies, including genetics, infections, environment, including the intra-uterine environment, drugs and toxins. It is an aggressive disease, more so in black children compared to white children. The rationale of performing a kidney biopsy at presentation is

that it confirms the histological subtype and may give clues to the stage and type of initial injury. It has been suggested that the different variants of FSG may respond differently to treatment (Valeri. et al. 1996: 1734). Immuno-histochemistry and electron microscopy may also help in differentiating primary and secondary forms of FSGS. It is well known that patients with extensive involvement of glomeruli, advanced tubulo-interstitial fibrosis and tubular atrophy are less likely to respond to corticosteroid treatment compared to those with no interstitial involvement. It is questionable whether it is justifiable to expose such a patient who may also happen to be malnourished and have poor social circumstances to aggressive immune suppression for a disease which may not have an immunological background. The possible departure of many of our black patients from a hostile intra uterine environment resulting in low birth weight and low glomerular endowment is an aspect which has not been investigated systematically.

9. Conclusions

Compared to the ISKDC report black children have a lower incidence of MCNS and a higher incidence of FSGS. Black children also have a more aggressive form of FSGS which responds poorly to corticosteroid and other immuno-suppressive treatment. More black children develop CKD stage 4 and 5 compared to white children and black children have a higher mortality compared to white children. The results of this study and similar evidence from the rest of Africa suggest that the ISKDC recommendation of empiric corticosteroid treatment in children with INS should not be followed in the management of black children with INS. We suggest that a kidney biopsy should be done at presentation to allow a definitive diagnosis and targeted treatment from the outset.

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Blood Pressure Control in Patients with Glomerulonephritis

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1. Introduction

Although the expanding prevalence of lifestyle-related diseases such as diabetes mellitus and hypertension which ultimately cause renal dysfunction, glomerulonephritis still remains as one of the major causes of end-stage renal failure in most countries all over the world. In addition to the immunological therapy using corticosteroids and immunosuppressants, management of non-immunological risk factors such as hypertension, obesity and disorders of glucose and lipid metabolism greatly affect the prognosis of renal function in the treatment of patients with glomerulonephritis. Especially, hypertension is a pivotal risk factor for the progression of renal injuries and the adequate blood pressure control is a matter of primary importance in order to prevent the development of renal dysfunction.
In this chapter, the importance of blood pressure control is stressed referring the evidence thus far, and current topics and future prospects are discussed as to the matters such as target blood pressure levels and choices of antihypertensive agents.

2. Target blood pressure

Generally, hypertension is diagnosed when the systolic blood pressure is higher than 140mmHg and/or the diastolic blood pressure is higher than 90mmHg. However, this is an arbitrary definition and the linear relation between the blood pressure level and the risk of renal dysfunction can be extended even in the normotensive range in epidemiological studies. Figure 1 shows the relations of blood pressure level categories and the risk of developing end-stage renal failure in 17-year follow-up study of Okinawa prefecture residents in Japan (1). Naturally, hypertension increases the risk of renal failure with elevating grade of blood pressure levels. Moreover, blood pressure levels lower than 140/90mmHg but higher than 130/85mmHg, namely the high-normal blood pressure, offers a significant risk for future development of renal failure.
As for the target blood pressure level in the treatment of glomerulonephritis patients, Figure 2 depicts the outcomes of Modification of Diet in Renal Disease (MDRD) study (2) in which the blood pressure control level less than 125/75mmHg brought about slower GFR reduction than the level less than 140/90mmHg in subjects with nondiabetic renal diseases especially when the proteinuria was prominent. Similarly, Figure 3 plots the annual decrease rates of GFR against achieved blood pressure levels in hypertensive subjects with

renal diseases (3). In patients whose hypertension was not treated, GFR decreased by more than 10mL/min per year. When the blood pressure was lowered to 140/90mmHg, the rate of annual GFR decline was reduced by half. However, considering that the physiological annual GFR decline with aging is about 1mL/min, the annual GFR decline in 140/90mmHg subjects is faster than the natural rate. As compared with this, strict blood pressure lowering to 130/85mmHg or 130/80mmHg yielded retardation of GFR decline to a nearly physiological level in subjects with renal diseases.

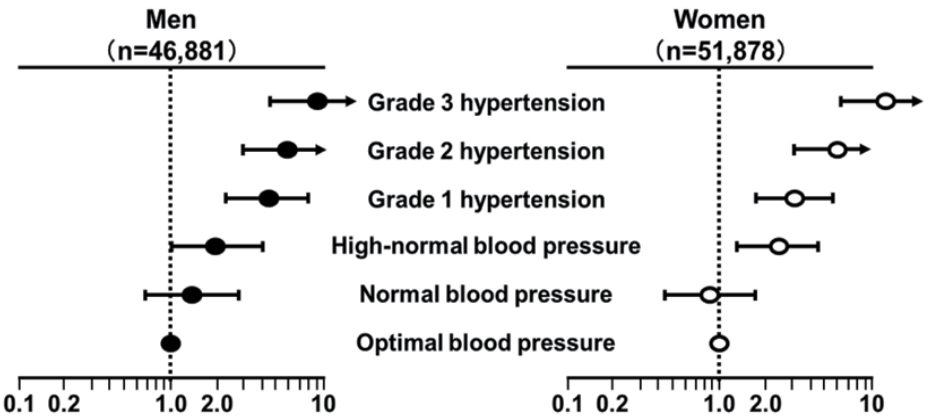

Fig. 1. Relationship between the incidence of end-stage renal failure and the blood pressure level (1). The incidence of end-stage renal failure is increased not only in hypertensive subjects but also in subjects with high-normal blood pressure ranging 130-139/85-89 mmHg as compared with lower normal blood pressure subjects.

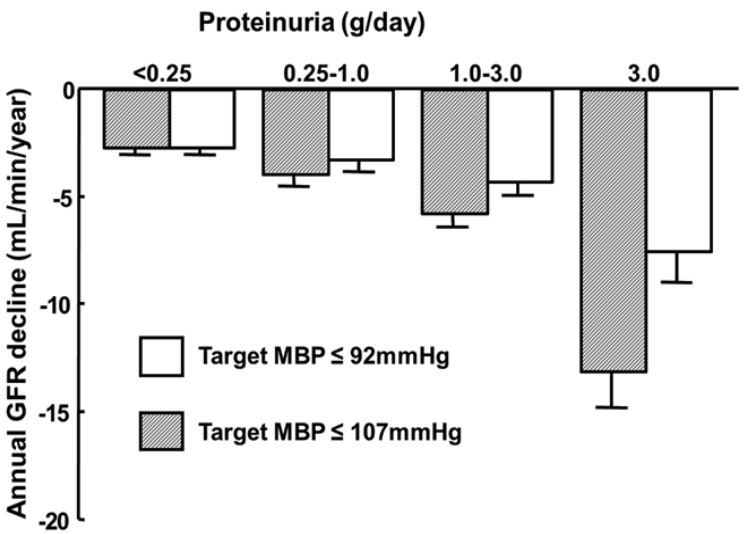

Fig. 2. The annual decrease in glomerular filtration rate (GFR) in nondiabetic renal disease patients of The Modification of Diet in Renal Disease(MDRD) Study. (2)

Thus, it is suggested that the blood pressure should be lowered below the high-normal level in glomerulonephritis patients in order to maximally slow the progression of renal dysfunction. Therefore, the American, European and Japanese guidelines for the management of hypertension recommend the target blood pressure level of less than 130/80mmHg in patients with chronic kidney disease (CKD) (4-6).

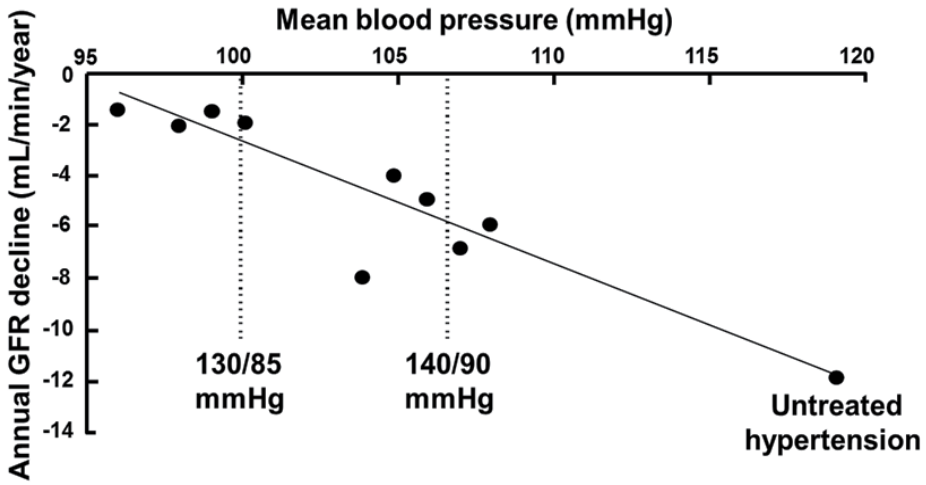

Fig. 3. Relationship between the annual decrease in glomerular filtration rate (GFR) and the achieved mean blood pressure level in studies treated hypertensive patients with renal diseases (3). The GFR decline rate was suppressed to the level near to the physiological decrease with aging in patients whose blood pressure was lowered under 130/85mmHg.

3. Glomerular hypertension and hyperfiltration

According to the hyperfiltration theory proposed by Hostteter and Brenner (7,8), increases in glomerular capillary pressure, referred to as glomerular hypertension, play an important role in the development and the progression of glomerular injuries ultimately resulting in glomerular sclerosis and the loss of its nephron. As indicated in Figure 4, not only high blood pressure but also increased salt intake and decreased urinary sodium excretion resulting in body fluid volume expansion raise intraglomerular capillary pressure and cause glomerular hypertension. In addition, the increases in protein intake and glomerular efferent arteriolar resistance are also the factors that contribute to the elevation of intraglomerular capillary pressure. Long-lasting of sustained glomerular hypertension impairs glomerular capillary endothelium and allows filtration of plasma protein molecules, followed by widening of mesangial area, obstruction of capillary lumen, hyalinosis of glomerular tuft and finally resulting in glomerular sclerosis, abolition of blood flow and filtration function. The loss of glomeruli brings about the atrophy of following renal tubules and nephrons themselves. Once a certain proportion of nephrons fall into atrophy, the intraglomerular capillary pressure and the single nephron filtration glomerular filtration rate of remaining glomeruli increase in order to compensate the reduced renal blood flow and maintain the glomerular filtration rate, which consequently promote further development of glomerular hypertension.

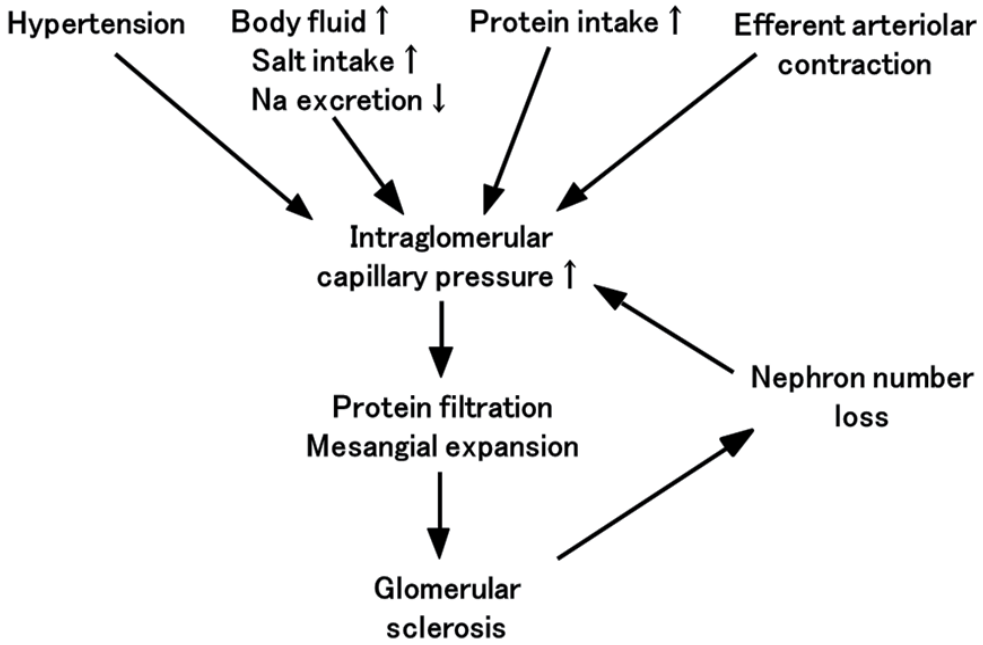

Fig. 4. Relations of factors contributing to the increase in intraglomerular capillary pressure and progression of glomerular sclerosis.

In order to stop the progression of this vicious cycle, comprehensive control of factors influencing the intraglomerular capillary pressure elevation such as arterial hypertension and intakes of salt and protein is needed. As mentioned concerning the target blood pressure, strict blood pressure control is important in patients with glomerulonephritis. In controlling the blood pressure, it should be kept in mind that reduction of intraglomerular capillary pressure as well as systemic arterial pressure is essential in order to achieve maximally effective inhibition of glomerular injuries and renal dysfunction. With regard to the hemodynamic aspect of renal microcirculation, intraglomerular capillary pressure is regulated by the balance between vascular resistances of afferent and efferent glomerular arterioles as depicted in Figure 5. A number of neural and humoral factors are known to affect the contraction and dilation of glomerular arterioles. Among them, the renin-angiotensin-aldosterone (RAA) system indicated in Figure 6 is assumed to play a pivotal role in the regulation of glomerular hemodynamics. Especially, angiotensin II, a peptide exhibiting prominent bioactivities in the RAA system, induce strong contraction of efferent rather than afferent glomerular arterioles. In addition, angiotensin II facilitates mesangial cell proliferation, increases oxidative stress by activating NAD(P)H oxidase, and induce proinflammatory transcription factor NF-κB (9,10). These versatile effects of angiotensin II also contribute to the progression of renal tissue injuries.

On the other hand, angiotensin II stimulates the adrenal cortex to secrete aldosterone, a major mineralocorticoid, which facilitates renal tubular reabsorption of sodium resulting in blood and body fluid volume expansion and blood pressure elevation. Besides this well-known effect, aldosterone has been shown to promote renal tissue fibrosis and production of extracellular matrices such as collagen (9-11). Moreover, aldosterone injures endothelial,

epithelial and mesangial cells of glomeruli. In addition, aldosterone, like angiotensin II, constricts the efferent arterioles preferably to the afferent arteriole and increase the intraglomerular capillary pressure and filtration of plasma protein molecules. Thus, aldosterone is also assumed to be a factor exerting detrimental effects to the progression of glomerular diseases.

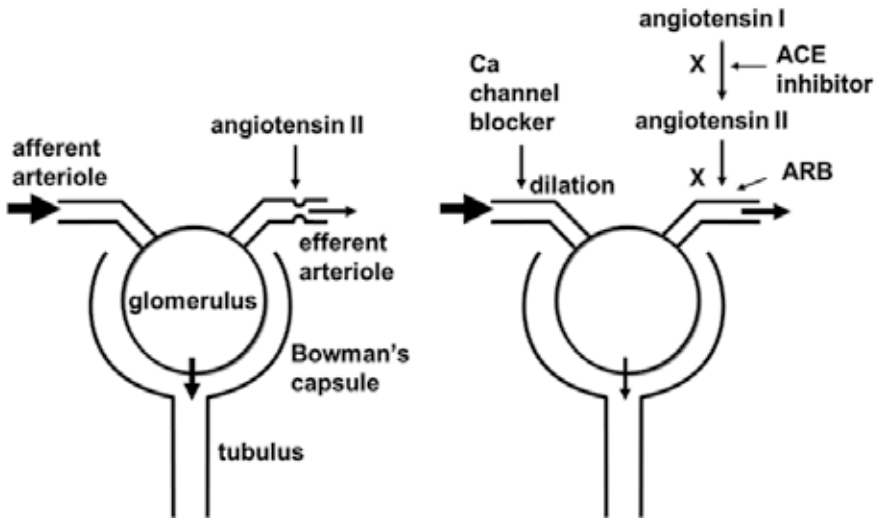

Fig. 5. The structure of glomeruli and factors relating to the hemodynamics and hydrauric pressure of glomeruli and glomerular arterioles.

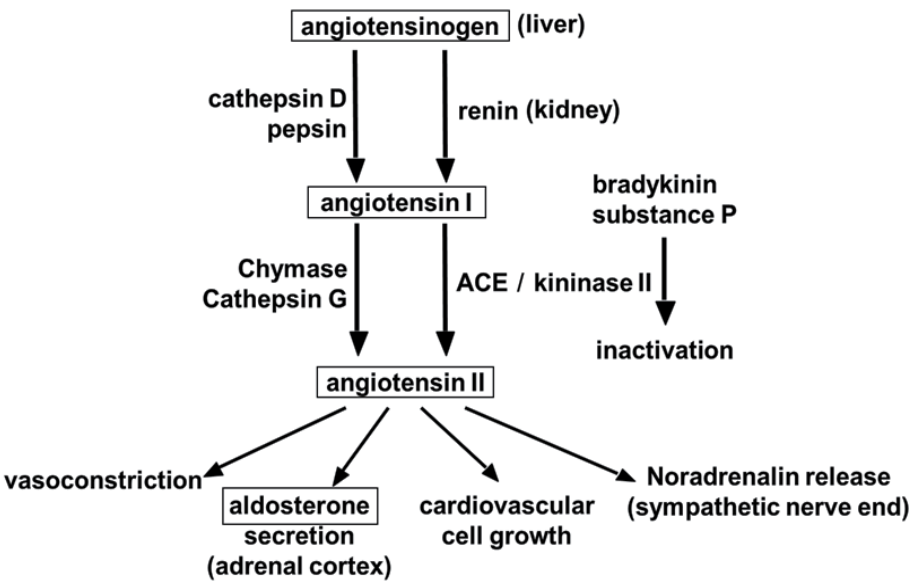

Fig. 6. Outlines of the renin-angiotensin-aldosterone system and the biological actions elicited by its components.

Several other hormones and autacoids are known to elicit dilation or contraction of the glomerular arterioles. Atrial natriuretic peptide (ANP), produced by the heart, dilates the afferent arteriole and preserves renal and glomerular blood flow in the state of heart failure (12). This action is supposed to work also in glomerulonephritis patients with reduced renal function because the plasma ANP level is increased by body fluid volume increase and reduced clearance in the kidney. Vascular endothelium produces vasoactive substances such as nitric oxide (NO) and endothelin (ET). NO preferentially dilates and ET preferentially contract the afferent arterioles (13,14). NO is supposed to participate in the mechanism of increased glomerular filtration in the early stage of diabetic nephropathy, however, the NO synthase inhibition has been shown to increase intraglomerular capillary pressure in experimental glomerulonephritis (15,16). The pathophysiological implication of ET in the glomerular circulation is not well understood. The kidney has abundant ability to produce prostaglandins (PG) from arachidonic acid and PGE_2 which facilitates natriuresis and dilates the afferent arterioles is the major PG produced in the kidney. As compared with this, PGI_2 produced by vascular endothelium dilates both the afferent and the efferent arterioles (17). Nonsteroidal anti-inflammatory drugs such as indomethacin, which inhibit cyclooxygenase and PG production, can cause renal dysfunction as the adverse effect. The inflammatory process in the pathogenesis of glomerulonephritis is supposed to stimulate PG production in the kidney. This possibly increases glomerular and renal blood flow on one hand, however, may rather increase intraglomerular pressure on the other hand by preferentially dilating the afferent arterioles. However, it has been reported that the long-term administration of PGI_2 analogue mitigated the progression of renal dysfunction without increasing intraglomerular capillary pressure in patients with chronic glomerulonephritis (18).

Taken these together into consideration, it is suggested that the enhancement of RAA system is harmful to the glomeruli and the kidney via the nocuous actions of angiotensin II and aldosterone. Reductions in renal function generally cause an increase in body fluid volume which inhibits plasma renin activity and concentrations of angiotensin II and aldosterone. Therefore, the circulating components of RAA system is supposed to be rather suppressed in patients with advanced glomerulonephritis. However, the renal and cardiovascular cells have been shown to produce components of RAA system such as renin, angiotensin converting enzyme (ACE) and aldosterone. In addition, angiotensinogen produced by the liver is abundant in plasma. Therefore, it is thought that angiotensin II and aldosterone are locally produced in the renal and cardiovascular systems and their concentrations in the tissues may be higher than in plasma. And, it is possible that the renal tissue RAA system is rather enhanced and contributes to the progression of renal injuries in patients with advanced glomerulonephritis although the circulating components of RAA system are suppressed.

4. Inhibitors of renin-angiotensin-aldosterone system in antihypertensive drug therapy for patients with glomerulonephritis

The precedent sections stressed the importance of strict blood pressure control and the implications of RAA system in the management of renal diseases in order to prevent the progression of renal dysfunction efficiently and effectively. In this context, inhibitors of RAA system such as ACE inhibitors and angiotensin II receptor antagonists (ARB) are supposed to provide renoprotective effects in addition to their hypotensive effects by inhibiting the detrimental actions of angiotensin II and aldosterone. Especially, these inhibitors of RAA system preferentially dilate the efferent arterioles as compared to the

afferent arterioles and thereby lower intraglomerular capillary pressure effectively (Figure 5). Anderson et al. (19) have shown that an ACE inhibitor lowers intraglomerular capillary pressure, reduces proteinuria and inhibits the progression of glomerular sclerosis more prominently than other antihypertensive drugs in rats with reduced renal mass in which the circulating RAA system is thought to be suppressed.

In human, it is a distinctive feature that the intraglomerular capillary pressure is elevated and the GFR is increased at the early stage of diabetic nephropathy. This glomerular hypertension facilitates the progression of diabetic nephropathy stages, namely, microalbuminuria, overt proteinuria, a GFR reduction, a serum creatinine increase and end-stage renal failure. Taguma et al. (20) have first reported that an ACE inhibitor reduces proteinuria in patients with diabetic nephropathy, and it is suggested that the suppression of angiotensin II generation brings about alleviation of glomerular hypertension and reduce hydrauric transcapillary filtration pressure of protein. After that, Lewis et al. (21) performed the multi-center collaborative prospective study evaluating the renoprotective effects of an ACE inhibitor in patients with type 1 diabetes mellitus presenting overt proteinuria and demonstrated that captopril inhibited the serum creatinine increase and the incidence of end-stage renal failure. As well as ACE inhibitors, multiple lines of later clinical studies have indicated that ARB are effective in retarding the progression of nephropathy at each stage in patients with type 2 diabetes (22-24).

With regard to the non-diabetic renal disease such as glomerulonephritis, the ACE Inhibition in Progressive Renal Insufficiency (AIPRI) study (25) and Ramipril Efficacy in Nephropathy (REIN) study (26) showed that ACE inhibitors delay the progression of renal insufficiency in European patients with non-diabetic renal disease. Furthermore, African American Study of Kidney Disease and Hypertension (AASK) (27) suggested that ACE inhibitors slow renal disease progression in African American patients with hypertensive renal disease. Also as for the Asian population, we have reported that an ACE inhibitor and an ARB are effective in reducing proteinuria and slowing the deterioration of renal function in Japanese patients with chronic glomerulonephritis (28,29). Namely, an ACE inhibitor, benazepril, or an ARB, valsartan, inhibited the increase in serum creatinine and reduced proteinuria by 30-40% as compared with placebo (Figure 7,8). In addition, there is another study reported that an ACE inhibitor improved renal outcomes in Chinese patients with advanced stage of non-diabetic renal disease whose serum creatinine ranged 3.1 to 5.0mg/dL (30).

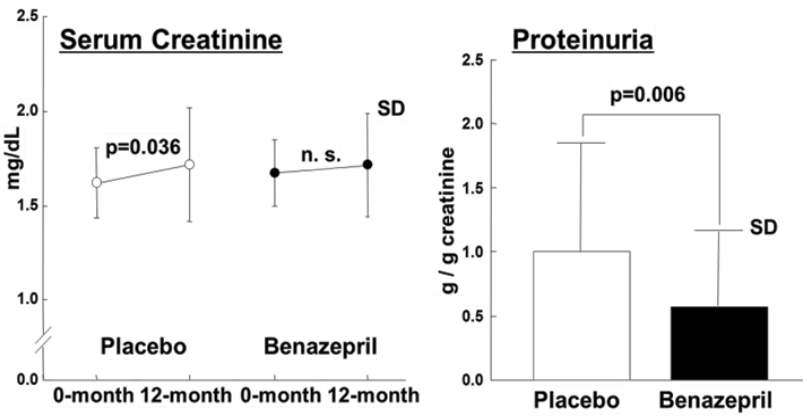

Fig. 7. Changes in serum creatinine concentrations and urinary protein excretions in glomerulonephritis patients given the ACE inhibitor or the placebo (28).

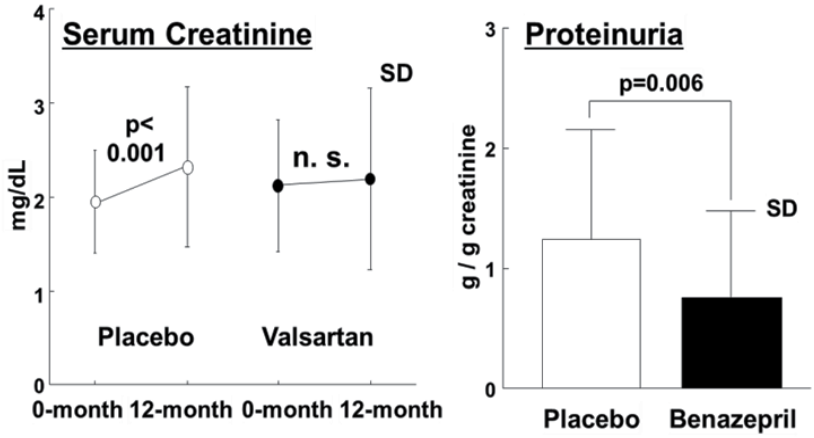

Fig. 8. Changes in serum creatinine concentrations and urinary protein excretions in glomerulonephritis patients given the angiotensin II receptor blocker (ARB) or the placebo (29).

As compared with ACE inhibitors and ARB, clinical evidence of other inhibitors of RAA system, such as renin inhibitors and aldosterone blockers seems less abundant regarding their renoprotecitve effects in patients with glomerulonephritis. In the cascade of RAA system indicated in Figure 6, conversion of angiotensinogen to angiotensin I by the enzymatic action of renin is assumed to be a rate-limiting step. Therefore, renin inhibitors such as aliskiren are thought to be theoretically effective in suppressing the activity of RAA system. Aliskiren, alone or in combination with ARB, has been shown to reduce albuminuria and proteinuria in patients with diabetic nephropathy (31,32), however, its efficacy in patients with glomerulonephritis is to be studied.

ACE inhibitors are widely used in the treatment of hypertension and renal disease. They reduce plasma levels of angiotensin II and aldosterone. However, it has been shown that the plasma aldosterone concentration rather increases in a certain portion of patients after month of long-term administration and this phenomenon is recognized as aldosterone breakthrough. Sato et al. (33) have reported that the long-term ACE inhibitor treatment failed to reduce albuminuria in patients with diabetic nephropathy who had developed aldosterone breakthrough, however, the albuminuria significantly reduced after adding spironolactone, an aldosterone blocker. Although spironolactone can cause adverse effects by its partially estrogenic actions such as gynecomastia and menstrual disorder which sometimes hamper the continuation of administration, eplerenone, a newly developed aldosterone blocker, is much more specific to the mineralocorticoid receptor and almost free from such estrogenic side effects. There is paucity of clinical evidence as to the effects of aldosterone blockers in glomerulonephritis patients, however, the use of an aldosterone blocker in addition to an ACE inhibitor or an ARB would be expected to exhibit protective effects against the progressions of glomerular injuries and renal dysfunction.

5. Calcium channel blockers in antihypertensive drug therapy for patients with glomerulonephritis

Although the guidelines for hypertension management recommend strict blood pressure control in order to prevent organ injuries and cardiovascular diseases, the target blood

pressure is generally achieved only in less than a half of hypertensive patients under treatment. In terms of lowering blood pressure, the hypotensive effect of CCB, directly dilating vascular smooth muscle, is consistently reliable in various conditions including glomerulonephritis patients. Therefore, the addition of CCB to RAA system inhibitors is expected to bring about effective blood pressure reduction with few chances to cause impeding adverse effects.

Ca channels residing in the plasma membrane of cells are composed five subunits; α1, α2, β, γ and δ. Among them, the α1 subunit conforming Ca^{2+} ion pathway has isoforms of L, N, P/Q, R and T. There are three isoforms of α1 subunit, L, N and T in the cardiovascular tissues, and Table 1 shows their distributions, functions and pharmacological blockers. Dihydropyridine (DHP) CCB, which are generally used as hypertensive drugs, blocks the L-type Ca channels existing in the arterial smooth muscle. With regard to the glomerular arterioles, because the afferent but not the efferent arterioles have the L-type channels, DHP CCB generally preferentially dilate the afferent arterioles. Therefore, it is supposed that the reduction in intraglomerular capillary pressure may not be so prominent as compared with the reduction in systemic arterial pressure. In this respect, the N-type and the T-type channels exist both in the afferent and the efferent arterioles and the blockers of these Ca channels are assumed to dilate both glomerular arterioles. This property is expected to contribute to the reduction in intraglomerular capillary pressure. Indeed, the N-type CCB, cilnidipine, and the T-type CCB such as efonidipine and azelnidipine have been shown to reduce proteinuria significantly in patients with glomerulonephritis as compared with L-type CCB (Figure 9), suggesting these CCB are effective in alleviating glomerular hypertension (34-36).

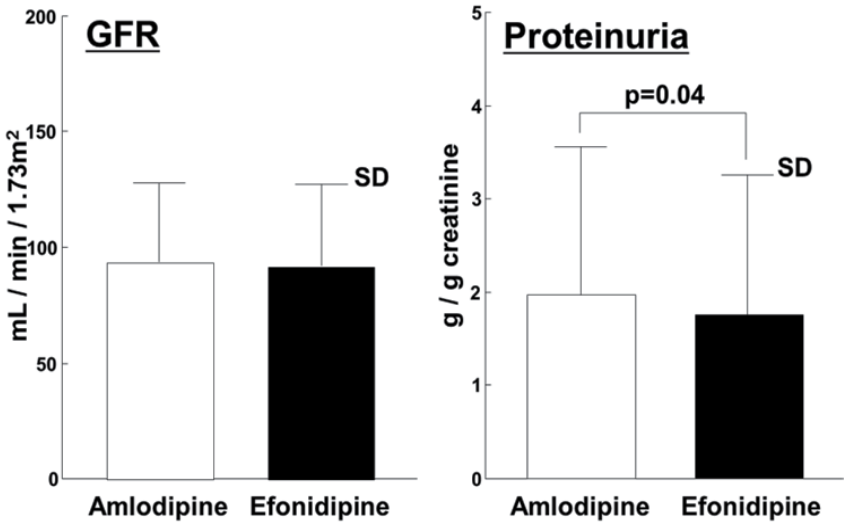

Fig. 9. The glomerular filtration rate (GFR) and the urinary excretions of protein in glomerulonephritis patients given the L-type Ca channel blocker (CCB), amlodipine, or the L- and T-type CCB, efonidipine (35).

As listed in Table 1, T-type Ca channels exist also in the adrenal and the in vitro experiments using cultured adrenal cells have shown that the T-type CCB suppress the expression of

aldosterone synthase gene (CYP11B2) and production of aldosterone (37,38). In harmony with this, clinical studies in healthy subjects and hypertensive patients have shown that the acute or the chronic administrations of T-type CCB lower plasma aldosterone levels (39,40). We have compared the effects of L- and T-type CCB efonidipine and L-type CCB amlodipine in patients with glomerulonephritis and observed that efonidipine reduces plasma aldosterone concentration as compared with amlodipine while the plasma angiotensin II concentrations were comparable (Figure 10)(35). It is mentioned in the previous section of this chapter that aldosterone is supposed to promote the progression of glomerular injuries and the aldosterone blocker can reduce albuminuria. Considering that the mechanism of aldosterone suppression by T-type CCB is different from those by ACE inhibitors, ARB and aldosterone blockers, this property of T-type CCB would be expected to provide an additive benefit, when combined with the RAA system inhibitors, against the progression of renal dysfunction in the antihypertensive treatment of glomerulonephritis patients.

Ca channel	Tissue distribution	Function	Blocker
L-type	vascular smooth muscle intestinal smooth muscle	vasocontraction intestinal contraction	nifedipine nicardipine nitrendipine amlodipine etc.
N-type	brain nerve end	facilitation of signal transmission	cilnidipine
T-type	vascular smooth muscle cardiac muscle adrenal	vasocontraction stimulation of excitement conduction aldosterone secretion	manidipine efonidipine benidipine azelnidipine mibefradil

Table 1. Subtypes of Ca channel and their locations, functions and blockers.

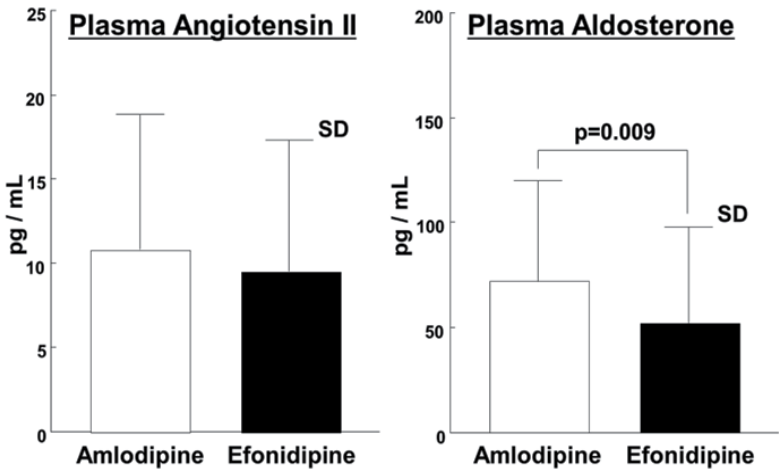

Fig. 10. The plasma concentrations of angiotensin II and aldosterone in glomerulonephritis patients given the L-type Ca channel blocker (CCB), amlodipine, or the L- and T-type CCB, efonidipine (35).

6. Summary and conclusions

Strict blood pressure control over 24 hours is of primary importance in preventing progression of renal injuries and deterioration of renal function in patients with glomerular diseases. In addition, it is important to lower not only systemic blood pressure but also intraglomerular capillary pressure in order to protect glomeruli from sclerosis because the increase in intraglomerular capillary pressure, glomerular hypertension, causes filtration of albuminuria and proteinuria which are dose-dependently related to the progression of renal injuries. Therefore, the antihypertensive therapy in patients with glomerulonephritis should aim not only the normalization of blood pressure but also the reduction of proteinuria and albuminuria. In order to lower intraglomerular capillary pressure, inhibitors of RAA system such as ACE inhibitors and ARB are effective as antihypertensive drugs because angiotensin II greatly contribute to the contraction of the efferent arterioles of glomeruli. In addition, interests are attracted as to the usefulness aldosterone receptor blockers and renin inhibitors as novel agents protecting the kidney. CCB are potent hypotensive agents, however, they rather dilate the afferent arterioles and may not be so effective as RAA system inhibitors in lowering intraglomerular capillary pressure. In this respect, some dihydropyridine CCB which block not only L-type Ca channel but also N- or T-type Ca channel have been shown to dilate efferent arterioles in addition to dilating afferent arterioles and are expected to be beneficial to protect glomeruli as well as lowering blood pressure effectively.

Prognosis of renal function in glomerulonephritis may be largely dependent on the nature of its pathohistological diagnosis and the therapeutic effects of immunosuppressive agents. In addition to these, efforts to lessen and minimize risk factors for renal injuries should be continuously made in order to inhibit the deterioration of renal function. Such efforts would be expected to contribute to inhibit not only the development of renal failure but also the incidence of cardiovascular diseases and to improve the prognosis of glomerulonephritis patients. Among the various risk factors for real injuries hypertension has great influence and the adequate blood pressure control is a pivotally important issue.

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