ANCA-ASSOCIATED VASCULITIS: STUDIES ON CLINICAL PRESENTATION AND FACTORS INVOLVING DISEASE ACTIVITY AND OUTCOME

Anna Salmela

Faculty of Medicine

University of Helsinki

2018

Nephrology
Abdominal Center
Helsinki University Hospital
University of Helsinki
Helsinki, Finland

ANCA-associated vasculitis:

studies on clinical presentation

and factors involving disease activity and outcome

Anna Salmela

ACADEMIC DISSERTATION

To be presented, with the permission of the Medical Faculty of the University of Helsinki,
for public examination in the auditorium Richard Faltin of the Surgical Hospital,
Helsinki University Hospital, Kasarmikatu 11-13,
on December 13th 2018, at 12 noon.

Helsinki 2018

Supervised by

Docent Agneta Ekstrand
University of Helsinki and Helsinki University Hospital, Helsinki, Finland

Reviewed by

Professor emerita Marjatta Leirisalo-Repo
University of Helsinki and Helsinki University Hospital, Helsinki, Finland
and
Docent Satu Mäkelä
University of Tampere and Tampere University Hospital, Tampere, Finland

Discussed with

Docent Laura Pirilä
University of Turku and Turku University Hospital, Turku, Finland

ISBN 978-951-51-4704-2 (paperback)
ISBN 978-951-51-4705-9 (PDF)
http://ethesis.helsinki.fi
Hansaprint Oy
Turenki 2018

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, referred to in the text by their Roman numerals.

I. Salmela A, Törnroth T, Poussa T, Ekstrand A. Prognostic factors for survival and relapse in ANCA-associated vasculitis with renal involvement: a clinical long-term follow-up study. Int J Nephrol 2018; 2018:6369814.

II. Salmela A*, Rasmussen N*, Cohen Tervaert JW, Jayne D, Ekstrand A on behalf of the European Vasculitis Study Group (EUVAS). Chronic nasal Staphylococcus aureus carriage identifies a subset of newly diagnosed granulomatosis with polyangiitis patients with high relapse rate. Rheumatology 2017; 56(6):965-972.
* equal contribution to the manuscript

III. Rasmussen N*, Salmela A*, Ekstrand A, de Groot K, Gregorini G, Cohen Tervaert JW, Gross W, Wiik A, Jayne D on behalf of the European Vasculitis Study Group (EUVAS). Changes in proteinase 3 anti-neutrophil cytoplasm autoantibody levels in early systemic granulomatosis with polyangiitis (Wegener's) may reflect treatment rather than disease activity. Clin Exp Rheumatol 2013; 31(1 Suppl 75):38-44.
* equal contribution to the manuscript

IV. Salmela A, Ekstrand A, Jousi-Korhonen L, Räisänen-Sokolowski A, Lassila R. Activation of endothelium, coagulation and fibrinolysis is enhanced and associates with renal ANCA associated vasculitis. Nephrol Dial Transplant 2015; 30(Suppl 1):53-59.

In addition, this thesis contains unpublished data.

ABBREVIATIONS

AAGN	glomerulonephritis in ANCA-associated vasculitis
AAV	ANCA-associated vasculitis
ACR	American College of Rheumatology
ANCA	anti-neutrophil cytoplasmic antibody
aPLAb	anti-phospholipid antibodies
AZA	azathioprine
BVAS	Birmingham vasculitis activity score
C-ANCA	cytoplasmic ANCA
CHCC	Chapel Hill Consensus Conference
CKD	chronic kidney disease
CNSAC	chronic nasal Staphylococcus aureus carriage
cPR3	complementary to proteinase 3
CRP	C-reactive protein
CV	cardiovascular
CYC	cyclophosphamide
CYCAZAREM	A Randomized Trial of Maintenance Therapy for Vasculitis Associated with Antineutrophil Cytoplasmic Autoantibodies
C5a	complement component 5a
DVT	deep venous thrombosis
EGPA	eosinophilic granulomatosis with polyangiitis
ELISA	enzyme-linked immunosorbent assay
EMA	European Medicines Agency
ENT	ear, nose and throat
ESRD	end-stage renal disease
EUVAS	European Vasculitis Study Group
F1+2	prothrombin fragments
FVIII	factor VIII
FVIIIC	factor VIII activity
GC	glucocorticoid
GFR	glomerular filtration rate
GN	glomerulonephritis
GPA	granulomatosis with polyangiitis
GWAS	genome-wide association study
HR	hazard ratio
IgG	immunoglobulin G
IIF	indirect immunofluorescence
iv	intravenous
LAMP-2	lysosomal membrane associated protein-2
LR	likelihood ratio
MMF	mycophenolate mofetil
MPA	microscopic polyangiitis

MPO	myeloperoxidase
MTX	methotrexate
NET	neutrophil extracellular trap
NORAM	A Randomised Trial of Cyclophosphamide Versus Methotrexate for Induction of Remission in Early Systemic Antineutrophil Cytoplasmic Antibody-Associated Vasculitis
OR	odds ratio
P-ANCA	perinuclear ANCA
PE	pulmonary embolism
PEX	plasma exchange
PR3	proteinase 3
RCT	randomised controlled trial
RLV	renal limited vasculitis
RTX	rituximab
S. aureus	Staphylococcus aureus
SIR	standardised incidence rate
SMR	standardised mortality rate
T/S	trimethoprim/sulfamethoxazole
TSST-1	toxic shock syndrome toxin-1
VTE	venous thromboembolic event
VWF	von Willebrand factor
VWF:Ag	von Willebrand antigen
VWF:RCO	von Willebrand ristocetin cofactor activity

.

ABSTRACT

Aims. The aim of this study was to investigate the factors associated with long-term prognosis and disease activity in patients with anti-neutrophil cytoplasm autoantibodies (ANCA)-associated vasculitis (AAV). An additional aim was to define the coagulation and fibrinolysis profile of renal AAV patients.

Methods. Four cohorts including patients with granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA) and renal-limited AAV were investigated to achieve these aims. Long-term prognosis and relapses were assessed retrospectively in a Finnish cohort of 85 patients with renal biopsy-proven AAV from a single centre (Study I).

The associations between chronic nasal Staphylococcus aureus carriage (CNSAC) and proteinase 3 (PR3) ANCA with relapse were studied in AAV patients who participated in two randomised controlled trials in Europe. To define nasal CNSAC status, monthly nasal swabs were obtained from 200 patients with early systemic or generalised disease during the 18-month trials. The patient was defined as a chronic carrier of Staphylococcus aureus (S. aureus) when ≥ 75% of at least four nasal cultures were positive for S. aureus (Study II).

PR3-ANCA levels, which were examined via nine different enzyme-linked immunosorbent assays (ELISAs), were obtained monthly during the 18-month trial in 28 patients with early systemic GPA. PR3-ANCA peaks were identified by the highest sum of logarithmic transformation values from all assays (Study III).

The coagulation profile was assessed prospectively in 21 Finnish patients with renal AAV in the active versus the remission phase of disease and further compared with that of 40 patients with other renal diseases. The laboratory analysis consisted of platelet count, thrombin time, antithrombin activities, fibrinogen, factor VIII activity (FVIIIC), von Willebrand factor antigen (VWF:Ag) and ristocetin cofactor activity (VWF:RCo), prothrombin fragments (F 1 + 2), D-dimer and antiphospholipid antibodies (Study IV).

Results. The 5-year and 20-year patient survival rates were 88% and 45%, respectively. Older age and presence of myeloperoxidase (MPO) ANCA were significantly associated with worsened survival. The 5-year and 20-year renal survival rates were 79% and 68%, respectively. Renal survival was best in a focal class and worst in the sclerotic class of AAV glomerulonephritis. Female sex was significantly associated with better renal survival, while a glomerular filtration rate < 30 ml/min and MPO-ANCA predicted worse renal survival. Relapse-free survival at 5 years was 47% while at 20 years it was only 10%. Patients with GPA had higher relapse risk compared with MPA patients. (Study I).

The frequency of CNSAC was 12% in the whole cohort. CNSAC was almost exclusively seen in GPA patients. In patients with generalised GPA, the association with CNSAC and relapse was observed. Also, in early systemic GPA, in those patients who were under immunosuppressive treatment, a similar trend for significant association was found (Study II).

A PR3-ANCA peak corresponded to relapse. However, the PR3-ANCA peak could also be identified in non-relapsing patients, and large overlaps in PR3-ANCA values prevented

drawing a distinction between relapsing patients and non-relapsing patients. The alterations of immunosuppression were reflected in PR3-ANCA levels (Study III).

F 1 + 2 and D-dimer were substantially elevated during active disease. During remission, their levels decreased considerably, even though D-dimer level remained above the reference value. FVIIIC, VWF:Ag and VFW:RCo levels were high during active AAV and remained elevated during remission. The load of coagulopathies during remission was comparable to that of patients with other renal diseases involving at least moderate renal impairment. No antiphospholipid antibodies were found. Among AAV patients, two thromboembolic complications were observed (Study IV).

Conclusions. In a long-term follow-up cohort, patient and renal survival were comparable with recent studies showing improved prognosis as compared to earlier reports. Both patient and renal survival were negatively predicted by the presence of MPO-ANCA. The development of end-stage renal disease was more common in men. In the long run, relapses were common, especially in patients with GPA. One special subgroup of individuals who were more prone to relapse among GPA patients were those with CNSAC. PR3-ANCA levels were not only affected by disease activity but also reflected the level of immunosuppressive treatment. Active renal AAV was characterised by enhanced coagulation and fibrinolysis, which failed to normalise completely during remission.

TIIVISTELMÄ

Tausta ja tavoitteet. ANCA-vaskuliitit ovat ryhmä verrattain harvinaisia sairauksia, joita luonnehtivat pienten verisuonten seinämän tulehdus ja kuolio. ANCA-vaskuliitit voidaan jaotella kliinisen ilmiasun perusteella alaryhmiin. Granulomatoottinen polyangiitti (GPA) ja mikroskooppinen polyangiitti (MPA) muodostavat valtaosan tapauksista. Tautivaiheiden vaihtelu, eli jo rauhoittuneen taudin uusiminen, on leimallista näille taudeille. ANCA-vaskuliittipotilailla on suurentunut riski saada veritulppa. Tämän väitöstutkimuksen ensisijaisena tarkoituksena oli selvittää GPA- ja MPA-potilaiden ennusteeseen ja taudin aktiviteettiin vaikuttavia tekijöitä. Tutkimuksessa selvitettiin myös ANCA-vaskuliittipotilaiden veren hyytymisprofiilia suhteessa tautiaktiviteettiin.

Menetelmät. Pitkäaikaisennustetta, eli potilaiden eloonjääntiä, munuaiskorvaushoitoon joutumista ja taudin uusimista, tutkittiin suomalaisessa munuaistautia sairastavassa ANCA-vaskuliittipotilaista koostuvassa aineistossa (N=85) (Tutkimus I).

Kroonista Staphylococcus aureus-nenäkantajuutta (KSANK) ja sen yhteyttä taudin uusiutumis- eli relapsiriskiin tutkittiin ANCA-vaskuliittipotilailla (N=200), jotka olivat osallistuneet kahteen eurooppalaiseen hoitotutkimukseen. KSANK-määritelmä edellytti, että vähintään 75 %:a kuukausittain nenän limakalvoilta otetuista bakteeriviljelyistä oli positiivisia Staphylococcus aureuksen suhteen 18 kuukautta kestävän tutkimuksen aikana (Tutkimus II).

Tautiaktiviteetin ja PR3-ANCA-vasta-ainetasojen yhteyttä tutkittiin niin ikään eurooppalaiseen hoitotutkimukseen osallistuneilla GPA-potilailla (N=28). Käytössä oli yhdeksän entsyymi-immunologista testiä ja PR3-ANCA-vasta-aineille määritettiin testikohtaisesti korkein taso (Tutkimus III).

Veren hyytymisaktiviteettia tutkittiin suomalaisessa aineistossa, joka koostui munuaistautia sairastavista ANCA-vaskuliittipotilaista (N=21) ja muita munuaistauteja sairastavista verrokkipotilaista (N=40). Tutkimuksessa määritettiin verihiutalepitoisuus, plasman trombiiniaika, antitrombiiniaktiviteetti, fibrinogeeni, hyytymistekijä VIII:n aktiviteetti, von Willebrand-tekijän antigeeni ja ristosetiinikofaktorin aktiviteetti, protrombiinifragmentit, D-dimeeri sekä fosfolipidivasta-aineet (Tutkimus IV).

Tulokset. Elossaolo-osuus oli 88 % viiden vuoden kuluttua ja 45 % kahdenkymmenen vuoden jälkeen. Kahdenkymmenen vuoden kuluttua 32 % potilaista oli pysyvästi munuaiskorvaushoidossa. Potilailla, joilla oli diagnoosivaiheessa todettavissa MPO-ANCA-vasta-aineita, oli heikompi ennuste sekä eloonjäännin että munuaistoiminnan suhteen. Miehet päätyivät naisia useammin munuaiskorvaushoitoon. Vain 10 % potilaista vältti taudin uusimisen kahdenkymmenen vuoden seurannassa ja relapsi oli tavallisempi GPA-potilailla (Tutkimus I).

Krooninen Staphylococcus aureus nenäkantajuus todettiin 12 %:lla potilaista ja valtaosa heistä oli GPA-potilaita. Nenäkantajilla oli suurempi relapsi- eli taudin uusimisriski (Tutkimus II).

Relapoivilla potilailla (N=16) PR3-ANCA-vasta-ainetason huippu osui taudin relapsiajankohtaan. Kuitenkin myös niillä potilailla, joilla vaskuliitti pysyi rauhallisena, todettiin

samankaltainen PR3-ANCA-vasta-ainetasojen nousu immunosupressiivisen lääkityksen vähentyessä (Tutkimus III).

Diagnoosivaiheen ANCA-vaskuliittia sairastavilla todettiin protromboottinen tila. Erityisesti trombiinin muodostus ja kohonnut D-dimeeri vallitsivat aktiivisessa taudissa. Taudin rauhoituttua hyytymisalttius väheni, mutta edelleen erityisesti hyytymistekijä VIII aktiviteetti oli koholla. Seuranta-aikana kahdella vaskuliittipotilaalla todettiin veritulppa (Tutkimus IV).

Johtopäätökset. Suomalaisten ANCA-vaskuliittipotilaiden pitkäaikaisseurannassa voitiin todeta viimeaikaisiin kansainvälisiin tuloksiin vertautuva, aiempiin vuosikymmeniin nähden parantunut ennuste sekä kuolleisuuden että munuaistoiminnan suhteen. Potilailla, joilla oli diagnoosivaiheessa todettavissa MPO-ANCA-vasta-aineita, oli heikompi ennuste sekä eloonjäännin että munuaistoiminnan suhteen. Miehet päätyivät naisia useammin munuaiskorvaushoitoon. Taudin uusiminen oli pitkäaikaisseurannassa yleistä erityisesti GPA-potilailla. Kroonisen Staphylococcus aureus-nenäkantajuuden perusteella voitiin tunnistaa GPA-potilaat, jotka olivat alttiita saamaan tautirelapsin. PR3-ANCA-vasta-ainetasot heijastivat tautiaktiviteetin lisäksi myös immunosupressiivisen lääkityksen kuormaa. Aktiivisessa, diagnoosivaiheen ANCA-vaskuliitissa veren hyytymisaktiviteetti oli korostunutta eikä se normaalistunut täydellisesti tautiaktiviteetin rauhoituttua.

INTRODUCTION

Granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA) and eosinophilic granulomatosis with polyangiitis (EGPA) are small-vessel vasculitides characterised by anti-neutrophil cytoplasm autoantibodies (ANCA). Together, they form an entity called ANCA-associated vasculitis (AAV).

Vasculitis refers to an inflammatory process in the blood vessel walls. In AAV, vessels of small or medium size are affected, leading to symptoms and signs of ischaemia or haemorrhage in the target organ. Fundamentally, any organ or even many organs of the body can be affected simultaneously, or the disease may be localised. Rapidly progressive glomerulonephritis (GN), one of the most hazardous conditions in nephrology, is often caused by AAV. Another life-threatening manifestation of AAV is vasculitis of the lung capillaries leading to alveolar haemorrhage. Due to the variable presentation of the disease and its rather low incidence, the diagnosis may be difficult and delayed. Thanks to effective immunosuppressive medication and decreased diagnostic delay, the prognosis of AAV patients has improved dramatically over the last decades. Today, AAV is a chronic disease commonly associated with relapse. Depending on the disease state and diagnostic subgroup, renal manifestation is common, and the need for permanent dialysis is frequent in patients with AAV. End-stage renal disease (ESRD) is not only a tragedy for an individual but also shortens patients' lifespan.

The first purpose of this study was to describe the long-term survival of renal patients with biopsy-proven AAV. The potential of a recently launched classification schema for AAV glomerulonephritis (AAGN) to act as a predictor of survival was validated retrospectively [1].

An additional goal of this study concerned relapse. In cases of relapsing disease, determining the factors related to disease activity is remarkably important in identifying those patients who are in need of extended immunosuppressive medication. Previously, the role of infectious agents, and Staphylococcus aureus (S. aureus) in particular, as disease triggers has been suggested [2-4]. Additionally, ANCAs play an undisputed role in the diagnosis of AAV. However, their role as a marker of disease activity has been a subject of controversy since their recognition. In two randomised controlled trials (RCTs) conducted by the European Vasculitis (EUVAS) Study Group in newly diagnosed AAV patients, the associations between chronic nasal S. aureus carriage (CNSAC) and proteinase 3 (PR3) ANCA levels and relapse were defined.

During the disease course, patients accumulate organ damage due to disease activity and other complications, such as infections and malignancies. An increased risk of thromboembolic events in AAV patients was recognised a decade ago [5, 6]. As a third component of this study, the activation of coagulation and fibrinolysis was addressed in a prospective cohort of newly diagnosed renal AAV patients during the acute and remission phases of the disease.

Patients with EGPA, the rarest form of AAV, were not included in the original publications of this study. That is, this thesis focuses on GPA and MPA, including AAV patients with renal limited vasculitis (RLV).

2 REVIEW OF THE LITERATURE

2.1. Pioneers and historic milestones in vasculitis

Historically, the first description of systemic vasculitis concerned polyarteritis (periarteritis) nodosa and was made by Adolf Kussmaul and Rudolf Robert Maier in 1866 [7]. In 1939, Friedrich Wegener published four cases "about a peculiar rhinogenic granulomatosis with marked involvement of the arterial system and kidneys", describing the classical triad of symptoms that carried his name for over 70 years (Wegener's granulomatosis) [8]. However, as early as 1931, Heinz Klinger reported a similar case, assuming that it was a "borderline variant of periarteritis nodosa" [9]. In 1951, Jacob Churg and Lotte Strauss presented allergic granulomatosis (Churg-Strauss syndrome) [10]. In 1985, Fokko van der Woude et al. discovered ANCAs in the blood of GPA (Wegener's) patients, and the era of ANCA vasculitides was launched [11].

2.2. Classification of AAV

The classification of vasculitides is based on the similar phenotypes observed in groups of patients and the size of the affected vessels. Since 1990, the American College of Rheumatology (ACR) classification criteria have mainly aimed to ensure uniformity of diagnosis for purposes of epidemiological studies [12]. In 1994, the Chapel Hill Consensus Conference (CHCC1994) nomenclature provided definitions for vasculitis based on the size of the affected vessels [13]. In the CHCC1994, MPA was detached from classical polyarteritis nodosa and came to be considered a separate disease. It was also distinguished from Wegener's granulomatosis and Churg-Strauss syndrome by the absence of granuloma. The importance of ANCA serology in diagnosis was noted but not included. The European Medicines Agency (EMA) algorithm (also known as Watts' algorithm), which combines the ACR and CHCC classifications, attempted to further classify patients by using surrogates for vasculitis and granuloma when histological biopsy was not possible [14].

In 2012, the CHCC nomenclature was revised (CHCC2012) [15]. In this version, the major feature was the phasing out of eponyms whenever pathophysiological knowledge allowed to propose descriptive names. Accordingly, Wegener's granulomatosis was renamed GPA, and Churg-Strauss syndrome was renamed EGPA. Together with MPA, they were combined in the form of AAV and placed within the small-vessel vasculitis group. The definitions of AAV subsets provided in CHCC2012 are given in Table 1.

An alternative perspective on diagnosis is the use of ANCA serology to classify patients. Instead of GPA and MPA, it is thus possible to use PR3-ANCA-positive disease or myeloperoxidase (MPO) ANCA-positive disease. A combination of both phenotype and ANCA-specificity was also suggested by CHCC2012 (for example, PR3-ANCA positive GPA) [15]. Indeed, grouping patients by ANCA specificity is increasingly discussed because serology, more so than phenotype, correlates with genetics, epidemiology and prognosis (as discussed later in this study).

To date, however, no diagnostic criteria are available, and the results of a large multinational study are awaited; the ongoing Diagnostic and Classification Criteria in Vasculitis study aims to develop diagnostic criteria and update the classification criteria for vasculitis [16].

Table 1. Definitions of AAV according to CHCC2012.

MPA	Predominantly small-vessel necrotising vasculitis Necrotising glomerulonephritis is very common Pulmonary capillaritis often occurs Granulomatous inflammation is absent
GPA	Granulomatous inflammation usually involving the upper and lower respiratory tract Predominantly small-vessel necrotising vasculitis Necrotising glomerulonephritis is common
EGPA	Eosinophil-rich granulomatous inflammation often involving the respiratory tract Predominantly small-vessel necrotising vasculitis Asthma and eosinophilia

Adapted from Jennette JC, Falk RJ, Bacon PA et al. Arthritis Rheum 2013; 65(1):1-11.

2.3. Epidemiology

From the 1980s to the 1990s, increasing incidences of AAV, particularly of GPA, were reported in several studies [17-19]. This increase in incidence was most likely due to better awareness of the disease by physicians due to the introduction of ANCA testing into clinical practice in the early 1990s [19]. Since the early 2000s, studies have suggested a stable annual incidence of AAV in Europe of 20/million inhabitants [20, 21]. The incidence of GPA is suggested to be higher than that of MPA in Northern Europe while MPA predominates in Southern Europe [20, 22, 23]. However, this North-South gradient is not confirmed in all cohorts [21, 24]. In Finland, the incidence of GPA in the late 1990s was 9.3/million [19].

Both GPA and MPA can occur at any age, but they are most often diagnosed after patients reach the age of 55 years [19, 21]. The incidence peaks after 65 and seems to be higher for MPA than GPA [19, 21]. In adults, both sexes are equally affected by AAV. However, in cases of GPA, the male-to-female ratio is 1.3–1.6, while in MPA, the opposite male-female ratio, 0.5–0.9, is reported [17, 25, 26].

The point prevalences of AAV in the 2000s in Europe are 146–160/million for GPA and 63-94/million for MPA [26, 27]. In Japan and China, on the other hand, MPA- and MPO-associated vasculitis are much more common than GPA [26, 27].

2.4. ANCA detection

ANCAs are autoantibodies directed toward multiple intracellular antigens in neutrophils and monocytes. Such neutrophil-specific autoantibodies (called anti-leukocyte factors at that time) were originally detected in the late 1950s via an indirect immunofluorescence (IIF) technique in patients with leucopenia and various autoimmune diseases [28]. These autoantibodies were first related to vasculitis patients with segmental necrotising GN in the early 1980s [29]. Soon after, antibodies revealing a cytoplasmic fluorescence pattern (referred to as C-ANCA) were detected

via IIF on ethanol-fixed neutrophils in patients with GPA [11]. A few years later, an alternative fluorescence pattern, perinuclear ANCA (P-ANCA), was recognised in vasculitis patients [30, 31].

The major antigen of C-ANCA is PR3, which is a 29-kDa serine protease of myeloid azurophilic granules consisting of 229 amino acids. PR3 can degrade phagocytosed bacteria and other extracellular material and cause tissue damage [32]. The main antigen for P-ANCA in AAV is MPO. Highly cationic MPO, with a molecular weight of 140 kDa, is an enzyme with bactericidal properties in neutrophils and monocytes that acts by catalysing the peroxidation of chloride into hypochlorite [32]. ANCAs (most often P-ANCA or atypical ANCA) may also be detected in patients with a variety of autoimmune, inflammatory and infectious diseases and in cases of drug exposure, which typically involves other antigens, such as lactoferrin, elastase, cathepsin G, bactericidal permeability-increasing protein and others. Thus, P-ANCA is not specific to AAV and must therefore be confirmed by determining the specific antigen for AAV (that is, PR3 or MPO) via an enzyme-linked immunosorbent assay (ELISA). Recently, circulating autoantibodies against MPO and PR3 have been found in asymptomatic individuals and designated as "natural" or non-pathogenic autoantibodies [33]. More than 20 different MPO epitopes bound by MPO-ANCA have been identified, and epitope specificities of healthy individuals were different from those recognised in patients with active disease [34]. Furthermore, it was suggested that a reaction against the natural epitope may become generalised from non-pathogenic to pathogenic epitopes through a process of epitope spreading when the disease develops [34].

Direct ELISA testing was originally performed by direct enzyme-linked assays using purified, solid-phase PR3 and MPO antigens bound to the wells of high-binding microplates, as illustrated in Figure 1. The second-generation assay, so-called capture ELISA, uses secondary antibodies to capture PR3 or MPO on the plate [35] (Figure 1). The capture method reduces the potential covering of epitopes by the plate and consolidates the conformation of epitopes, leading to better recognition of the antigen by the autoantibodies. This allowed for better sensitivity in PR3 detection [36, 37]. In the third-generation assays for ANCA detection, so-called anchor ELISAs or high-sensitivity ELISAs, the antigen is immobilised on the plate by a bridging molecule (Figure 1). This allows preserving the epitopes for antibody binding, resulting in better diagnostic performance as compared to direct ELISAs and some capture ELISAs [38]. However, in other comparisons, the second (capture) and third (high-sensitivity) generation PR3-ANCA assays have performed equally well as conventional direct ELISAs [39, 40]. Most commonly, anchor assays are still used in the detection of PR3-ANCA. Another technology for ANCA ELISA uses recombinant autoantigens instead of human antigens to further improve antibody binding [41]. In addition, several new developments in ANCA-specificity detection are available: fluorescent-enzyme immunoassays, addressable laser bead immunoassays, chemiluminescent immunoassays and dot or line immunoassays [41]. Because the interpretation of conventional IIF is highly subjective and time-consuming, automated systems that can perform computer-aided analysis of IIF images using pattern-recognition algorithms are promising techniques [42].

A consensus statement from 1999 holds that positive IIF results must be confirmed with antigen-specific tests for PR3-ANCA and MPO-ANCA [43]. However, this two-phase procedure has been questioned during the last years. In 2016, a European multicentre study

confirmed that due to the equal or even improved diagnostic performance (measured by the area under the receiver operating characteristics curve) of novel PR3- and MPO-ANCA immunoassays (including first-, second- and third-generation assays) as compared to IIF, double-checking with IIF and antigen-specific immunoassays will no longer be necessary [44]. Finally, based on the European multicentre study [44], the revised international consensus statement on testing ANCAs in GPA and MPA concluded that high-quality immunoassays for PR3- and MPO-ANCA should be used as a primary screening method for patients suspected of having AAV, without categorical testing via IIF [45].

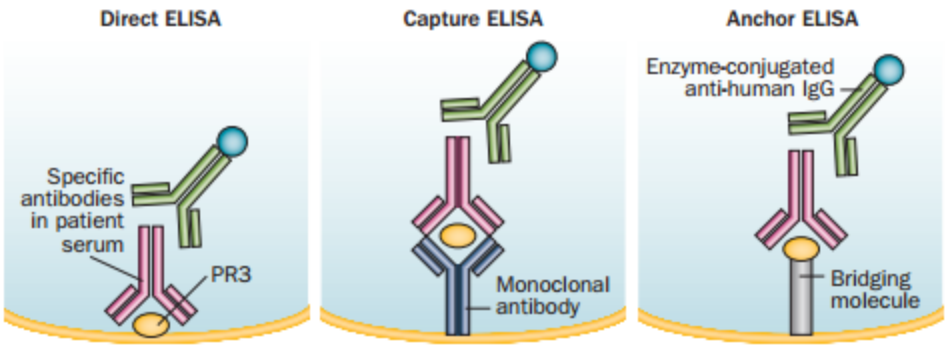

Figure 1. ANCA detection by antigen-specific assays.
(Csernok E and Moosig F. Nat Rev Rheum 2014; 10(8):494-501. Reprinted with permission from the copyright holder.)

2.5. Aetiopathogenesis

The pathogenesis of AAV remains incompletely understood. However, intensive research during the last decades has provided increasing knowledge about this multifactorial process involving genetics and environmental triggers, combined with both the innate and adaptive immune systems. The potential factors that may contribute to the immune mechanisms underlying interrupted self-tolerance and the induction of autoimmunity as well as the anticipated main mechanism leading to vascular damage are discussed below.

2.5.1. Genetic associations

Even though the risk of GPA in first-degree relatives is low, family clusters of GPA and MPA have been described, suggesting a genetic influence on the pathogenesis of AAV [46, 47]. AAV rarely occurs in African Americans. However, the HLA-DRB1*15 allele was over-represented (odds ratio 73) in African American patients with PR3-ANCA-positive disease as compared to community-based controls, and the frequency of this allele was clearly higher than in Caucasians with PR3-ANCA positive disease [48]. Two recently published genome-wide association studies have confirmed genetic associations with AAV and concluded that these are different from those observed with GPA and MPA [49, 50]. GPA was associated with HLA-DP, SERPINA 1 (encoding for serine protease inhibitor alpha 1 antitrypsin) and PRTN3 (encoding

PR3), and MPA was associated with HLA-DQ polymorphisms. Moreover, these genetic associations were stronger for ANCA specificity than for AAV clinical phenotypes [49].

2.5.2. Infections, with a special emphasis on S. aureus

The role of microbial infection as a cause of granulomatous disease in GPA was suggested by Wegener himself in his original article [8]. The most convincing evidence of microbiological associations in the aetiology of AAV is derived from S. aureus. The role of S. aureus as a potential trigger of disease activity in GPA was suggested by several groups during the early 1980s [2, 3]. High CNSAC percentages, up to 60–72%, have been reported in cases of GPA [4, 51, 52], whereas in the normal population, 20–30% of individuals are chronic carriers [53, 54]. In cases of GPA, a seven-fold relapse risk was reported in patients with CNSAC by Stegeman et al. [4]; others have also reported increased risks, but not to this degree [51, 52, 55]. GPA patients have been shown to have epithelial nasal barrier dysfunction due to their altered antimicrobial peptide response and dysregulated cytokine expression, which could facilitate S. aureus colonisation and contribute to disease activity [56, 57].

Staphylococcal super-antigens, such as toxic shock syndrome toxin-1 (TSST-1), are extremely potent T-cell activators that lead to polyclonal T-cell proliferation and a massive release of cytokines [58]. Indeed, TSST-1-positive S. aureus was especially implicated in an increased risk of relapse among GPA patients, suggesting that staphylococcal super-antigens may mediate S. aureus pathogenicity [59]. However, recent data from studies on humoral immune responses and genetic studies of S. aureus in GPA patients do not support the pathogenic role of TSST-1 or other staphylococcal super-antigens in GPA [60, 61]. Surprisingly, sera from GPA patients contained lower anti-staphylococcal IgG levels than sera from healthy controls, even though the total immunoglobulin G (IgG) levels were not different [60]. In the same study, no particular S. aureus genotype in nasal isolates taken from GPA patients was found to be different from that of healthy controls, suggesting that GPA patients may have poorer humoral immune protection against S. aureus than healthy persons. Low levels of anti-staphylococcal antibodies are also found in MPO-ANCA patients; these levels are comparable to those seen in PR3-ANCA patients [61]. As shown via a DNA microarray-based approach, several genetic loci of S. aureus are associated with either MPO- or PR3-ANCA AAV; a gene encoding leucocidin, a pore-forming toxin, was detected in S. aureus nasal isolates taken from PR3-ANCA patients, implying the potential role of this virulence factor in the survival of S. aureus in the nasal mucosa [61].

The theory of molecular mimicry has been postulated to interpret the relationship between S. aureus and PR3-ANCA formation: the genetic sequences of antisense regions complementary to PR3 (cPR3) are found in S. aureus, implying that cPR3 could serve as antigenic target for PR3-ANCA formation through anti-idiotypic antibodies in GPA [62, 63]. Nevertheless, anti-cPR3-reactivity was not increased in PR3-ANCA-associated vasculitis patients [64]. The theory of molecular mimicry is implicated in another context related to the infection-associated background of AAV as well. Kain et al. found antibodies against lysosomal membrane-associated protein-2 (LAMP-2) in individuals with active pauci-immune focal necrotic GN [65]. LAMP-2 shares sequence homology with FimH (a gram-negative bacterial adhesion molecule), and LAMP-2-ab is capable of cross-reacting with FimH [65]. Furthermore,

antibodies to human LAMP-2 can induce focal necrotic GN when injected into rats. However, these observations could not be reproduced by another group [66].

2.5.3. Environmental factors and drugs

Environmental exposures have also been incriminated as triggers of vasculitis: silica, as a risk determinant, is linked to AAV in several case-control studies [67-69]. A recent meta-analysis found an odds ratio (OR) of 2.56 for the association between silica exposure and the risk of developing AAV [70]. High levels of silica dust released into air during the 1995 earthquake in Kobe, Japan, and the subsequent increase of MPO-ANCA-associated vasculitis suggest a causal relationship [71].

Farming at the year of diagnosis was associated to GPA (OR 2.7), and this risk was particularly associated with exposure to livestock [69]. However, a case-control study of GPA from Sweden found no occupational risk associations [72]. In addition, various drugs, such as propylthiouracil, hydralazine, minocycline, sulfasalazine, penicillamine, allopurinol and rifampicin, are associated with AAV [73, 74]. These drugs may induce ANCA seroconversion, mainly against MPO but also against other antigens (elastase, lactoferrin). In most cases, no treatment other than discontinuing the drug is needed to cure the vasculitis.

2.5.4. The anticipated main mechanism of ANCA-induced neutrophil activation and endothelial damage

Neutrophils are believed to be the key players orchestrating the multiple steps leading to tissue damage in AAV. Neutrophil activation by ANCA seems to be the primary event. *In vitro* studies have shown that MPO and PR3-ANCA are able to activate neutrophils that have been pre-activated through a process called priming by pro-inflammatory stimuli, such as tumour necrosis factor-α, bacterial lipopolysaccharide, interleukin 18 or complement component 5a (C5a) [75-78]. In resting neutrophils, PR3 and MPO are located in the lysosomal compartment and thus inaccessible to antibody binding. However, priming causes the translocation of ANCA antigens to the cell surface and leads to a microenvironment in which interactions with ANCAs may take place [79]. The engagement of the Fc receptor on neutrophils with ANCA-antigen immunocomplexes and the binding of the $F(ab')_2$ of ANCA to the antigens expressed on the neutrophil surface complete neutrophil activation [79-82]. These ANCA-activated neutrophils adhere and transmigrate through the vessel wall and undergo respiratory burst with the release of reactive oxygen species and lytic enzymes, causing apoptosis, NETosis (see later) and the necrosis of the neutrophils and adjacent vessel wall [75, 83, 84]. Ultimately, inflammatory endothelial cell damage as a result of endothelium-neutrophil interaction leads to a loss of anti-thrombogenic activity of endothelium, resulting in haemorrhage and the release of plasma proteins, including coagulation factors and tissue factor, leading to fibrin formation [79, 85].

Increasing evidence suggests that an alternative complement pathway plays an important role in the pathogenesis of AAV. The generation of C5a, which is a potent chemoattractant for neutrophils and capable of priming them, was also found in the supernatants of ANCA-activated neutrophils [78]. Thus, the interaction between C5a and the C5a receptor can result in a pro-inflammatory amplification loop. In a mouse model, researchers found that

the ablation of complement component C5 and the depletion of complement component C3 prevented disease after the transfer of anti-MPO IgG [86]. Later, observations of patients have supported the role of the activation of the alternative complement pathway in the pathogenesis of AAV [87, 88]. Furthermore, the pharmacological blockade of the C5a receptor by oral C5a receptor antagonist protected transgenic mice from AAV [89].

Yet another novelty suggested in the pathogenesis of AAV is NETosis, as mentioned above. As a defensive strategy against pathogens, activated neutrophils have been shown to release webs of decondensed fibres of chromatin called neutrophil extracellular traps (NETs) into the extracellular space [90]. In the kidneys of AAV patients, NETs containing PR3 and MPO have been found, indicating that NETosis could be one of the mechanisms via which these autoantigens are presented to the immune system [91]. This simplified sequence of pathogenic events in vascular inflammation during AAV is illustrated in Figure 2.

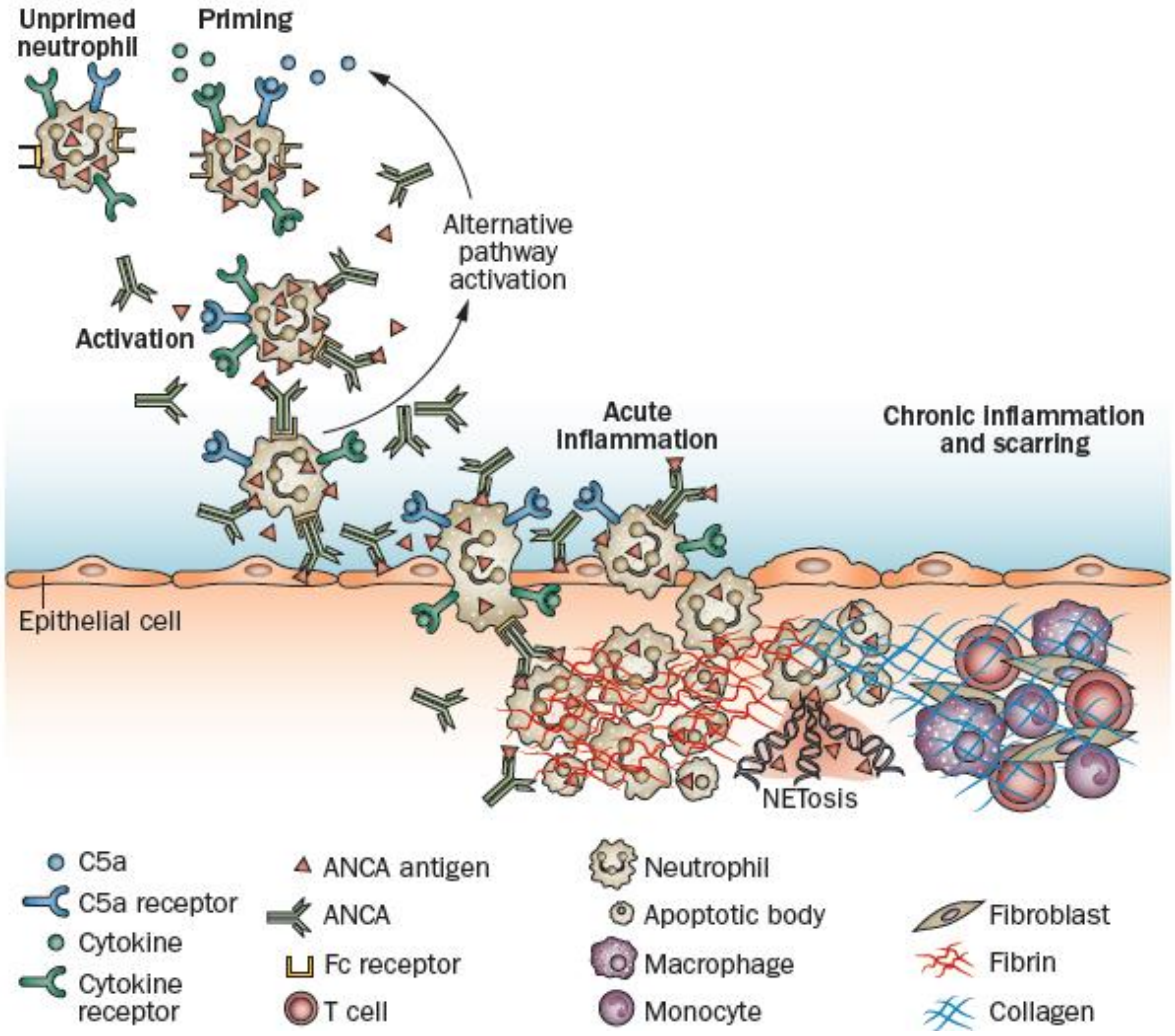

Figure 2. Supposed sequence of pathogenetic events in AAV.
(Jennette JC and Falk RJ. Nat Rev Rheumatol 2014; 10(8):463-7. Reprinted with permission from the copyright holder.)

Excessive NETosis also seems contribute to thrombosis in AAV. In a case study, NETs were identified in a thrombus and glomerular crescents from a MPA patient. In this patient, the number of NETs in this thrombus was also higher compared to those in other thrombi unrelated to MPA [92]. Furthermore, in active AAV, neutrophils activated by inflammatory mediators and ANCA induced the release of tissue-factor-expressing NETs and microparticles [93]. *In vitro*, neutrophil-derived microparticles can promote the generation of thrombin [94]. This was also shown *in vivo* in children with active vasculitis complicated by venous thromboembolic events (VTE) [95].

2.6. Clinical characteristics and diagnosis

2.6.1 Symptoms and findings

Suspicion of AAV is based on a combination of symptoms and clinical findings, in addition to abnormal results on blood and urinary tests. A typical patient presents with general symptoms such as fever, weight loss and arthralgia, which may have continued for months. Non-specific haematological signs of acute inflammation, such as elevated C-reactive protein (CRP), hypersedimentation, anaemia and thrombocytosis, are commonly observed in the acute phase of generalised AAV.

Upper respiratory tract disease is particularly common in GPA, including the involvement of vessels supplying the cartilage of the septum, which may lead to septal perforation and saddle nose deformation. In MPA, ear, nose and throat (ENT) involvement may occur, but without granuloma and destruction. The inflammation of the trachea, often in the subglottic region, is also typical of GPA.

Lung involvement is common in AAV. The symptoms may vary from cough to haemoptysis, and radiograph findings may reveal nodules, pulmonary infiltrates and hilar adenopathy.

Renal involvement is frequent in GPA and MPA. In urine, unspecific findings, such as microscopic haematuria and proteinuria, which is usually non-nephrotic (i.e. <3 g/day), can be detected in case of active renal AAV. Renal insufficiency, and even rapidly progressive GN, is commonly seen in AAV.

The clinical picture of AAV varies, and the most common organ-specific presentations are shown in the Table 2, which is organised according to the Birmingham vasculitis activity score (BVAS) [96]. BVAS summarises AAV symptoms and findings into nine different organ systems, giving points for each item attributable to active vasculitis (maximum total score of 63). BVAS serves as a valuable aid when evaluating disease activity in AAV patients, although some training is needed to apply this instrument correctly.

Occasionally, AAV is limited to one organ group: RLV is often regarded as a form of MPA. GPA, in the diagnostic phase, may be limited to the ENT, although the disease tends to generalise later.

2.6.2. Diagnosis

A definite diagnosis is based on a typical histologic finding obtained from a biopsy of the affected organ. The biopsy of purpuric skin lesions reveals leucocytoclastic vasculitis, although this is a non-specific finding. Nasal biopsies are rarely diagnostic, whereas open lung biopsies have a high sensitivity, but because this is an invasive procedure, its use is limited in clinical practice. Renal biopsy is the main diagnostic approach in nephrology, as well as the most important tool for establishing a diagnosis in patients who have signs of renal involvement. However, a biopsy is not always possible or safe (for example, in a patient with only one functioning kidney), in which case diagnosis must be based on the typical findings, ANCA-positivity and the exclusion of other aetiologies.

The diagnosis is supported by positive ANCA testing. MPA patients are predominantly P and/or MPO-ANCA-positive (61.4–90.4%), whereas GPA patients are often C and/or PR3-ANCA-positive (73.9–78.5%) [97, 98]. However, even with modern ANCA assays, up to 16% of MPA and GPA patients proved ANCA-negative at diagnosis [44]. ANCA-negative patients often have limited GPA.

Table 2. Major clinical characteristics and their occurrence in microscopic polyangiitis and granulomatosis with polyangiitis.

Clinical manifestations	MPA	GPA
Constitutional symptoms fever, malaise, myalgia, arthralgia, weight loss	+++	+++
Ear, nose and throat involvement purulent/bloody nasal discharge, crusting rhinitis, sinusitis, otitis media, subglottic stenosis	+	+++
Lung involvement hoarseness, haemoptysis/alveolar haemorrhage, pneumonia, pleural effusion, nodules	++	+++
Renal involvement asymptomatic haematuria, sub-nephrotic proteinuria, renal insufficiency	+++	+++
Skin involvement purpura, ulceration, livedo reticularis	++	++
Eye involvement conjunctivitis, corneal ulceration, episcleritis, optic neuropathy	+	++
Neurological involvement mononeuritis multiplex, sensory peripheral neuropathy, stroke, cranial nerve palsy	++	++
Cardiac involvement pericarditis, cardiomyopathy, ischemia, congestive heart failure	+	+
Gastrointestinal involvement ischaemia, bloody diarrhoea, peritonitis	+	+

MPA = microscopic polyangiitis; GPA = granulomatosis with polyangiitis; + = rare (occurrence <15% of patients); ++ = common (in 15–50%); +++ = frequent; (in >50%)

Adapted from Mahr A, Katsahian S, Varet H et al. Ann Rheum Dis 2013; 72(6):1003-1010 (including 277 MPA and 396 GPA patients) and Solans-Laqué R, Fraile G, Rodriguez-Carballeira M et al. Medicine (Baltimore) 2017; 96:e6083 (including 167 MPA and 184 GPA patients).

2.6.3. Renal histology

Fibrinoid necrosis in the vascular wall is a hallmark of vasculitis. The presence of fibrinoid necrosis and/or extracapillary proliferation upon light microscopy, combined with the absence (or weak staining) of immune deposits upon immunofluorescence microscopy, is called pauci-immune necrotising glomerulonephritis, which enables the histopathological diagnosis of renal AAV. Glomerular extracapillary proliferation refers to cell proliferation into the urinary space of Bowman's capsule, giving the appearance of a crescent. Although a crescentic lesion *per se* is nonspecific and observed in a few other types of GN, pauci-immune GN is the most common cause of crescentic GN [99]. A cellular crescent is a sign of active disease whereas a fibrocellular crescent refers to chronicity, and both can be found in the same biopsy. The typical findings of renal histology upon light microscopy are illustrated in Figure 3.

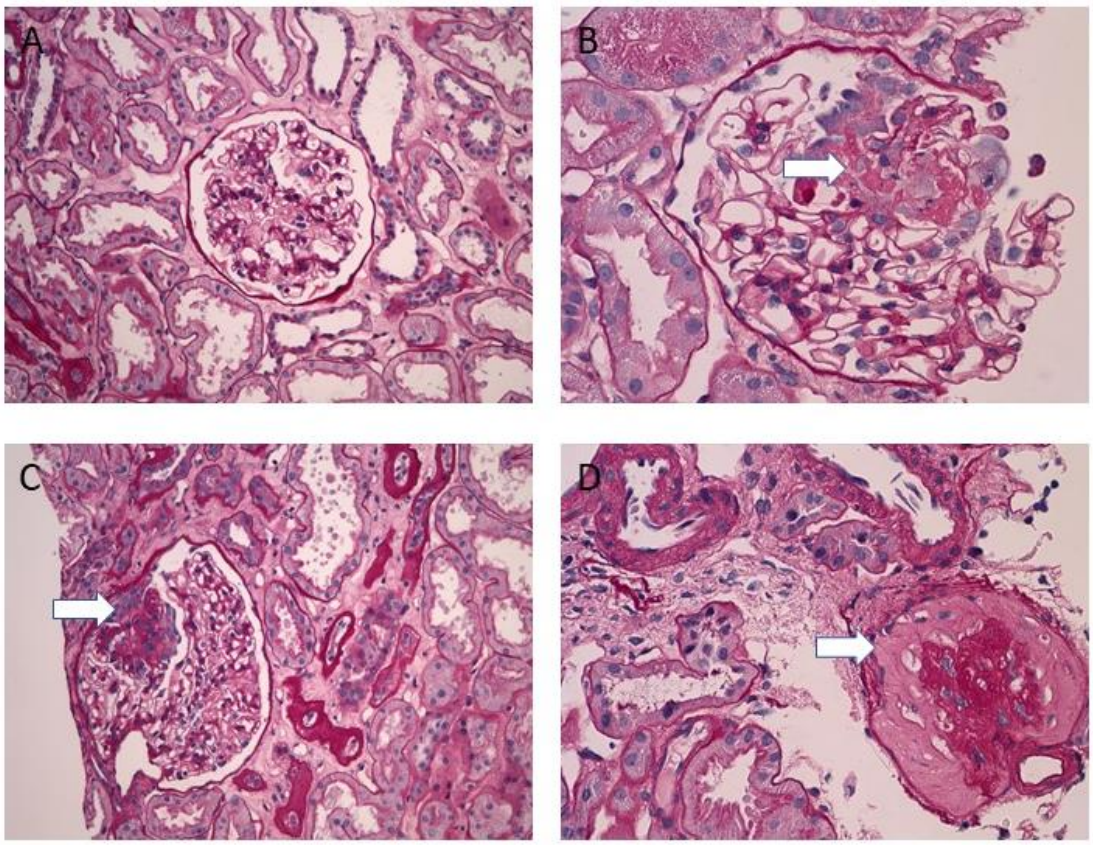

Figure 3. A normal glomerulus surrounded by normal interstitial tissue (A). A cellular crescent with fibrinoid necrosis (arrow) (B). A fibrocellular crescent (arrow) with fibrosis and some cellularity left (C). A globally sclerotic glomerulus (D).

Vasculitis of the arteries and arterioles is quite seldom seen (~25%) in renal biopsies of AAV patients. Renal granulomas are rare and present in 5% of patients with GPA [100]. Patients with MPA and especially those positive for MPO-ANCA often have advanced injury in renal histology (glomerulosclerosis, interstitial fibrosis, tubular atrophy and arteriosclerosis) as compared to GPA or PR3-ANCA-positive patients [101].

In 2010, a histopathological classification schema for AAGN was introduced [1]. This classification schema is based on four classes–focal, crescentic, sclerotic and mixed–depending on the predominance of the affected glomeruli upon light microscopy, as shown in Table 3.

Table 3. Classification of AAGN.

Class	Distribution of glomeruli
Focal	≥ 50% normal glomeruli
Crescentic	≥ 50% glomerular with cellular crescents
Sclerotic	≥ 50% globally sclerotic glomeruli
Mixed	< 50% normal, < 50% crescentic, <50% globally sclerotic glomeruli

Adapted from Berden AE, Ferrario F, Hagen EC et al. J Am Soc Nephorol 2010; 21(10):1628-1636.

2.7. Treatment of AAV

To control disease activity and avoid organ damage, treatment should be begun promptly after diagnosis. Occasionally, a patient's condition worsens within a few days (for example, in the case of rapidly progressive GN or pulmonary haemorrhage), and treatment cannot be delayed until a definite diagnosis based on a biopsy of the affected organ is available. According to criteria used in EUVAS trials, the disease state is classified as localised (no systemic features outside ENT or lung, no constitutional symptoms), early systemic (absence of disease features that would threaten the organ's function, constitutional symptoms present), generalised (systemic disease, threatened vital organ function) or severe (organ failure present or the patient's life threatened) [102]. However, disease state can change over time, and patients with early systematic disease may develop severe manifestations, in which case they should be treated accordingly [103]. The treatment of AAV is comprised of two phases: induction treatment to achieve remission and, when remission is achieved, maintenance treatment. In addition, relapses must be treated.

2.7.1. Induction of remission

For over 40 years, the induction treatment of severe AAV has been based on glucocorticoids (GC) and cyclophosphamide (CYC) [104]. This combination of oral CYC and GC induces remission in 90% of patients within 3–6 months [105]. GC can be started with one to three intravenous (iv) pulses of methylprednisolone in cases of generalised disease, followed by oral predniso(lo)ne. Alongside GC, CYC can be administrated orally or iv with a similar remission induction success rate [106]. The iv pulse strategy, as compared to the oral, cuts down the cumulative dose of CYC, thus reducing the drawbacks associated with the medication (cytopenia, infection and infertility). Although this approach achieves more relapses, there is no difference in survival or kidney function in the long run [107]. To avoid the occurrence of uroepithelial toxicities of CYC, 2-mercaptoethanesulfonate sodium, either iv or orally, is recommended for patients receiving CYC [108]. A daily dose of between 7.5 and 10 mg of prednisolone (or equivalent) after 3 months was recently recommended [108].

As an induction medication, rituximab (RTX) has been shown to be equally effective as CYC in two RCTs (the RAVE and RITUXVAS trials) [109, 110]. Notably, patients with severe kidney disease were scarce in these studies. In the RAVE trial, patients with creatinine ≥ 4 mg/dl (corresponding to ~350 µmol/l) were excluded, whereas in the RITUXVAS trial, patients with low glomerular filtration rates (GFR) were allowed, while there were fewer than ten patients on dialysis [109, 110]. In a small retrospective study of AAV patients with severe renal disease (median GFR 13 ml/min), however, no differences in remission, renal recovery, ESRD or death were observed in RTX- and GC-treated patients with or without concomitant CYC [111]. In sub-analyses of the RAVE trial, RTX was more effective than CYC in patients experiencing relapse and also in PR3-ANCA-positive patients [109, 112]. Thus, RTX can be recommended for relapsing patients, those with plans for pregnancy and those who have already received large cumulative doses of CYC.

Prophylactic doses of trimethoprim/sulfamethoxazole (T/S) against Pneumocystis jirovecii pneumonia is recommended in patients treated with CYC, as well as those receiving RTX [108].

The addition of plasma exchange (PEX) is recommended for patients with rapidly deteriorating renal function and advanced kidney failure (creatinine > 500 µmol/l), as well as those suffering from pulmonary haemorrhage [108]. Thus far, the evidence regarding PEX is restricted to the MEPEX trial, which showed that in comparison to iv GC pulses, the risk of progression to ESRD at 12 months was lower with PEX [113]. The multinational PEXIVAS trial included 700 GPA and MPA patients with renal (GFR < 50ml/min) or severe pulmonary disease [114]. Patients were randomised to receive PEX or no PEX, and to receive standard or reduced oral GC dosing. All patients received immunosuppression with either CYC or RTX. The preliminary results of the PEXIVAS trial concluded, however, that all-cause mortality and the risk of ESRD were not lower in patients treated with PEX (a lecture by Michael Walsh on 25th of May 2018 at the 55th Meeting of the European Renal Association – European Dialysis and Transplant Association). In addition, the preliminary results of PEXIVAS concluded that the reduced dose of GC (i.e. < 60% of the cumulative oral dose of a standard regimen by six months) was non-inferior to a standard dose and resulted in fewer serious infections.

To avoid CYC toxicity in cases of non-severe AAV, alternative treatments have been tested. In the NORAM trial (A Randomised Trial of Cyclophosphamide Versus Methotrexate for Induction of Remission in Early Systemic Antineutrophil Cytoplasmic Antibody-Associated Vasculitis), methotrexate (MTX) was compared with oral CYC and found to be equally successful in inducing remission among patients with early systematic disease, although this came at the expense of a higher early relapse rate with MTX [115]. In the follow-up of the NORAM trial, which lasted a median of 6 years, patients in the MTX group received immunosuppressive medications for longer periods of time than those in the CYC group, with no difference in the occurrence of major adverse events (ESRD, cancer or serious infections), but again, there was a tendency toward shorter relapse-free survival with MTX [116]. According to evidence from the two small RCTs comparing mycophenolate mofetil (MMF) with CYC mainly in terms of their effects on MPA patients, MMF and GC can be used as an induction treatment for patients with non-severe AAV [117, 118].

2.7.2. Maintenance of remission

Remission is often defined by the absence of vasculitis disease activity, which in renal AAV is determined as the absence of microscopic haematuria and stable or improved proteinuria and GFR. Remission treatment with a lower-level immunosuppression, often including GC, is used to avoid relapses. However, in dialysis-dependent patients with no extrarenal disease activity, immunosuppression should be terminated after 4 months [119].

Azathioprine (AZA) has been shown to be equally effective as oral CYC in remission maintenance after oral CYC-based induction, as shown in the CYCAZAREM trial (A Randomised Trial of Maintenance Therapy for Vasculitis Associated with Antineutrophil Cytoplasmic Autoantibodies) [105]. More recently, in the MAINRITSAN trial, RTX was found to prevent major relapses better than AZA after CYC-induced remission during a 2-year follow-up [120]. Furthermore, in the 60-month follow-up of the MAINTRISAN trial, the rate of sustained remission remained superior on RTX-based maintenance regimen [121]. MTX performed as well as AZA in terms of maintaining remission and may be an alternative to AZA in patients intolerant of this drug who have mild or no renal disease [122].

Leflunomide could be a more effective alternative to MTX in terms of remission maintenance, as shown in German GPA patients; however, more adverse events occurred in the leflunomide group [123]. Recently, the results of an RCT performed with AAV patients and comparing MTX to oral CYC after induction treatment with oral CYC and GC were published: relapse rates after 12 months (when both medications were stopped) and 24 months did not differ between the treatment arms [124].

In terms of remission maintenance, MMF was inferior to AZA according to the IMPROVE trial [125]. In the placebo-controlled WEGT trial, etanercept, when given in addition to standard therapy based on CYC and GC for remission induction and maintenance to GPA patients, increased the occurrence of solid malignancies [126].

T/S, when used as an adjunctive therapy alongside of remission treatment, significantly reduced ENT relapses in GPA patients [127].

To date, the optimal duration of maintenance treatment remains an open question; suggestions vary been 12 and 24 months after remission, although the length of five years has been suggested for GPA patients and those who remain PR3-ANCA-positive [108, 128, 129]. On the other hand, for MPO-ANCA-positive patients, who are less likely to relapse than PR3-ANCA-positive patients, maintenance treatment with only low-dose GC for 12 months is proposed [130].

In a meta-analysis, longer courses of low-dose glucocorticoids for longer than 12 months were associated with fewer relapses [131]. Relapse-free survival was not better in PR3-ANCA-positive patients given extended AZA for up to 4 years in comparison to those who received AZA for one year [132]. Furthermore, the data from six EUVAS studies with 380 patients comparing the length of AZA remission treatment indicate that 5-year relapse-free survival was not significantly increased in those who stopped receiving AZA at 18 months [133].

2.7.3. Treatment of relapses and refractory disease

Severe relapses should be treated according to the same protocol as used in the induction treatment, whereas minor relapses should be treated with the intensification or modification of the immunosuppressive medication provided during the maintenance regimen [108]. For patients with refractory disease activity, switching the induction medication from CYC to RTX or vice versa is recommended [108].

Deoxyspergualin in combination with GC has shown effects in GPA patients with refractory or relapsing disease; however, adverse events were also common [134]. Leflunomide or iv immunoglobulins can be used as an alternative for refractory disease [123, 135].

2.8. ANCA and disease activity

The diagnostic value of ANCA testing, as discussed above, is unquestioned, whereas the clinical utility of serial ANCA testing has been debated since its recognition. A meta-analysis of 15 studies and 828 AAV patients concluded that serial ANCA testing during remission has limited value in predicting AAV relapses [136]. In that analysis, pooled positive likelihood ratio (LR) of a rise in ANCA predicting a subsequent relapse was 2.84, and positive LR was even lower (1.97) if ANCA was persistently positive. In addition, the absence of an ANCA increase could not exclude the possibility of relapse because the negative LR of an ANCA increase in predicting a later relapse was 0.49. In the subgroup analysis, an increase in P/MPO-ANCA (i.e. P-ANCA or MPO-ANCA) was better able to predict future relapse, with positive LR of 10.0 vs 1.4 for C/PR3-ANCA (i.e. C-ANCA or PR3-ANCA) (p=0.01). However, substantial between-study heterogeneity was found, including various types of ANCA tests, frequencies of ANCA measurement and definitions of ANCA increase, making it difficult to draw clear conclusions [136]. Relapse-free survival was significantly longer for patients who remained PR3-ANCA-negative as compared to PR3-ANCA-positive patients [hazard ratio (HR) of 0.60] [137]. PR3-ANCA reappearance may also correlate more closely with relapse than persistently positive PR3 -ANCA [137, 138].

Different disease phenotypes seem to behave differently with regards to disease activity and its relationship with ANCA levels. GPA patients with active, limited disease are more often ANCA-negative than patients with severe disease [44, 139]. In a large AAV cohort (N=166) from the Netherlands, in patients with renal involvement, ANCA increases (measured with ELISA ANCA methods) were better correlated with relapses than in patients who presented with non-renal AAV (HR) of 11.09 vs 2.79, respectively) [140]. This finding was confirmed in a PR3-ANCA-positive cohort (N=131) from the RAVE trial, in which ANCA titre increased when analysed with direct ELISA (but not with capture ELISA), suggesting an increased risk of severe relapse, particularly in patients with renal involvement (HR 7.94) and alveolar haemorrhage (HR 24.19) [141].

The ANCA response to different treatments may also be different. In the RAVE trial, PR3-ANCA-negativity was more likely after RTX than after CYC treatment (47% vs 24%, respectively) despite the similar clinical efficacy of these treatment arms [109]. Furthermore, a follow-up of the same study cohort revealed that increased PR3-ANCA titre was associated with an increased risk of relapse in RTX-treated but not in CYC/AZA-treated patients [141].

Quite recently, the results from the MAINRITSAN2 trial were published [142]. In that trial, the efficacy of two rituximab regimens in terms of maintaining remission did not differ significantly: rituximab redoses were administrated either pre-emptively, as determined by ANCA status or the reappearance of CD19 lymphocytes, or systematically every 6 months.

2.9. Outcomes of AAV

2.9.1. Patient outcomes

If left untreated, patient life expectancy after a diagnosis of AAV is dismal; historical data from the era before GC and CYC treatment reported a one-year mortality of 80% for GPA [143]. Combined treatment with GC and CYC improved the prognosis of GPA patients dramatically [104]. The mortality of AAV patients has been declining continuously during the last decades [144-146]. The reported 1- and 5-year survival rates based on the surveys of both GPA and MPA patients have been 77–91% and 66–78%, respectively, although even better 5-year survival rates of up to 89–93% were found in patients diagnosed after 2000 [21, 145, 147]. In renal patients, however, 5-year patient survival is 72–84%, even in cohorts diagnosed after 2000 [118, 146]. In the recent literature, long-term survival at 10 years has varied between 49 and 81% [145, 148-150], and survival is better in patients without renal involvement than in those with renal disease (HR 0.55) [148]. The improved prognosis is mainly attributed to shorter diagnostic delay and therapeutic improvements, especially regarding the lower cumulative dose of CYC [144].

Recently, a meta-analysis of mortality in AAV patients indicated a 2.7-fold increase in mortality as compared with the general population [151]. No difference in mortality risk between the sexes was found. Studies with only GPA patients indicated a similar risk as studies with mixed diagnoses of GPA, MPA and EGPA. In addition, a trend toward improved survival over time was found [151]. In a study from Norway conducted on patients with AAGN (diagnosed 1981–2012), the standardised mortality ratio (SMR) was 2.8; however, in those patients who survived the first year and did not develop ESRD, the age- and gender-adjusted SMR was equivalent to that of the general population [152].

The most frequently reported negative factor regarding patient survival is advanced age [21, 145, 147]. In addition, higher mortality has frequently been associated with decreased kidney function at baseline [21, 145, 147]. In contrast, ENT involvement has been associated with better patient survival [153]. In a cluster analysis of 673 patients with GPA or MPA, patients with renal disease without PR3-ANCA (HR 5.87), patients with cardiovascular AAV (HR 6.41) and those with AAV with gastrointestinal involvement (HR 6.74) had a higher risk of death than non-renal AAV patients [154]. In patients on chronic dialysis, the 5-year survival rate for AAV was only 53%; however, this was comparable to that of non-AAV controls [155].

In the long-term EUVAS study, the main causes of death within one year after diagnosis were active vasculitis (19%) and treatment-associated infections (48%), whereas deaths occurring later were mainly attributed to cardiovascular disease (26%), malignancy (22%) and infection (20%) [147].

2.9.2. Renal outcomes

In recent studies on AAV patients with renal involvement, the 5- and 10-year renal survival rates, i.e., survival without ESRD, were 66–77% and 60%, respectively [149, 156, 157]. According to the histopathological classification schema for renal AAV, its subclasses are as follows: focal, crescentic, mixed and sclerotic AAGN. In the original article, the predictive value of this classification was validated in 100 patients, showing an increased risk of ESRD in those with a sclerotic histology [1]. Later, several studies validated the AAGN histology classification [149, 156-165]. The results of these studies are shown in Table 3. Overall, renal survival was best in patients with focal AAGN, whereas ESRD occurred most often in those with sclerotic AAGN. Mixed and crescentic AAGN typically had intermediate outcomes. As anticipated, baseline renal function was the most frequently reported independent prognostic factor for ESRD [1, 149, 158, 160, 162-164]. Histology class was also found to have an independent prognostic effect on renal survival in several studies [158, 160-162]. In three validation studies, patients with MPO-ANCA were shown to have an increased likelihood of chronic injuries in histology [158, 163, 165].

2.9.3. Relapse

AAV typically presents with fluctuating disease activity, and relapses are common. The different definitions of relapse, disease stage, the therapies given, and study settings affect the relapse rate. The recommended definition of relapse is the re-occurrence or new onset of disease activity attributable to active inflammation caused by AAV [102]. Relapse rates of 34–49% within 5 years of follow-up have been reported for AAV patients [98, 146, 166-169]. Recently, in the long-term follow-up data for the 10 years after the WEGENT trial, only 30% of patients remained relapse-free [150].

Several studies have reported that patients with C/PR3-ANCA positivity at baseline are more prone to relapse [4, 98, 145, 150, 166-169]. Furthermore, C/PR3-ANCA-positivity at the time of the switch from induction to remission maintenance therapy is associated with a higher relapse rate [170]. In a recent study, however, positive C-ANCA status at stable remission was not associated with an increased relapse risk [132]. According to some studies, a diagnosis of GPA along with PR3-ANCA-positivity was also a predictor of relapse [98, 166, 169].

Recently, genetic associations with relapse risk have been proposed; the carriage of HLA-DPB1*04:01 (more often found in PR3-AAV than in MPO-AAV) was associated with an increased risk of relapse [171]. Organ involvement at baseline seems to affect disease activity later. Upper respiratory, lung and cardiac involvement are associated with a higher relapse risk [167-169]. Kidney function at diagnosis was found to be predictive of the subsequent risk of relapse: advanced renal insufficiency was associated with a lower relapse risk, while a better GFR was associated with a higher level of risk [150, 168, 169].

The CNSAC significantly increased the occurrence of relapse in GPA patients [4, 51]. The impact of AAGN histology on relapse risk has not been widely studied. In one study, no difference in relapse rate was found [157], whereas in another study, sclerotic AAGN and the absence of interstitial infiltrates were associated with an increased risk of renal relapse [172].

Table 3. Summary of studies on renal survival according to AAGN histology and prognostic factors for ESRD

	Country	**Period**	**Age** years	**Number** GPA/MPA/ EGPA %	**ANCA** PR3/MPO/ negative %	**Follow-up** years	**Histology** F/C/M/S %	**ESRD %** F/C/M/S %	**Renal survival %** F/C/M/C %	**Predictors of ESRD ****
Berden [1]	European	nd	62.6	100 39/41/0	45/47	nd	16/55/16/30	31% all 7/24/46/70%	1y: **93**/84/69/*50* 5y: **93**/76/61/*50*	GFR at entry; Histology
Chang [158]	China	1997-2010	57.2	121 41/59/0	11/89	2.9	27/44/20/9	25% all 9/28/17/77%	1y: **100**/73/83/*29*	Baseline creatinine; Histology
Hilhorst [159]	The Netherlands	1979-2011	61.0	164 nd	51/49	8.5	49/26/24/<1 N=1 in S	nd	5y: **91**/*64*/69/- *	nd
Ellis [160]	USA	1995-2011	58	76 57/43/0	40/42/18	nd	32/28/43/17	nd	1y: **90**/78/81/*73**	Baseline GFR; Age; Histology
Iwakiri [161]	Japan	2000-2010	66	102 3/95/2	5/84/11	3.4	45/31/18/6	22.5%	1y: **98**/78/89/*67* FU: **98**/72/56/*44**	Histology: trend for significance
Ford [162]	Australia	1993-2011	66	120 nd	23/71/ 6% atypical	3.4	28/28/28/17	33%	ESRD or death **32**/42/39/*80*	Baseline GFR; Histology; Chronic interstitial injury
Quintana [163]	United Kingdom Spain	1996-2012	62	136 32/65/0 (3% undiag)	37/56/7	2.5	26/23/39/13	23.5%	5y: **96**/86/81/*61**	Baseline GFR
Tanna [164]	United Kingdom	1997-2011	62.2	104 nd	47/47/6	4.2	22/25/46/7	nd	1y: **100**/74/85/*50** 5y: **100**/74/77/*25**	Baseline GFR
Moroni [149]	Italy	1995-2011	58.8	93 41/47/0 (12% nd)	39/46/15	5.2	21/30/39/10	1y: 27%; 5y: 34% 10y: 40%	1y: **88**/*66*/94/69 5y: **82**/*37*/81/51 10y: **82**/37/75/*25*	Baseline creatinine; Arterial hypertension; < 20% normal glomeruli;
Córdova-Sánchez [157]	Mexico	2004-2013	52	62 55/45/0	56/34/10	3.4	N=55 9/2/45/44	1y: 20% 5y: 25%	1y: **100**/-/92/*66** 5y: **100**/-/86/*58**	RRT at presentation; proteinuria
Kristensen [165]	Denmark	1999-2001	62.9	87 nd	44/56/0	3	24/28/35/3	18%	FU: **96**/*67*/87/*67**	nd
Bjørneklett [156]	Norway	1991-2012	59	250 nd	54/46	3.5	38/28/24/9	1y: 14% 5y: 23%	1y: 96/81/86/56* 5y: 90/69/75/51*	0-1y: histology

GPA = granulomatosis with polyangiitis; MPA = microscopic polyangiitis (including RLV = renal limited vasculitis); EGPA = eosinophilic granulomatosis with polyangiitis; ANCA = anti-neutrophil cytoplasmic antibody; PR3 =proteinase 3 (or C =cytoplasmic); MPO = myeloperoxidase (or P = perinuclear); F = focal; C = crescentic; M = mixed; S = sclerotic; ESRD = end stage renal disease; nd= no data; * statistically significant difference; ** predictors in multivariate analysis;

2.10. Complications of AAV

2.10.1. Thrombotic complications

During the last decade, accumulating data demonstrating a significant increase in VTEs in cases of AAV have been presented. The high number of VTEs in adult GPA patients was first recognised in a sub-analysis of the WGET trial; during the two-year trial, 8.9% of patients were diagnosed with VTE, yielding an incidence of 7.0 per 100 person-years [6]. Soon afterward, high incidence rates of VTEs in AAV patients were shown in retrospective cohort studies [173-175]. In the largest retrospective cohort of 845 patients with AAV, VTE occurred in 7.6% of MPA patients and 8.0% of GPA patients during a mean follow-up of 58 months [175]. A long-term follow-up study from EUVAS found a VTE occurrence rate of 9.8% during a mean follow-up of 72 months in patients with AAV [176]. The risk of VTE is clearly higher during the active phase of the disease [6, 174, 175, 177]. During the first two years after diagnosis, the incidence rate ratio was 25.7 for pulmonary embolism (PE) and 20.2 for deep venous thrombosis (DVT) in GPA patients as compared to matched population controls [177]. However, after two years, the risk for PE disappeared while the risk for DVT remained elevated by 4.5-fold during a median follow-up of 7.2 years [177].

Older age at diagnosis has shown to be a risk factor for VTE in AAV patients [6, 175, 176]. Higher baseline creatinine (per 100 μmol/l increase, OR 1.17), higher baseline CRP (per 10mg/l increase, OR 1.05) and previous VTE (OR 3.48) were also associated with higher VTE risk [175, 176]. In addition, in AAV patients, the following forms of organ involvement were related to VTE risk: stroke with motor deficit (OR 5.82), cutaneous (OR 4.83) and gastrointestinal (OR 6.27) [175, 176]. VTE was found less commonly in GPA and PR3-ANCA-positive patients [174], but this was not confirmed in two other studies [175, 176].

The pathogenetic background of the increased frequency of VTE in AAV is still poorly understood. Recently, NETs, as a potential bridge between autoimmunity and coagulation, have shed light on the pathogenetic background of this phenomenon, as discussed above. Common risk factors for thrombosis (such as immobilisation, major surgery, malignancy, hormone replacement, oral contraceptive use, diabetes mellitus, obesity, smoking, nephrotic range proteinuria and deficiencies of protein, protein S or antithrombin) were not recognised in AAV patients with VTE [173, 174]. The prevalence of mutations in factor V Leiden, prothrombin and methylenetetrahydrofolate reductase was also not higher in patients with GPA and VTE as compared to the general population [178]. In GPA patients, the prevalence of antiphospholipid antibodies (aPLAb) was somewhat higher than in the general population, although there was no association with VTE [179, 180].

Disturbances in coagulation markers in AAV patients have been recognised. Elevated D-dimer and thrombin-antithrombin complexes were observed in active as compared to remission patients in an AAV cohort with creatinine clearance over 25ml/min [181]. In the same study, elevated levels of von Willebrand factor (VWF) activity were shown regardless of disease activity [181]. Recently, another study showed that because both elevated endogenous thrombin potential and increased levels of factor VIII (FVIII) were detected during stable remission, AAV patients remain in a hypercoagulable state [171]. In the non-AAV population, high levels of FVIII constitute an independent and dose-dependent risk factor for VTE [182, 183]. VWF,

having prothrombogenic properties through its involvement in platelet adhesion, activation and aggregation, serves as a carrier protein for FVIII and has been suggested as a marker of generalised endothelial dysfunction.

Anti-plasminogen antibodies were identified in patients with AAV, suggesting that the fibrinolytic system may be dysregulated; these antibodies also correlated with the presence of glomerular fibrinoid necrosis, cellular crescents and more severe renal dysfunction [184]. Patients with systemic AAV frequently have impaired kidney function, and renal patients are especially at risk of VTEs [185]. Patients with chronic kidney disease (CKD) exhibit increased pro-coagulants and a loss of anticoagulant factors, that is, increased levels of tissue factor, VWF, factor XIIa, factor VIIa, activated protein C, fibrinogen, plasminogen activator inhibitor-1 and D-dimer and reduced tissue plasminogen activator [186-188].

The data concerning the effect of medication use during AAV on coagulation and fibrinolysis are scarce. At the population level, the risk of VTE is increased among GC users, particularly during the first three months after initiating the treatment [189]. In a rather small study, no differences in endogenous thrombin potential were found between AAV patients who were given or went without immunosuppressive medication [190]. In a long-term follow-up study from EUVAS, treatment with CYC was not significantly associated with VTEs [176]. PEX is recommended for patients with severe AAV in order to effectively remove ANCAs [108]. In theory, PEX may, *per se,* have beneficial effects on coagulation, such as the removal of procoagulant agents and the use of anticoagulation and replacement fluids during the treatment. However, VTE risk was not affected by PEX in a EUVAS cohort [176]. To date, there are no significant data regarding the use of antiplatelet or anticoagulant therapy in AAV patients.

2.10.2. Infections

In the EUVAS cohort of 524 AAV patients, 24% of patients developed infections, mostly bacterial infections of the respiratory tract, during the first year after diagnosis [191]. Infection-related mortality in this cohort after 1 year was 5.6%. Infection is not only the main cause of death during the first year after diagnosis in AAV patients; it also remains an important factor contributing to excess mortality later as well [147]. Among 113 French GPA patients, major infections (such as bronchopneumonia, herpes zoster recurrence) were reported in 31.0%; of these, 39.6% occurred during the first year [192]. However, infection-related mortality in this cohort, which had a median follow-up of 3 years, was only 1.8%. Patients who developed infections had significantly higher cumulative doses of CYC and GC [192]. Notably, infection rates were not different in RCTs comparing RTX and CYC in regimens based on GCs [109, 110].

2.10.3. Malignancy

Patients with AAV experience an excess risk of cancer development. Historically, the standardised incidence ratios (SIR) for cancer at all sites in GPA patients have been about double those of the background population [193-195]. The risk of bladder cancer was highlighted, with a 3.6–33-fold increased risk. An increased risk of non-melanoma skin cancer (SIR 4.7–7.3), lymphoma (SIR 4.2–11.0) and leukaemia (SIR 5.7–5.9) were reported [193-195].

The risk of bladder carcinoma has been associated with the cumulative dose of CYC in particular. However, the safe cumulative dose of CYC is not known precisely, and the dose thresholds above which bladder cancer risk is increased have varied from 25 to 36 g in GPA patients [194, 195]. Another problem is the latency of cancer development after CYC administration.

In 2011, the cancer data of 535 patients from EUVAS trials were published: during a 2,650 person-year follow-up, the SIR for cancers at all sites was 1.58 [196]. This result was mainly driven by an increased risk of non-melanoma skin cancer (SIR 2.78), and the cancer risk for other organs, including bladder cancer, was not statistically significantly increased. These results were mainly explained by the reduced cumulative dose of CYC administered to the patients, while the role of AZA was also brought up, along with sun-exposure, as a possible contributor to the increased SIR seen for non-melanoma skin cancers. Notably, the SIR for all-site cancers was significant for GPA but not for MPA [196].

Recently, a retrospective study found that the malignancy risk in CYC-treated patients was 4.6-fold higher than in RTX-treated patients, in whom the malignancy risk was comparable with that of the general population [197].

2.10.4. Cardiovascular disease

Patients with AAV are at excess risk of cardiovascular (CV) events. The risk of acute myocardial infarction in Danish GPA patients was 2.5-fold higher as compared to the background population [198]. In the EUVAS cohort of 535 patients, including both GPA and MPA patients, the age-standardised annual CV mortality was 3.7-fold higher than in the background population [199]. Within five years of diagnosis, 11.7% of patients with GPA and 16% of those with MPA undergo at least one CV event. The independent determinants of CV outcomes were older age, diastolic hypertension and positive PR3-ANCA status, which was inversely related to CV risk [199]. Moreover, CV disease is a major cause of death after the first year from diagnosis [147]. Both disease-specific and therapeutic factors likely contribute to the increased CV risk in the AAV population. CV risk in this population is also increased by traditional CV risk factors, systemic inflammation and kidney impairment [198, 200, 201].

3 AIMS OF THE STUDY

The objectives of the study were as follows:

1. To describe the clinical presentation of biopsy-proven renal AAV at diagnosis, explore the outcomes for up to 20 years and assess prognostic factors for renal and patient survival and relapse (I).

2. To investigate the association between chronic nasal SA carriage and relapse in AAV patients (II).

3. To assess the value of monthly measurements of PR3-ANCA, as detected with nine different ELISAs, in monitoring disease activity in GPA patients with early systemic disease (III).

4. To characterise the coagulation profile in patients with renal AAV at the time of diagnosis and in remission as compared with patients with other renal diseases (IV).

4 PATIENTS AND METHODS

4.1. Patients and study designs

4.1.1. Study I (Long-term outcome study of renal AAV)

Patients

The cohort consisted of all consecutive patients diagnosed with renal biopsy-proven ANCA-positive vasculitis between the years 1996 and 2005 at the Division of Nephrology, Helsinki University Hospital. During that time, 85 patients with AAV were diagnosed, including 47 patients with MPA and 38 patients with GPA.

Study design

The study design is a retrospective, single-centre, observational cohort study of AAV patients with renal involvement. This study is a longitudinal study with a follow-up period of 20 years.

4.1.2. Study II (CNSAC in relation to relapse in AAV patients)

Patients

The patients in Study II were originally recruited from the two RCTs conducted by the EUVAS vasculitis study group. The newly diagnosed AAV patients in these studies were from twelve European countries, and the studies were carried out between 1995 and 2002.

The NORAM trial, including 95 patients, was designed to determine whether oral MTX could replace oral CYC as an induction treatment in patients with early systemic GPA or MPA without overt renal involvement [115]. The inclusion criteria were as follows: a new diagnosis of active GPA or MPA with involvement of one or more organ systems together with constitutional symptoms. Patients with organ or life-threatening manifestations of AAV and those with creatinine > 150 µmol/l or urinary red cell casts or proteinuria > 1 g/day were excluded.

The CYCAZAREM trial, including 155 patients, compared AZA with oral CYC for the maintenance of remission after CYC and steroid-based induction therapy in patients with generalised AAV [105]. The inclusion criteria were as follows: a new diagnosis of GPA, MPA or RLV with either renal involvement and/or other threatened loss of a vital organ. Patients with creatinine > 500 µmol/l were excluded.

Of the original 250 patients in the NORAM and CYCAZAREM trials, 200 were eligible for Study II. Patients with fewer than four nasal cultures, those with early withdrawal or death before remission and those lost to follow-up were excluded.

Study design

This is a prospective, multicentre study of 200 patients with early systemic and generalised AAV. The duration of the study was 18 months.

4.1.3. Study III (PR3-ANCA in monitoring disease activity)

Patients

The patients in Study III were obtained from the NORAM trial (described above) [115]. Of the original 100 patients, 95 were actively treated, and 28 were eligible for the study because they were PR3-ANCA-positive and had complete sera series.

Study design

This study is a prospective, multicentre study of 28 PR3-ANCA-positive, early systemic cases of AAV. The duration of the study was 18 months.

4.1.4. Study IV (Coagulation profile of renal AAV patients)

Patients

A cohort of 21 consecutive patients with newly diagnosed active renal AAV at the Division of Nephrology at the Helsinki University Hospital from 2008 to 2011 were included. Of these patients, 14 were classified as having MPA, including three patients with renal-limited AAV; seven patients had GPA, including one with overlapping GPA and anti-glomerular basement membrane disease. A diagnosis of AAV was based on renal biopsy in all but one individual, from whom a biopsy could not be safely obtained due to acute coronary syndrome but who otherwise had clinically evident MPA. Patients on immunosuppressive treatment for longer than one week were excluded.

Two control groups were created from patients undergoing renal biopsy during the same time period. Control Group 1 consisted of 20 patients with mild chronic kidney disease (eGFR $\geq$ 60 ml/min/1.73m^2) and no chronic findings in their renal histology. Control Group 2 included 20 patients with moderate or severe chronic kidney disease (eGFR < 60ml/min/1.73m^2) and/or sclerotic findings in their renal histology. Patients with diabetes mellitus, a history of previous thromboembolic disease, nephrotic syndrome or anticoagulant treatment other than low-dose aspirin were excluded from the control groups.

Study design

This is a prospective, single-centre, observational cohort study conducted with AAV patients with renal involvement and patients with other kidney diseases (different glomerulonephritides and other nephropathies), who served as controls. All patients were evaluated at diagnosis, and AAV patients were followed until remission when the laboratory assessment was repeated.

An overview of the patients in Studies I–IV is provided in Table 4.

Table 4. Summary of the patients in Studies I–IV.

Study	Patients' Origin	Number of patients (male/female)	Age, years median (range)
I	Renal AAV Helsinki	85 (53/32)	58 (22-80)
II	AAV patients Europe, multicentre	200 (97/103)	54 (18-78)
III	AAV patients Europe, multicentre	28 (12/16)	50 (26-78)
IV	Renal AAV Helsinki	21 (16/5)	60 (18-84)
	Control Group 1	20 (11/9)	44 (21-66)
	Control Group 2	20 (14/6)	58 (25-77)

4.2. ETHICAL ASPECTS

Because Study I was a retrospective study and all information was gathered from the patients' medical records, approval from the ethics committee was not required. However, research permission from the Department of Medicine in Helsinki University Hospital was granted. The approvals for Studies II and III were obtained from local ethics committees of each participating centre for the original RTCs, that is, NORAM and CYCAZAREM. Study IV was approved by the co-ordinating ethical committee of Helsinki University Hospital. Every patient provided written informed consent.

4.3. METHODS

4.3.1. Data collection

Study I

The patients were classified as having GPA or MPA according to the EMA algorithm. Patients' medical records, including baseline clinical data, were systematically reviewed from diagnosis until December 31, 2017, death or loss to follow-up. At diagnosis, patients' ANCA status was defined by MPO- or PR3-ELISA. AAV disease activity and organ involvement were assessed by BVAS at diagnosis and relapse. The following variables were obtained: kidney function (at time of diagnosis, at 3, 5, 10, 15 and 20 years), measured with plasma creatinine and eGFR using the CKD-EPI formula [202]; the amount of protein excreted in 24-hour of urine collection; dialysis treatment and the occurrence of ESRD.

Studies II and III

The clinical data were collected from the EUVAS database, in which they were recorded during the original NORAM and CYCAZAREM trials. The clinical data were obtained at diagnosis,

then monthly until 6 months, and then every three months until the end of the study and at relapse. For the purposes of Study II, nasal swabs were obtained at the time of diagnosis and then monthly and at relapse during the studies. Of the original 95 patients included in the NORAM trial, 84 completed the trial [115]. Of the original 155 patients in the CYCAZAREM trial, remission was achieved in 144 patients [105]. Of these, 138 completed the trial. After exclusion of patients with fewer than four nasal cultures, 200 patients remained eligible to Study II.

For the purposes of Study III, serum samples were collected locally in the centres and then collected and stored centrally at the EUVAS Serum Bank at the Statens Seruminstitut, Copenhagen, Denmark. During the NORAM trial, the samples were obtained at entry and then monthly and at any clinically suspected relapse. Of the 84 patients who completed the NORAM trial, 70 patients were PR3-ANCA-positive. Sera from 45 patients were sent to Statens Seruminstitut. Of these patients, 17 were either PR3-ANCA negative or had incomplete series of sera. Finally, 28 patients with complete series of sera could be included in Study III. After ANCA analyses (described in detail later), the PR3-ANCA results were merged into the original NORAM database.

Study IV

The patients were classified as having GPA or MPA according to the EMA algorithm (also known as Watts' algorithm). The baseline clinical data were recorded, including previous history of hypertension, coronary artery disease, smoking and the use of statin or acetylsalicylic acid. AAV disease activity and organ involvement were assessed by BVAS at diagnosis and in remission.

The recorded renal variables included kidney function measured by plasma creatinine level and GFR (as determined by the CKD-EPI formula [202]), the amount of 24-hour urinary protein excretion and dialysis treatment. The blood sample results regarding the activation of coagulation and fibrinolysis (described in detail below) were obtained at baseline from each patient. In AAV patients, blood samples were re-collected in stable remission. Blood samples were available from all 21 AAV patients at baseline whereas after death of two patients and removal of one patient to another hospital, blood samples at remission were available from 18 patients.

Thromboembolic events during induction treatment were recorded.

4.3.2. Measurement of disease activity

Vasculitis disease activity and organ involvement were scored with the Birmingham vasculitis disease activity score (BVAS) [96] (I-IV).

4.3.3. Definitions

Remission was defined as absence of disease activity (BVAS 0) in Studies I and IV. In Studies II and III, remission was defined as the absence of new or worse BVAS items allowing minor persistent activity in one affected organ (Studies II and III). Relapse was defined as new or worsening clinical disease activity requiring the augmentation of immunosuppressive medication (Studies I–IV). ESRD was defined as need for permanent dialysis (Study I).

4.3.4. Treatment schedules

Studies I and IV

Induction treatment was based on a combination of GC and CYC, which was given either orally (Study I) or iv (Studies I and II). The CYC dose began at 2 mg/kg/d orally and 0.75g/m^2 iv at 2–3 weeks intervals. Dose adjustments were performed based on age and renal insufficiency. CYC was given until stable remission was achieved. The initial GC dose was 1 mg/kg, aiming at 10 mg/day at 6 months, followed by the tapering of the dose. Iv pulses of 500–1000 mg of methylprednisolone were given for three days in case of rapidly deteriorating renal function. Plasma exchange was considered for patients with alveolar haemorrhage or severe renal failure (eGFR < 15 ml/min/m^2). Maintenance treatment was mainly based on AZA combined with low-dose GC. AZA was started at 2 mg/kg, with down-adjustment in elderly patients. However, the treatment protocol was not strict, and the medication could be considered individually according to the clinical situation.

Studies II and III

Patients in the NORAM trial were randomised to receive GC combined with either weekly oral MTX or daily oral CYC. [115]. MTX was started at 15 mg/week, escalating the dose to 20-25 mg/week by 12 weeks, which was then maintained until 10 months and then tapered and discontinued at 12 months. CYC was started at a daily dose of 2 mg/kg (maximum 150 mg/day). At remission the dose was reduced to 1.5 mg/kg/day until month 10 and then discontinued by month 12. Both treatment groups received prednisolone 1 mg/kg/day; the dose was tapered to 15 mg/day by 3 months and 7.5 mg by 6 months and stopped by 12 months. After discontinuation of all treatment at 12 months, patients were followed up for a further 6 months.

In the CYCAZAREM trial, all patients received the oral CYC-based remission-induction therapy combined with a tapering course of daily oral prednisolone until remission (doses as in NORAM) [105]. Patients in remission between 3 and 6 months were randomly assigned to either stop CYC and start AZA (2 mg/kg) or continue CYC (1,5 mg/kg) along with prednisolone treatment until 12 months. All patients received daily AZA (1.5 mg/kg) and prednisolone (7.5 mg/day) between months 12 and 18 after the trial ended.

T/S, at prophylactic doses (960mg three times a week) against Pneumocystis jirovecii pneumonia, was given according to the local policy of the centre.

4.3.5. Renal biopsy

The renal biopsy indications (Studies I and IV) were microscopic haematuria, proteinuria and/or declining renal function. The purpose of renal biopsy was to verify the diagnosis of AAV and/or assess the activity of renal disease.

In Study I, kidney biopsy histology was re-evaluated and classified into four classes of AAGN. Those with at least 50% normal glomeruli were classified as focal, those with at least 50% sclerotic glomeruli were classified as sclerotic, those with at least 50% glomeruli with cellular crescents were classified as crescentic and those not in line with the above-mentioned criteria were classified as mixed [1].

In Study IV, accessory immunohistochemistry from renal biopsies was produced with antibodies against thrombomodulin, CD42b, CD61 and CD39. Renal biopsies were fixed in 10% neutral-buffered formalin and embedded in paraffin. Paraffin-embedded specimens were cut at 3 μm using conventional histological techniques and stained for Haematoxin-Eosin, Jones Methenamine Silver, Masson Trichrome and Congo for histological analysis. For immunohistochemistry the sections were transferred to electrostatically charged glass slides (SuperFrost® Plus, Menzel-Gläser, Braunschweig, Germany). Slides were stained with a fully automatic immunohistochemical system Benchmark LT (Ventana Medical Systems, Illkirch, France) using standard cell conditioning with a biotin-free multimer-based detection system (*ultra*View™ Universal DAB, Ventana Medical Systems). Antigen expression was examined using the following mouse monoclonal primary antibodies against thrombomodulin (Novocastra, clone 15C8, diluted 1:100, incubated 48 min at 25°C), CD42b (Novocastra, clone MM2/174, diluted 1:100, incubated 32 min at 37°C), CD61 (Novocastra, clone 2f2, diluted 1:100, incubated 32 min at 37°C), and CD39 (Novocastra, Newcastle Upon Tyne, UK, clone 22A9, diluted 1:25, incubated 44 min at 37°C). To achieve a darker signal, Amplification Kit (Ventana Medical Systems) was used for CD39, tissue factor and thrombomodulin. Haematoxylin (Ventana Medical Systems) and Bluing Reagent (Ventana Medical Systems) were both used as counterstains (4 minute + 4 minute).

Scoring of the immunochemistry was done semiquantitatively. Thrombomodulin identifies endothelial cells and leukocytes. It was scored as follows: 1 indicates < 10% capillary positivity; 2 indicates 10–50% positivity; and 3 indicates > 50% positivity. CD42b identifies platelets and megakaryocytes and thrombin receptor. It was scored as follows: 1 indicates single platelets in glomeruli or interstitial tissue or peritubular capillaries; 2 indicates several platelets scattered around; and 3 indicates platelet aggregates in capillaries and glomeruli. CD61 identifies platelets, megakaryocytes, monocytes, macrophages and endothelial cells. It was scored in a similar way as CD42b, with the addition of class 0 which indicates negative staining. CD39 is a transmembrane glycoprotein that also identifies lymphocytes. It was scored as positive (class 1) or negative (class 0) in endothelial cells.

4.3.6. S. aureus nasal carriage

In Study II, nasal cultures were taken at diagnosis and then planned to be re-examined monthly and at relapse. The swabs were taken by firmly rotating a sterile cotton-tipped swab along the anterior nares and then incubating such swabs for 48 h at 35°C. The identification of coagulase-positive isolates was based on typical appearance upon slide coagulase testing and the ability to leave DNA. The patient was defined as a chronic carrier of S. aureus when ≥ 75% of at least four nasal cultures were positive for S. aureus. When all cultures we negative for S. aureus, the patient was defined as S. aureus-negative. All other patients received intermittent carrier status.

4.3.7. PR3-ANCA detection, identification of peak value and definitions of increments

In Study III, a serum sample to examine PR3-ANCA was taken at entry, then monthly and at the time of clinically suspected relapse. Nine PR3-ANCA ELISA assays were applied to measure the levels of PR3-ANCA: three direct ELISAs, four capture ELISAs using human native PR3

only and two direct ELISAs using human recombinant PR3 (human cDNA expressed in human cells, Euroimmun), one of which used a mixture of human native and human recombinant PR3. All assays were performed in accordance with the manufacturers' instructions, and all values were recorded as exact values. In cases with values exceeding the upper reading limit, dilutions were used to obtain exact values.

Phadia performed the aliquoting and labelling of all samples for the study. Phadia also performed the Varelisa PR3 ANCA and Varelisa PR3 capture ELISAs. All samples were examined by Statens Seruminstitut using a third-generation anchor anti-PR3 assay (Anti-PR3 HS ELISA, ORG 618, ORGENTEC Diagnostika GmbH, Germany). EuroDiagnostica performed direct ELISA and capture ELISA. Euroimmun performed four different ELISAs: three direct ELISAs coated with human PR3, human recombinant PR3 or a mixture of human and human recombinant PR3, as well as one capture ELISA using human PR3.

Because the PR3-ANCA values from the different assays were based on different units and reference sera, the PR3-ANCA values from all nine assays were transformed into logarithmic values. The sums of the logarithmic values at each month for each patient were then calculated. The highest sum value was then identified as the 'PR3-ANCA peak value'. This peak value could be identified in relapsing and non-relapsing patients, making it possible to compare increments related to the peak values in both groups.

For each assay, the increments in absolute PR3-ANCA values were calculated A) from time of remission until the time of the peak value defined above, B) from the previous month until the time of the peak value or C) from the previous month for all timepoints after month 4. The two increments prior to the peak values (A and B) were compared between the relapsing and non-relapsing groups. The largest monthly increment after remission (C) was recorded in order to determine whether it coincided with the time of relapse in each of the nine assays for each of the relapsing patients.

4.3.8. Markers of activation of coagulation and fibrinolysis

In Study IV, blood samples were collected from the AAV patients twice, first during the active phase of the disease and again when the patient was in remission. Blood samples were obtained from the control patients only at baseline, i.e., at the time of the renal biopsy.

Citrate-anticoagulated (109 mM sodium citrate) samples were centrifuged at 2,500 g for 10 min, and the separated plasma samples were frozen at -70°C. Laboratory analysis consisted of plasma prothrombin time (Nycotest PT, Axis-Shield PoC As, Oslo, Norway), activated partial thromboplastin time (Actin FSL), thrombin time (BC Thrombin Reagent), antithrombin activities (a chromogenic assay, Berichrom Antithrombin III), fibrinogen (a modification of the Clauss method, Multifibren U), FVIII activity (FVIII:C) (one-stage clotting assay, Pathromtin SL and Coagulation Factor VIII Deficient Plasma), vWF antigen (VWF:Ag with VWFag Latex Reagent) and ristocetin cofactor activity (VWF:RCo with BC von Willebrand Reagent), all of which were analysed with a BCS XP analyser (Siemens Healthcare Diagnostics, Marburg, Germany) and prothrombin fragments (F1+2) (enzyme immunoassay Enzygnost F1+2, monoclonal). All reagents were obtained from Siemens Healthcare Diagnostics (Marburg, Germany). D-dimer was measured with an immunoturbidimetric assay (Tina-quant D-Dimer, Roche Diagnostics, Mannheim, Germany).

Lupus anticoagulant was detected using two screening tests based on activated partial thromboplastin time (IL Test APTT-SP, Instrumentation Laboratory, Italy) and Russell Viper Venom activated clotting time (DVVtest 10, American Diagnostica Inc). Anti-cardiolipin and anti-beta2 glycoprotein I (specific phospholipid antibodies of the IgG class) were detected with immunological assays (Varelisa cardiolipin IgG antibodies and beta-2-glycoprotein I IgG antibodies, Phadia GmbH, Freiburg, Germany, respectively) with reference values of < 15 U/ml.

To assess the accumulation or load of acquired coagulation abnormalities, a score was calculated, including the abnormality of the following variables: thrombocytosis, short TT, low AT, high fibrinogen, FVIII:C, VWF:Ag, VWF:RCo, F1+2 and D-dimer (one point for every variable), providing a total score of 0–9.

4.3.9. Other assays

In Studies I and IV, plasma creatinine was measured by a photometric, enzymatic method. Plasma CRP was measured by a photometric immunochemical method. Blood haemoglobin was measured photometrically and blood cells with an impedance measurement. Urine protein was measured from a 24-hour collection with photometric method using benzetonium chloride. MPO- and PR3-ANCA were measured with direct ELISA methods.

In Study III, analyses other than ANCA, and in Study II, all analyses were done locally.

4.3.10. Statistical methods

Analyses were performed using IMB SPSS Statistics for Windows (versions 22.0-25.0) (Studies I, II and IV) and S-PLUS program (Study III). P-values < 0.05 were considered statistically significant.

The variable distributions were checked using Kolmogorov-Smirnov test and/or graphical plots. For normally distributed baseline characteristics and primary response variables, means and standard deviations (SD) were provided. For non-normally distributed data, variables were described as medians with ranges or medians with interquartile ranges (IQR, 25th–75th percentiles). The logarithmic transformation was used for variables skewed to the right, when appropriate, to normalise the distributions (IV).

Relations between two categorical variables were analysed by Chi-squared test or Fisher's exact test (I, II). Correlations between continuous variables were calculated using Pearson's correlation (r) for normally distributed (IV) and Spearman's rank (rho) coefficients for non-normally distributed variables (I, IV).

Associations with categorical and continuous normally distributed variables were analysed with analysis of variance (ANOVA) or t-test for independent samples depending on the number of categories: more than two categories or two categories, respectively (II, IV). Association between categorical variables and continuous non-normally distributed variables was analysed with Kruskal-Wallis test or Mann-Whitney U-test, also depending on the number of categories (more than two categories or two categories, respectively) (III, IV). After analysis of variance and Kruskal-Wallis test, Bonferroni corrections were used when appropriate (I, IV). Non-parametric Friedman's two-way ANOVA was used to analyse more than two repeated

measurements (I). Paired samples t-test and non-parametric Wilcoxon signed-ranks test were used to evaluate change between two repeated measurements (III, IV).

Univariate logistic regression was performed to study associations between prognostic factors and binary endpoints (II). The results were given as odds ratios (ORs) with 95% confidence intervals. Kaplan-Meier method was used to draw event-free survival curves and to estimate event-free survival rates (I, II). Univariate Cox-proportional hazards models were used to assess the associations between potential prognostic factors and the time without event (I, II). Multivariate analyses were performed to find the most important predictors, and predictors of significance $P < 0.10$ in univariate model were introduced into the models by forward stepping (I). The results were given as hazard ratios (HRs) with 95% confidence intervals.

5 RESULTS

5.1. Clinical and histological presentation of renal AAV patients and outcomes of 20-year follow-up (Study I)

5.1.1. Patient characteristics at baseline

Of 85 patients, 53 (62.4%) were men, and 47 (55.3%) had MPA. Of MPA patients, 87.2% were MPO-ANCA-positive, whereas 89.5% of GPA patients were PR3-ANCA-positive. GPA patients were younger than MPA patients [medians of 52 years (range 22–77) vs 62 years (28–80), respectively, p=0.004], and their GFR at diagnosis was better preserved than in MPA patients [medians of 35 ml/min/1.73m^2 (3–120) vs 17 ml/min/1.73m^2 (1–91), respectively, p=0.01]. In most patients (76.5%), the amount of proteinuria was non-nephrotic (i.e. ≤ 3g/day), with no difference between diagnostic subgroups. The diagnostic delay was four months (IQR 2–6), without any difference between MPA and GPA patients.

5.1.2. Histopathological classes of AAGN

Kidney biopsy was available from 84 patients. The histopathological class of AAGN was focal in 34.5%, crescentic in 26.2%, mixed in 20.2% and sclerotic in 19.1% of patients. The demographic and clinical data of patients in various histopathological classes are given in Table 5.

Table 5. Patients' characteristics by histopathological class

	Focal N=29 (%)	**Crescentic** N=22 (%)	**Mixed** N=17 (%)	**Sclerotic** N=16 (%)	**All** N=84 (%)	**P**
Diagnosis						0.01
MPA	11 (23.9)	10 (21.7)	11 (23.9)	14 (30.4)	46	
GPA	18 (47.2)	12 (31.6)	6 (15.8)	2 (15.8)	38	
Males	17 (58.6)	15 (68.2)	9 (52.9)	11 (68.8)	52 (61.9)	0.71
GFR ml/min/1.73m^2	60 (5-120)	14 (2-86)	21 (5-58)	12 (1-38)	24 (1-120)	<0.001
Age, years	55 (23-78)	50 (23-76)	60 (22-80)	67 (39-80)	58 (22-80)	0.02
Proteinuria >3 g/day	2 (6.9)	4 (18.2)	5 (29.4)	8 (50.0)	19 (22.6)	0.01

Data are presented as the median (range) for continuous non-normally distributed variables and as the number (%) for categorical variables.

5.1.3. Induction and maintenance treatment

Follow-up data were available on 82 patients and the median follow-up time was 16.2 years (95% CI 14.9–17.7). Regarding induction medication, 81.7% (67/82) of patients were given a combination of CYC and GC. CYC was given iv to 61.2% and orally to 38.8% of the patients. The cumulative dose of oral CYC was higher than that of iv CYC [median 13.3 g (IQR 7.4–21.0) vs 9.5 g (7.0-12.0), p=0.01]. Almost all GPA patients (97.3%) and 68.9% of MPA patients

were treated with CYC (p=0.001). CYC treatment was given to all patients with crescentic AAGN, 85.2% of patients with focal AAGN, 82.4% of patients with mixed AAGN and 53.3% of patients with sclerotic AAGN (crescentic vs sclerotic, p=0.002). PEX was given to 8.5% of the patients and 3.7% were treated with iv immunoglobulin. Altogether 96.3% of patients received maintenance treatment. A combination of AZA and GC was the most common medication and was given to 79.2% of the patients. The other maintenance treatments used were GC alone (10.4%) or in combination with CYC (3.9%) or MMF (5.2%).

5.1.4. Patient survival and causes of death

Forty deaths occurred during the follow-up, yielding a patient survival rate of 45% at 20 years (95% CI 31.2–59.2). The median survival time was 18.2 years (95% CI 13.2–23.2). The 1-, 5-, 10-, and 15-year patient survival rates were 96% (95% CI 92.3–100), 88% (95% CI 80.7–94.9), 71% (95% CI 60.9–80.6) and 55% (95% CI 44.3–66.2), respectively (Figure 4).

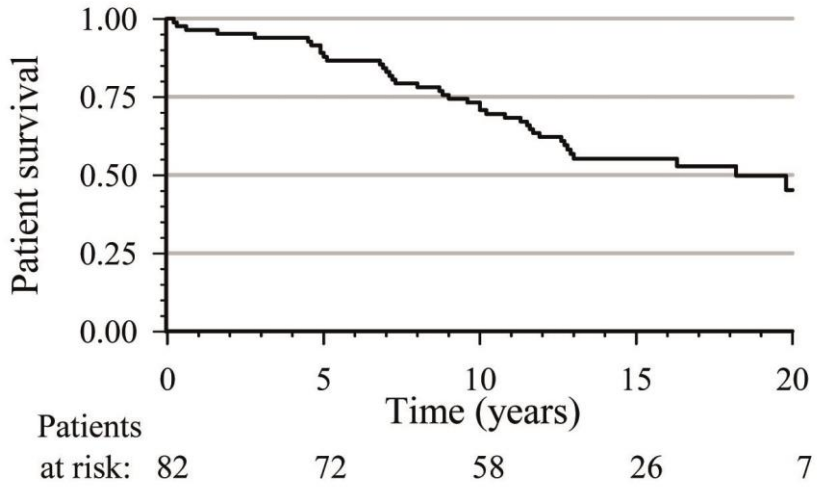

Figure 4. Patient survival.

(Salmela A, Törnroth T, Poussa T, Ekstrand A. Int J Nephrol 2018; 2018:6360814. Reprinted with permission from the copyright holder.)

Two patients died within 3 months, without achieving remission; one death was related to active disease (intracerebral haemorrhage), and another was related to treatment (opportunistic infection with cytomegalovirus and Pneumocystis jirovecii-pneumonia). A third death within the first year after diagnosis occurred at 7 months and was due to neutropenic sepsis following immunosuppressive medication. The cause of death was available for 82.5% of patients. The major causes of death were infections (27.3%), cardiovascular diseases (24.2%) and malignancies (21.2%). In 9.1% of patients, death was directly related to vasculitic disease activity. In a multivariate analysis, age ≥ 58 years (HR 7.64; 95% CI 3.44–16.95; p<0.001) and MPO-ANCA (HR 2.12.; 95% CI 1.08–4.17, p=0.03) were significant predictors of poor patient survival.

5.1.5. Kidney function and renal survival

Among patients who required dialysis within one month after diagnosis, 38.5% experienced reversible renal failure. During the follow-up period, a total of 25 patients developed ESRD, yielding an overall renal survival rate of 68% (95% CI 57.1–78.2). One-year renal survival was 84% (95% CI 76.2–92.0), 5-year renal survival was 79% (95% CI 70.1–87.9), and 10-year renal survival was 71% (95% CI 60.5–80.8). Two cases of ESRD occurred after 10 years, the last of which occurred at 11.3 years, as shown in Figure 5.

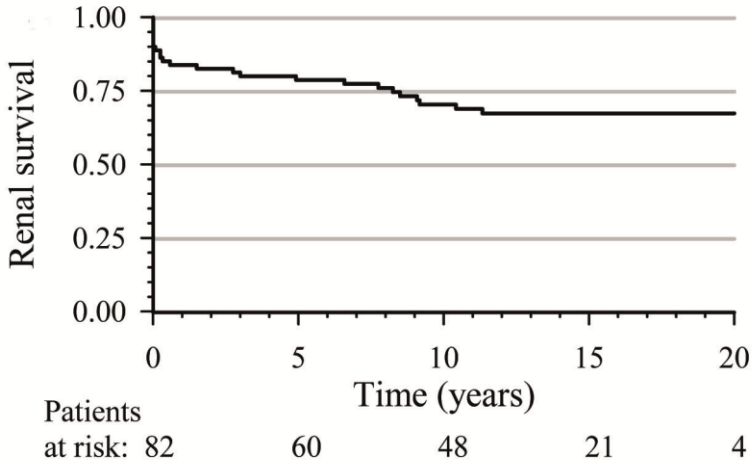

Figure 5. Renal survival.
(Salmela A, Törnroth T, Poussa T, Ekstrand A. Int J Nephrol 2018; 2018:6360814. Reprinted with permission from the copyright holder.)

In a multivariate analysis, male gender, GFR < 30 ml/min/1.73m^2 and presence of MPO-ANCA were related to worsened renal survival (HR 0.026; 95% CI 0.10–0.73, p=0.01 for female gender, HR 4.10; 95% CI 1.35–12.49, p=0.01 for GFR < 30 ml/min/1.73 m^2, and HR 3.10; 95% CI 1.21-7.95, p=0.02 for MPO-ANCA, respectively). Across histopathological classes, renal survival was as follows: 88% in focal, 71% in crescentic, 56% in mixed and 37% in sclerotic AAGN (p=0.01, Figure 6).

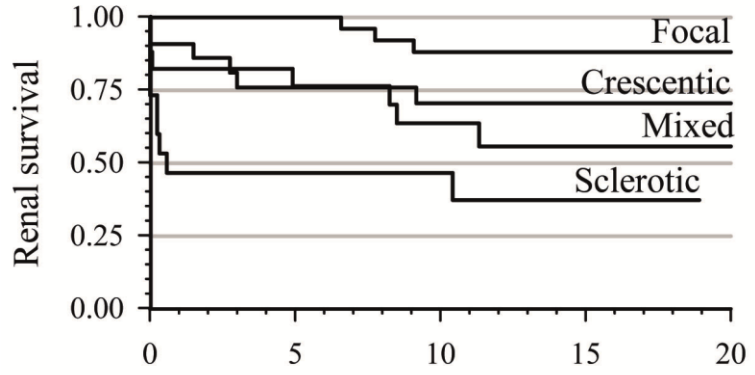

Figure 6. Renal survival according to histopathological classes.
(Salmela A, Törnroth T, Poussa T, Ekstrand A. Int J Nephrol 2018; 2018:6360814. Reprinted with permission from the copyright holder.)

5.1.6. Relapses

Relapse-free survival was 47% (95% CI 36.1–58.4) at 5 years, 30% (95% CI 19.1–40.8) at 10 years, 26% (95% CI 15.0–36.6%) at 15 years and only 10% (95% CI 0.0–24.7) at 20 years. The median time to the first relapse was 4.6 years (95% CI 3.0–6.2). In a multivariate analysis, diagnosis was the only significant predictor of relapse-free survival; in patients with GPA, the time to relapse was shorter than in patients with MPA (3.4 years, 95% CI 1.3–5.5 vs 5.9 years, 95% CI 0.9–10.9; HR 0.48, 95% CI 0.28–0.82; p=0.01).

During the 20-year follow-up period, 68.3% of patients had at least one relapse, 39.0% had more than one relapse and the total number of relapses was 129. Of these relapses, 40.3% were renal relapses. Across histopathological classes, the time to first relapse was 3.4 years (95% CI 1.9–5.0) in crescentic, 4.8 years (3.3–6.2) in focal, 5.5 years (0.0–15.7) in mixed and 7.4 years (3.6–11.3) in sclerotic AAGN (p=non-significant). The median number of relapses according to AAGN class was two (range 0–7) in focal, 1.5 (0–7) in crescentic, one (0–4) in mixed and 0 (0–3) in sclerotic AAGN (p=0.02).

At the time of the first relapse, 55.4% of the patients were without immunosuppression, 35.7% used AZA, and 8.9% received only GC. No difference in relapse rates was observed with respect to CYC administration route (iv vs oral, p=0.11).

AZA combined with GC was used as a relapse treatment in 41.1%, CYC in 23.3%, GC alone in 13.9%, MMF in 12.4% and MTX or cyclosporine in 2.3% of the relapses. From the year 2011 onward, the relapses of five patients (nine relapses altogether) were treated with RTX.

5.1.7. Severe infections during the first year

Infections requiring hospitalisation during the first year after diagnosis occurred in 32.0% of patients. Three patients had more than one event, and altogether 34 events were recorded. The most common infections were pneumonia (N=11) and septicaemia (N=10). Leucopenia (defined as leucocytes <3.5 $x10^9$/l) was associated with eight events. In a multivariate analysis, female gender (HR 0.32; 95% CI 0.12–0.84, p=0.02) and GFR < 60 ml/min (HR 9.04; 95% CI 1.22–66.78, P=0.03) were significant predictors of infection.

5.2. CNSAC in relation to relapse in AAV patients (Study II)

5.2.1. Patient characteristics

Of 200 patients, 48.5% were men, and 75.5% had GPA, 18.0% had MPA and 6.5% were diagnosed with a renal-limited form of AAV. In 58.5% of the patients, AAV was generalised (patients from the CYCAZAREM trial), and 41.5% had early systemic AAV (patients from the NORAM trial). ANCA specificities were distributed as follows: 71.5% of the patients were PR3-ANCA-positive, 25.0% were MPO-ANCA-positive and 3.5% were ANCA-negative. Patients with early systemic AAV were younger and exhibited nasal disease activity at entry more often than patients with generalised AAV [median 50.5 (range 18–78) vs 55.9 (20–76) years, p = 0.01; 85% vs 52.1%, p<0.001, respectively].

5.2.2. S. aureus nasal carriage status

The total number of nasal swabs was a median 12 (range 4–19) per patient. In the entire cohort, 12.0% were chronic nasal S. aureus carriers, 51.0% were intermittent carriers and 37.0% were negative for S. aureus. The distribution of nasal S. aureus carrier status was not significantly different in patients with early systemic as compared to generalised AAV (p=0.57). However, CNSAC was almost solely seen in GPA patients so that 23 out of 24 with CNSAC were GPA patients. Thus, the risk of CNSAC was higher in GPA patients (OR 8.63, 95% CI 1.13–65.6, p=0.04). Furthermore, CNSAC was more common in patients with nasal SA positivity at entry (OR 6.90, 95% CI 2.51–18.97; p<0.001).

Prophylactic T/S treatment was used in 27.5% of patients and was associated with a reduced risk of CNSAC (3.6% among T/S-treated vs 15.2% among non-T/S-treated patients, OR 0.21, 95% CI 0.05–0.93; p=0.04). Of the GPA patients who were not treated with T/S, 19.3% were chronic nasal carriers of S. aureus.

5.2.3. Relapse rate and associations with nasal S. aureus carriage

Relapse occurred in 32.5% of patients. The relapse rate was higher in early systemic patients than in those with generalised AAV (55.4% vs 16.2%; OR 6.41, 95% CI 3.33–12.34, p< 0.001). Relapse occurred in 41.7% of patients with CNSAC (all GPA), in 31.4% patients who were intermittent SA carriers and in 31.1% of non-carriers (p=0.59). Thus, for all AAV patients, no association between CNSAC and relapse was observed (OR 1.57, 95% CI 0.66–3.76; p=0.31).

However, the relationship between CNSAC and relapse differed based on disease severity: in patients with generalised AAV and CNSAC, a higher risk of relapse was observed (OR 4.64, 95% CI 1.29–16.67; p=0.02) in contrast to patients with early systemic (i.e. milder) AAV and CNSAC (OR 0.52, 95% CI 0.15–1.81; p=0.31). The same was true in GPA patients: in those with generalised disease, an association between CNSAC and relapse was observed (HR 4.10, 95% CI 1.37–12.25; p=0.01) (Figure 7). In GPA patients with early systemic disease, no association was found (HR 0.76, 95% CI 0.30–1.92; p=0.56) (Figure 8). In the NORAM trial (that is, in patients with early systemic disease), immunosuppression was terminated early, at 12 months, resulting in a high number of relapses afterwards. When the time to the first relapse was analysed for the first 12 months only, CNSAC in GPA patients was associated with a trend to an increased relapse risk (HR 2.73, 95% CI 0.95–7.87; p=0.06).

5.2.4. Relapses related to prophylactic T/S treatment

Prophylactic T/S treatment was not associated with reduced relapse rate because in the patients who were treated with the prophylactic T/S, relapse occurred in 27.3% of cases, as compared to 34.5% of those not treated with T/S (OR 0.71, 95% CI 0.36–1.41; p=0.33).

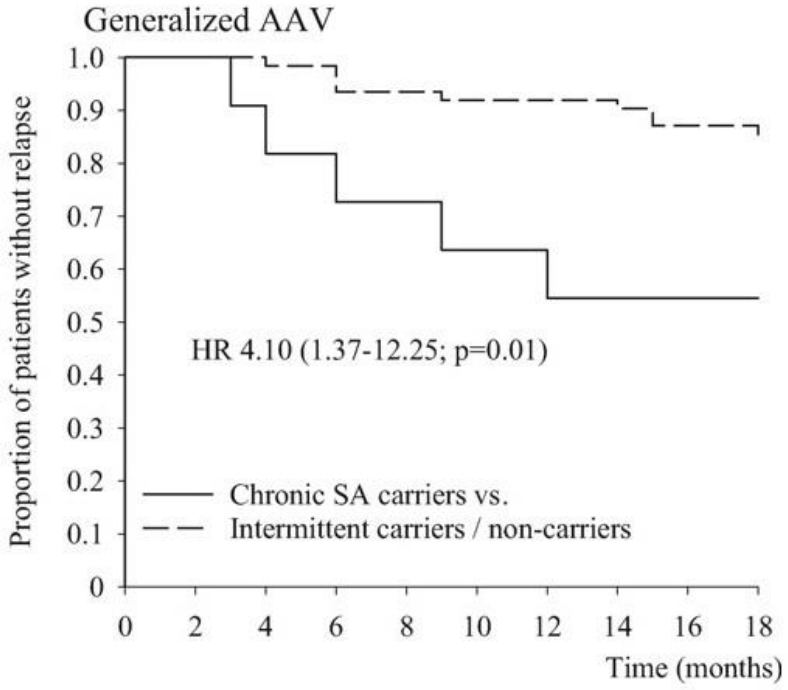

Figure 7. Time to first relapse in GPA patients with generalised disease.
(Salmela A, Rasmussen N, Cohen Tervaert J-W, Jayne D, Ekstrand A. Rheumatology 2017; 56(6):965-72. Reprinted with permission from the copyright holder.)

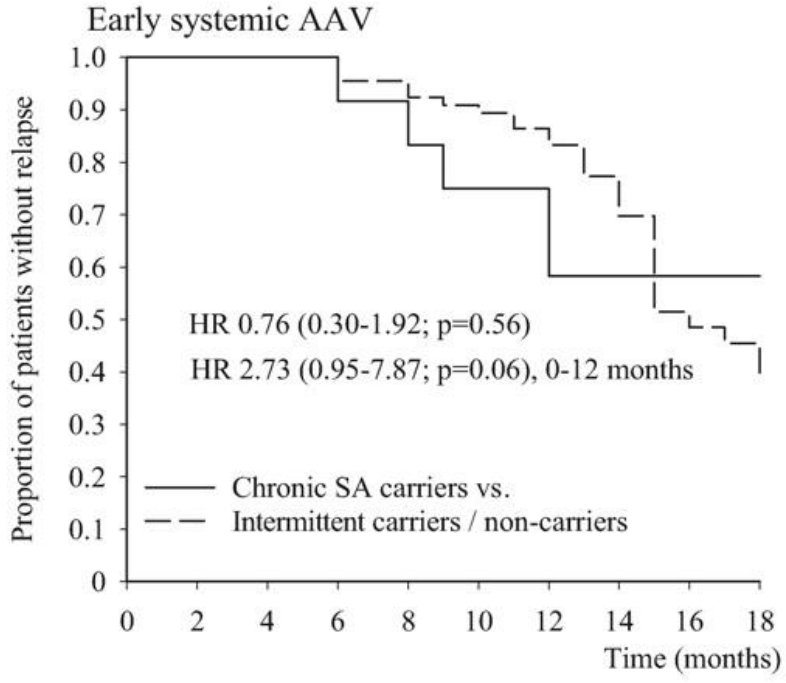

Figure 8. Time to first relapse in GPA patients with early systemic disease.
(Salmela A, Rasmussen N, Cohen Tervaert J-W, Jayne D, Ekstrand A. Rheumatology 2017; 56(6):965-72. Reprinted with permission from the copyright holder.)

5.3. PR3-ANCA in monitoring disease activity (Study III)

5.3.1. Patient characteristics

In this cohort, all 28 patients had GPA and were PR3-ANCA-positive. Of these patients, 42.9% were males. MTX was given to 53.6%, and 46.4% of patients were treated with CYC. Of these, 16 experienced relapse (relapsing patients), whereas twelve maintained remission (non-relapsing patients). Of the relapses, 68% included ENT activation, and renal activation was observed in 32% of these relapses.

5.3.2. PR3-ANCA profiles during the study

In general, in all assays, both in relapsing and non-relapsing patients, PR3-ANCA values decreased from entry to remission (by month four), when the lowest values were recorded, and then increased again when medication was reduced. We observed that in relapsing patients, PR3-ANCA declined again after relapse when medication was re-induced or augmented, while in non-relapsing patients, PR3-ANCA values continued to rise or remained unchanged. No significant differences were observed in PR3-ANCA profiles between treatment arms.

5.3.3. PR3-ANCA values in relation to relapse status

To compare changes in PR3-ANCA after remission between the relapsing and non-relapsing patients, the PR3-ANCA peak value was calculated from all assays performed for each patient. In relapsing patients, the peak value was reached at a median of 14 months (range 6–17 months), which corresponded with relapse in 14 patients and was within 1 month of relapse in two patients based on imputed values. In non-relapsing patients, the peak value occurred at a median of 12 months (median, range 6–17).

In general, the median PR3-ANCA value at relapse in relapsing patients was higher compared to the median peak value in non-relapsing patients. However, this difference was significant only in two capture assays (Phadia's, p=0.002 and EuroDiagnostica's, p=0.033, respectively). Nevertheless, due to a large overlap between the values at relapse and the peak values in non-relapsing patients, the assays performed could not identify a relapse.

Regarding the increments in PR3-ANCA values from remission to relapse or peak value (in non-relapsing patients), these increments were significantly higher in relapsing as compared to non-relapsing patients upon Euroimmun's direct ELISA using human recombinant PR3 and Phadia's capture ELISA assays (p=0.002 and p=0.034, respectively). The increments in PR3-ANCA since the month previous to relapse in relapsing patients as compared to such increments since the previous month until the peak value was reached in non-relapsing patients did not differ in any assay. The largest monthly PR3-ANCA increment after remission was not significantly related to relapse. Entry values were significantly higher in relapsing patients as compared to non-relapsing patients in three assays (Euroimmun's direct ELISA, p=0.019; EuroDiagnostica's direct ELISA, p=0.03; and Phadia's capture ELISA, p=0.045, respectively).

5.4. Coagulation profile in renal AAV patients as compared with other renal patients (Study IV)

5.4.1. Characteristics of and outcomes for AAV patients

Of 21 AAV patients, 16 (76.2%) were men, and 14 (66.7%) had MPA. One such patient had overlapping GPA and anti-glomerular basement membrane disease. The ANCA specificities in the diagnostic groups were distributed as follows: among MPA patients, 92.9% were MPO-ANCA-positive, and among GPA patients 85.7% were PR3-ANCA-positive. Their median GFR at diagnosis was 21 ml/min/1.73m^2 (range 7–45), and while in remission (at a median of 6.1 months, range 3.2–18.3), this was 35 ml/min/1.73m^2, p=0.15. Eighteen patients reached remission, two patients died before remission and one patient was moved to another hospital soon after the diagnosis. Altogether seven patients required dialysis; of these, kidney function was reversible in two (28.6%).

Two thromboembolic events occurred in AAV patients: one MPA patient was diagnosed with a DVT (3 months after diagnosis), and one GPA patient was diagnosed with a PE (4 months after diagnosis). In addition, one lethal, acute myocardial infarct occurred during active AAV (2 months after diagnosis).

5.4.2 Laboratory markers of coagulation and fibrinolysis in AAV patients

To assess the activation of coagulation and fibrinolysis, blood samples were obtained during the active phase of the disease and re-collected during remission (Table 6).

Table 6. CRP, haemoglobin, platelet count and coagulation variables in AAV patients during the active phase of disease and remission

	Active N=21	**Remission** N=18*	**P**	**Reference**
CRP	113 (19-134)	2 (2-5)	<0.001	<3 mg/l
Haemoglobin	105 (91-134)	122 (119-141)	0.01	134-167 g/l**
Platelets	290±116	224±59	0.01	150-360 E9/l
Prothrombin time	123±19	145±19	0.001	70-130%
Thrombin time	17 (16-17)	17 (16-19)	0.11	17-25 s
Antithrombin activity	99±15	116±14	<0.001	4-108%
Factor VIII	228 (187-266)	191 (177-256)	0.56	52-148%
VWF:Rco	198 (164-292)	196 (170-304)	0.86	44-183%
VWF:Ag	222 (168-266)	193 (165-236)	0.21	50-169%
Fibrinogen	6.9±2.6	5.2±1.5	0.007	1.7-4.0 g/l
F1+2	563 (385-730)	212 (184-318)	0.001	69-229 pM/l
D-dimer	3.0 (1.1-6.6)	0.6 (0.2-0.8)	0.001	≤ 0.5 mg/l

CRP = C-reactive protein; VWF:Rco = von Willebrand factor ristocetin cofactor activity; vWF:Ag = von Willebrand factor antigen; F1+2 = prothrombin fragments. Data are presented as means ± standard deviations or medians (25th–75th percentiles).

* Two AAV patients died before blood samples were obtained in remission and one patient was moved to another hospital; **in men; 117-155g/l in women.

F1+2 was markedly elevated at diagnosis. It was high (above the median) in all dialysis patients (one of whom died) and in two out of three patients who needed dialysis within one month after diagnosis, as well as in the two patients who developed thromboembolic complications later (Figure 9). The four patients with the lowest F1+2-values (< 25% quartile) had low disease activity without complications.

D-dimer levels were substantially elevated during the acute phase of the disease. Only four patients, all with lower disease burdens and uncomplicated disease courses, had normal D-dimer levels at diagnosis (Figure 8). Furthermore, D-dimer levels were positively correlated with disease activity (BVAS) (r=0.52, P=0.02). Both D-dimer and F 1+2 values decreased toward remission; however, the median D-dimer level remained above the normal range during remission. In active AAV, F1+2 and D-dimer levels were negatively correlated to GFR, although there was no such correlation during remission.

Fibrinogen levels decreased from the active phase of disease to remission although they did remain elevated during remission as well. High levels of FVIII, VWF:RCo and VWF:Ag were found in both the active and remission phases of AAV. No aPLAbs were observed.

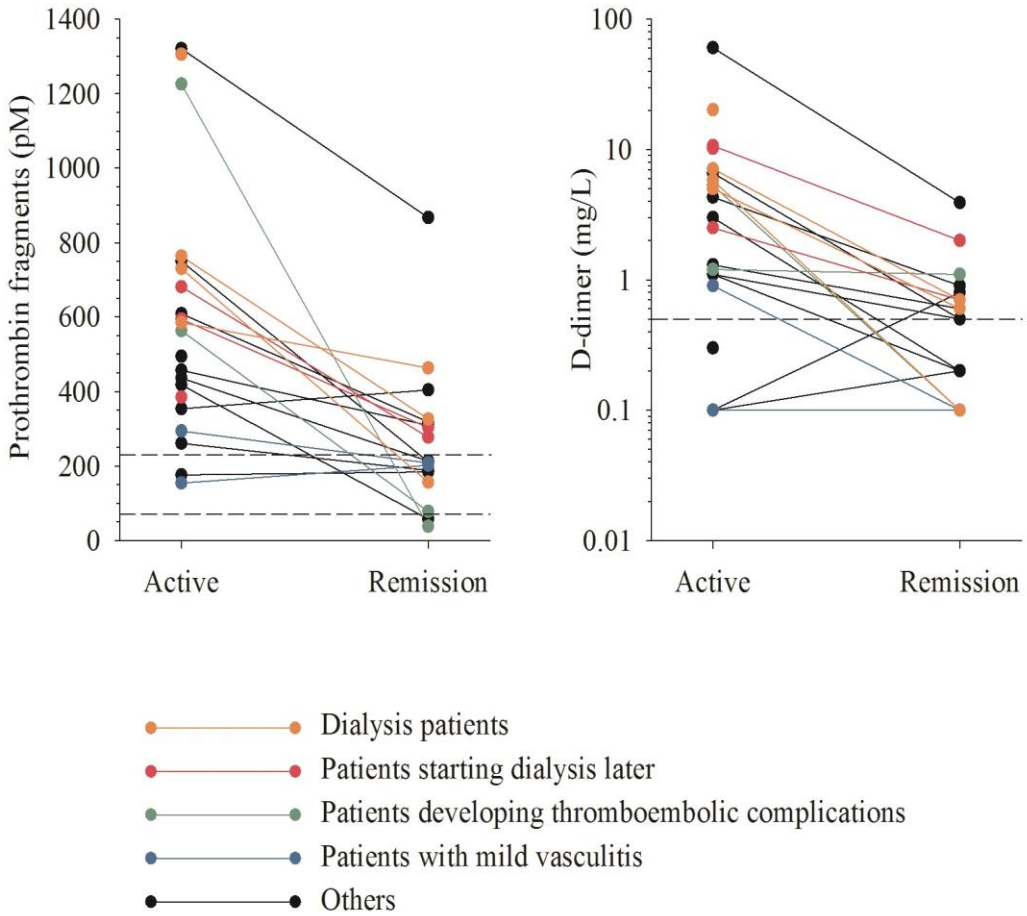

Figure 9. F 1 + 2 and D-dimer levels in AAV patients during active AAV and remission.
(Salmela A, Ekstrand A, Joutsi-Korhonen L, Räisänen-Sokolowski A, Lassila R. Nephrol Dial Transplant 2015; 30(Suppl 1):53-9. Reprinted with permission from the copyright holder.)

5.4.3. Patient characteristics in the control groups

The patients in Control Group 1 were younger than the AAV patients [median 44 years (range 21–66) vs 60 (18–84), p=0.03]. The kidney function was clearly better in these control patients than in the AAV patients at diagnosis [91 ml/min/1.73m^2 (IQR 66–107) vs 21 (7–45), p<0.001]. In Control Group 2, the median age was 57.5 years (range 25–77), and median GFR was 44 ml/min/1.73m^2 (IQR 31–50), with no significant differences as compared to AAV patients. The most common diagnosis in both control groups was IgA GN (N=12 in Control Group 1, and N=8 in Control Group 2). In Control Group 2, chronic changes were found in seven patients (nephrosclerosis N=4, chronic tubulointerstitial nephritis N=2 and ESRD N=1).

In the control groups, follow-up data were available for up to one year for 32 (80%) patients, and no VTE was detected.

5.4.4. Laboratory markers of coagulation and fibrinolysis in the control groups

In Control Group 1, the median/mean values for all tests related to coagulation activity and fibrinolysis were within the normal range, with the exception of fibrinogen, which was elevated (mean 4.5g/l, SD ±1.3).

In Control Group 2, the values of plasma prothrombin time (138%, ±24), FVIII (median 169%, IQR 141–194), fibrinogen (5.2g/L, ±2.0) and F1+2 (244pM/l, 188–283) were above the normal range. When patients in Control Group 2 were compared to patients with active AAV, significant differences were seen in VWF:RCO [median 165% (IQR 95–182) vs 198% (164–292), p=0.04], VWF:Ag [160% (IQR 107–192) vs 222% (168–266), p=0.04] and fibrinogen (mean 5.2 g/l ±2.0 vs 6.9 ±2.6, p=0.02) and even more clearly in F1+2 [median 244 pM/l (IQR 188–283) vs 563 (385–730), p=0.002] and D-dimer [median 0.3 mg/l (IQR 0.2–0.5) vs 3.0 (1.1–6.6), p=0.002], whereas in comparison with the remission values, no significant differences were found.

5.4.5. Load of coagulation abnormalities for the AAV patients and control groups

The load of acquired coagulation abnormalities decreased in AAV patients from the active phase to the remission phase of the disease (p=0.04), as shown in Figure 10. During remission, the load of acquired coagulation abnormalities was comparable to that of patients in Control Group 2.

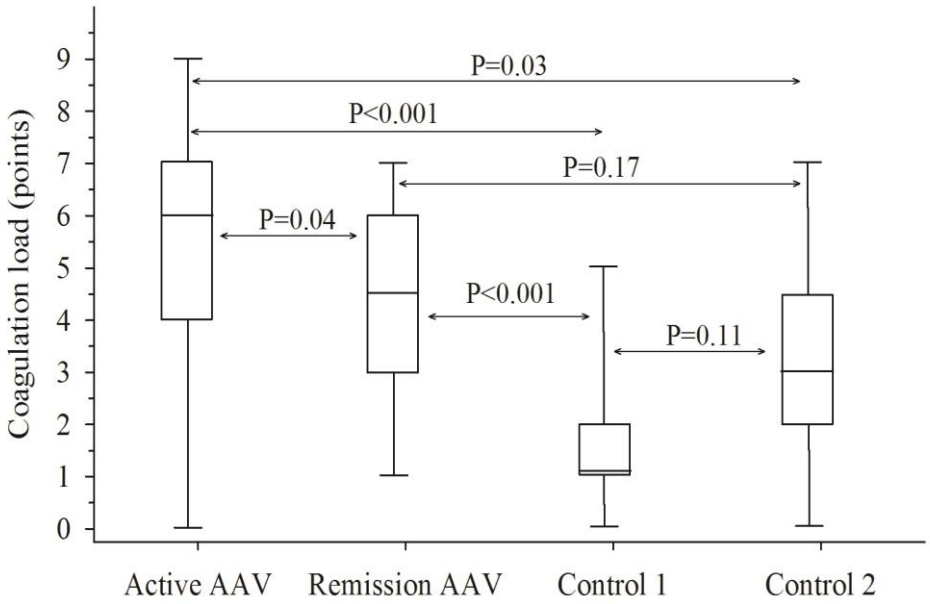

Figure 10. The cumulative load of coagulation abnormalities during the active and remission phases in AAV patients and in control patients.
(Salmela A, Ekstrand A, Joutsi-Korhonen L, Räisänen-Sokolowski A, Lassila R. Nephrol Dial Transplant 2015; 30(Suppl 1):53-9. Reprinted with permission from the copyright holder.)

5.4.6. *Immunohistochemistry from renal biopsies*

The results of immunochemistry from renal biopsies are given in Table 7. No significant differences between AAV and control patients were found in the staining patterns of thrombomodulin, CD42b, CD61 or CD39. Typical staining patterns are shown in Figure 11.

Table 7. Immunohistochemistry in AAV and control patients

Staining and scoring		**AAV patients** N=20 (%)	**Control patients** N=40 (%) *	**P**
Thrombomodulin	1	3 (15.0)	4 (10.3)	0.30
	2	6 (30.0)	20 (51.3)	
	3	11 (55.0)	15 (38.5)	
CD42b	1	9 (45.0)	12 (30.0)	0.12
	2	4 (20.0)	19 (47.5)	
	3	7 (35.0)	9 (22.5)	
CD61	0	1 (5.0)	2 (5.4)	0.86
	1	14 (70.0)	27 (73.0)	
	2	2 (10.0)	5 (13.5)	
	3	3 (15.0)	3 (8.1)	
CD39	0	10 (50.0)	17 (42.5)	0.58
	1	10 (50.0)	23 (57.5)	

AAV = ANCA-associated vasculitis. *in Thrombomodulin N=39; in CD61 N=37 due to the shortage of biopsy material. Scoring of immunohistochemistry is given in detail in the Methods section.

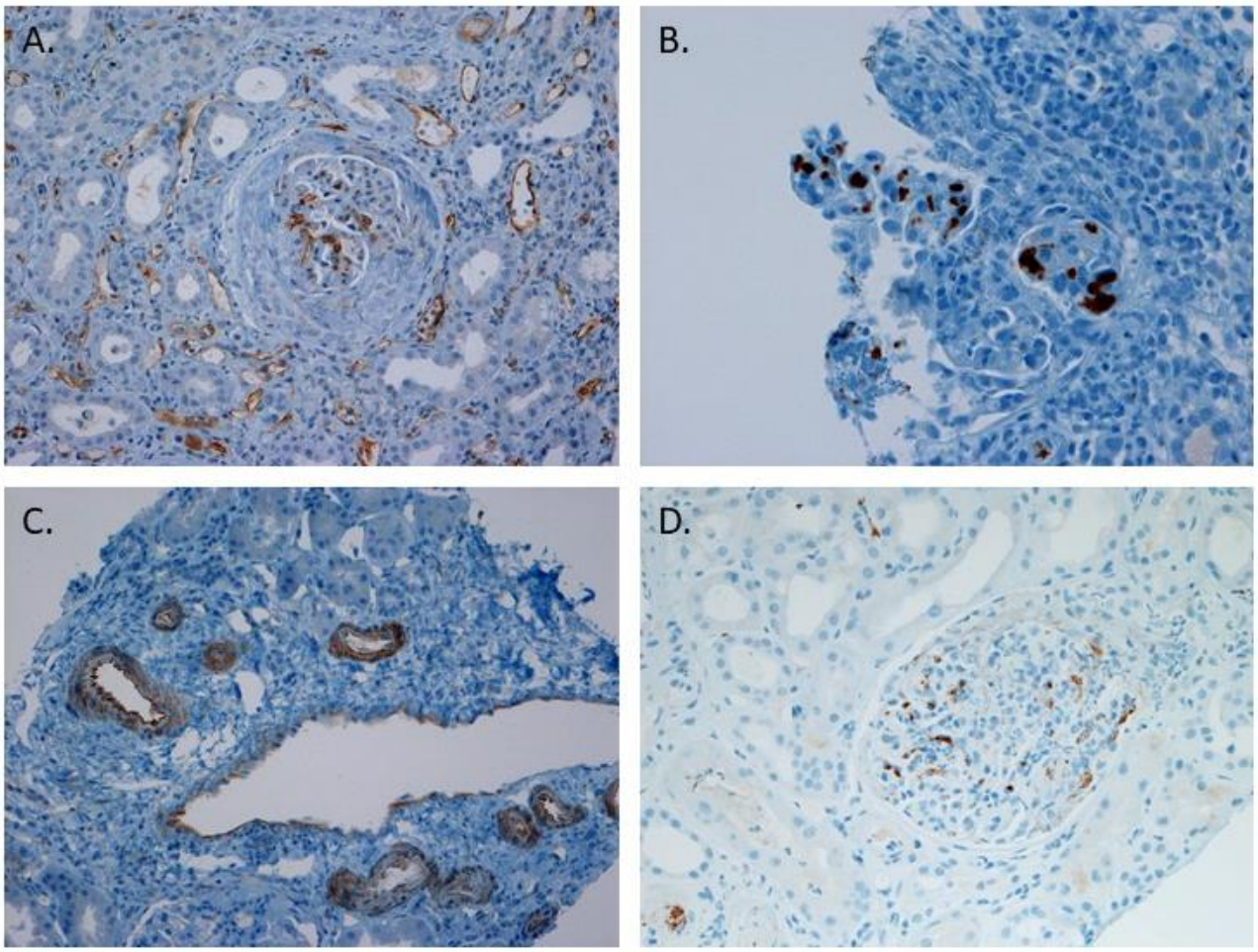

Figure 11. Typical staining patterns in immunohistochemistry. Endothelium of peritubular capillaries and injured glomeruli tuffs are stained positively with thrombomodulin (A). In CD42b staining, clusters of platelets are shown in the injured areas (crescents) (B). CD39 was expressed in the endothelium of all arterial walls and in some capillaries (C). CD61 was expressed in inflammatory cells and platelets in the injured glomerulus (D).

6 DISCUSSION

This study concerns the outcomes of AAV. Factors related to prognosis and disease activity are dealt with in a series of studies. In addition, special attention was placed on measuring alterations of coagulation status and ANCA levels in relation to disease activity. These issues were studied in four populations. Two of these included patients with AAV with renal involvement from a single Finnish centre, and two included AAV patients recruited for two clinical trials performed by the EUVAS group.

6.1. Patient survival

Due to induction treatment based on GC and CYC, the survival rate for AAV patients has improved dramatically over time. Our results suggesting a patient survival of 88% at five years (Study I) are in accordance with recent studies reporting a 5-year patient survival rate of about 90% [21, 145, 147], although this value is usually lower in renal cohorts [119, 146]. Previously, a lower 5-year survival of 74% was reported in a Finnish population of GPA patients by Takala et al. [203]. In that cohort of 492 GPA patients diagnosed between 1981 and 2000 from all over Finland, prognoses were unchanged between the first and the second 10-year period, the latter of which (1991–2000) overlaps with our study period. In recent renal AAV cohorts, the 10-year patient survival rate has varied between 49 and 81% [145, 149], and we found a survival of 71%. At 20 years, 45% of the patients in our cohort were alive, and to our knowledge, the survival rates at 20 years in renal AAV cohorts diagnosed after 1995 have not been reported elsewhere. The main potential reasons for improved patient survival are shorter diagnostic delay and lower cumulative CYC doses [144]. Both these facts were observed in our cohort. The superior survival as compared to the Finnish multicentre study could be due to the improved routine developed in a larger centre treating many patients with this rare disease annually. The latest recommendations regarding the management of AAV by EULAR and ERA-EDTA emphasise that AAV patients should be managed at or in close collaboration with centres of expertise [108].

As anticipated, older age is the most commonly reported factor contributing to higher mortality [21, 145, 147], and we confirmed this result (Study I). We also found that MPO-ANCA-positive patients had a lower survival rate than PR3-ANCA-positive patients. The mortality risk for MPO-positive patients was twice as high as in PR3-ANCA-positive patients. However, the statistical difference (HR 2.12, 95% CI 1.08–4.17) supplied by multivariate analysis was hampered by the large confidence interval. Previous studies have not clearly confirmed this association. However, in a cluster analysis of 673 patients, a nearly 6-fold increase in mortality was found in renal patients without PR3-ANCA, as compared with patients with PR3-ANCA [154].

6.2. Renal survival

Patients with MPO-ANCA had worse renal outcome than PR3-ANCA-positive patients (Study I). Even though it is not consistent, this association has been confirmed in many recent cohorts

[148, 152, 204]. In line with a recent Norwegian study, we found that males were more prone to developing ESRD [152]. This relationship is not widely recognised among AAV patients, but in CKD cohorts, the prevalence of renal replacement therapy is usually higher among men, specifically 1.6-fold higher according to the latest European registry data [205]. We confirmed the anticipated association between lower baseline renal function and the risk of ESRD [1, 149, 158, 160, 162-164].

Indeed, in diseases affecting the kidneys, avoiding dialysis is the main goal. Rapidly progressing GN is frequently seen in AAV and can lead to renal failure requiring dialysis within a short time. In Study I, 39%, and in Study IV, 29% of those who needed dialysis within a short time period after diagnosis regained renal function and were able to discontinue dialysis. After five years, renal survival was 79%. This result is in accordance with recent data from renal AAV cohorts reporting a 5-year renal survival of 77–66% [149, 156, 157]. Overall, the 20-year renal survival rate in Study I was 68%.

Since the introduction of the AAV renal histology classification system, many groups have validated the schema of dividing renal histology into focal, crescentic, mixed and sclerotic subclasses. The composition of classes has been variable, but one common finding is reported: renal survival is best in the focal class [1, 149, 156-165]. This underscores the importance of early diagnosis. Renal survival has been lowest in the sclerotic class [1, 156-158, 160, 161, 163, 164], while a few studies have found the lowest survival rate (or equal to that of the sclerotic group) in the crescentic class [149, 159, 165]. In Study I, renal survival was significantly better in the focal (88%) and in the crescentic (71%) classes than in the sclerotic class (37%). However, in a multivariate analysis, the histopathological class was not predictive of renal survival.

6.3. Relapses

Relapse is a major concern in patients with AAV. As described above, the survival rate of these patients has improved, which in turn has allowed more relapses to occur. We found (Study I) a relapse-free survival rate of 47% at 5 years, which is in agreement with prior studies showing relapse rates of 34–49% [98, 146, 166-169]. Recently, the long-term follow-up of the WEGENT trial reported a 30% relapse-free survival rate at 10 years, which is comparable to our findings [150]. At 20 years, the relapse-free survival rate in our cohort was only 10%. Data concerning follow-up for that long a period are not available for comparison purposes in recent literature. According to our findings, the clear majority of renal patients suffer from a relapse in the long run. Previously, advanced renal insufficiency has been shown to be related to lower relapse risk [168, 169]. We could not confirm this; however, we found a trend toward lower relapse risk in patients with baseline GFR < 30 ml/min. This could be due to patients remaining on dialysis, during which no new renal relapses could occur.

PR3-ANCA-positivity is the most commonly indicated risk factor for relapse in AAV [4, 98, 145, 150, 166-169]. In Study I, we also found a similar trend, but the difference was significant only between the diagnostic subgroups; specifically, there were fewer relapses in MPA. However, in Study III, the risk of relapse was higher in PR3-ANCA-positive patients than PR3-ANCA-negative patients (that is, MPO-ANCA-positive and ANCA-negative patients

pooled together), and the magnitude of this risk was higher based on ANCA-specificity as compared to the diagnostic subgrouping (GPA vs MPA, including patients with renal-limited disease).

6.4. Role of S. aureus in relapse

The aetiological role of S. aureus as a disease trigger in AAV has been proposed, and up to 72% of GPA patients have been found to be chronic carriers of SA in their noses [4, 51, 52]. Recently, however, a lower rate of CNSAC of 20–28% was reported in Norwegian GPA patients during RTX treatment [55]. In Study II, we found an even lower number, i.e., 15% of CNSAC in AAV patients (who were not treated with T/S). We also found that CNSAC was nearly exclusively observed in GPA patients, who had a CNSAC rate of 19%, which is comparable to that in the general population [53, 54]. Furthermore, CNSAC was associated with a higher relapse rate (HR 4.1) in generalised GPA, and the trend toward a higher relapse rate was also seen in patients with early systemic GPA who were using immunosuppressive medication. This finding is in line with the seminal study of Stegeman et al., which reported a seven-fold increase in the risk of relapse in outpatient clinic GPA patients with CNSAC [4], even though the magnitude of the risk we reported is closer to the later report of Zyzinska et al., who found a 4.5-fold increase in risk in cases of limited GPA [51]. We also found that CNSAC was strongly associated with S. aureus positivity at entry (OR 6.90). To define chronic carriage in our study, at least four swabs were required with an S. aureus positivity of at least 75%. Instead of performing multiple nasal swabs to confirm the carriage state, two nasal cultures within one week, combining both qualitative and quantitative tests, can predict persistent S. aureus carriage in healthy volunteers [206]. Applying this method of assessing carriage would facilitate the process of identifying this specific subgroup of GPA patients who are at higher risk of relapse.

However, the association between chronic S. aureus carriage and increased relapse risk does not necessarily imply causality. Indeed, there is no evidence of a direct role on the part of S. aureus in AAV disease activity, even though the mechanisms that contribute to S. aureus colonisation and eventually lead to the outbreak of disease have been brought to light during the last decade [56, 57, 60]. It could also be postulated that both CNSAC and higher relapse risk are consequences of the same underlying factor, which could be genetically determined. Despite the perspective, patients with CNSAC are the subgroup of GPA patients more prone to relapse (Study II). Defining the S. aureus nasal carrier status in the early phases of disease could be valuable when considering the length of maintenance treatment in GPA patients.

6.5. Role of ANCA levels in relapse

The predictive value of sequential ANCA determinations in relation to relapse has been one of the most discussed subjects within the AAV community. In Study III, this issue was addressed in PR3-ANCA-positive patients with early systemic GPA via monthly measurements of PR3-ANCA with nine ELISAs during an 18-month clinical trial. By using a new method of defining the peak value for PR3-ANCA, we were able to confirm that PR3-ANCA increase was closely

related to relapse [11]. Even though all assays identified the median peak value being higher in relapsing than in non-relapsing patients, the comparison was statistically different in only 2/9 assays, and the large overlap of PR3-ANCA values between relapsing and non-relapsing patients made the recognition of relapse impossible. Furthermore, the largest monthly increment in PR3-ANCA was not able to predict relapse, and the increment in PR3-ANCA a month before relapse or peak (in non-relapsing patients) did not differ between the two groups, either. Moreover, the monthly monitoring of PR3-ANCA during the clinical trial clearly showed that these values are influenced by the modification of immunosuppressive medications. Thus, the findings of Study III are largely consistent with those of a meta-analysis concluding that serial ANCA monitoring was of limited use in predicting future relapse [136].

Recent data on the effect of disease state and treatment on ANCA responses in relation to relapse could offer further explanations for the observations in Study III. In patients with severe disease (that is, renal involvement or alveolar haemorrhage), ANCA increase seems to correlate with relapses to a greater degree than in patients with milder AAV [140, 141]. As described above, the patients in Study III had early systemic AAV, and severe disease was an exclusion criterion. Likewise, the therapeutic regimen seems to contribute to ANCA responses: after RTX induction, an increase in PR3-ANCA was associated with increased relapse, whereas in CYC/AZA-treated patients, such an association was not observed [141]. The patients in Study III were treated with either CYC or MTX in addition to GC.

6.6. Coagulation and fibrinolysis activity

The increased risk of VTE among AAV patients is well-established, and the risk is highest during the active phase of the disease [6, 173-177]. This was confirmed in Study IV, in which 9.5% of AAV patients developed VTE within four months of diagnosis.

In general, we observed that patients with active AAV present a state of enhanced coagulation and fibrinolysis (Study IV). In particular, thrombin formation (measured with F1+2) and fibrin degradation (measured with D-dimer) were predominating during the acute phase of the disease. This is in agreement with the earlier observations showing elevated D-dimer levels during active AAV [181, 207]. Interestingly, D-dimer levels were correlated with BVAS (Study IV), suggesting that the activation of coagulation and fibrinolysis is linked with multi-organ AAV. Both F1+2 and D-dimer levels showed the potential to differentiate between patients with mild disease and those with more complicated disease. Furthermore, both F1+2 and D-dimer levels were negatively correlated with GFR during the acute phase of the disease, referring to the pathogenic process of active necrotising glomerulonephritis, and also to the enhanced thrombin formation and fibrin degradation in patients with advanced kidney disease.

High levels of FVIII, VWF:RCo and VWF:Ag were found during both the active and remission phase of AAV (Study IV). Previously, elevated levels of VWF were found regardless of disease state, and more recently, high levels of FVIII were detected during stable remission [181, 190]. Both earlier observations were derived from AAV cohorts excluding patients with advanced renal impairment, and the Dutch cohort comprised mainly GPA patients. High levels of VWF and FVIII could reflect ongoing endothelial involvement during the remission phase of the disease. In Study VI, D-dimer levels remained above the normal reference values in half of the patients in whom remission was achieved, whereas in another study, D-dimer levels during

remission were comparable to those of healthy controls [190]. This difference could be explained by the timepoints of the measurement: in Study IV, D-dimer was measured at a median of 6 months from diagnosis, whereas in another study, the patients had quiescent AAV that had been diagnosed 6 years earlier. Thus, D-dimer might represent a marker of disease activity in AAV.

To examine the association between renal function and coagulation activity and fibrinolysis, the AAV patients in Study IV were compared with patients with other renal diseases, who were divided into two groups based on kidney function. In general, the cumulative load of coagulopathies (defined as the sum of coagulation variables) was clearly lower in patients with mild renal impairment than in patients with AAV, regardless of disease state, whereas in patients with more advanced renal disease, the coagulopathy load was different only in comparison with active AAV. This suggests that AAV patients with renal disease could be a special group regarding the risk of thrombotic events. In fact, this assumption was recently shown to be true: the risk of VTE was increased in AAV patients with higher baseline creatinine levels [176].

The increased risk of VTE is recognised in the treatment guidelines for AAV. However, no controlled trials have been conducted, and there is an overall lack of knowledge of the potential benefits and risks of anticoagulation in AAV patients. Such data are needed, not only to reduce VTE comorbidity but also to address the question of whether anticoagulation therapy could affect renal function and improve renal survival in AAV patients.

6.7. Strengths and limitations

The main strength of Study I was the long follow-up; outcome data spanning 20 years are exceptional in the AAV literature. In addition, all patients were followed from the diagnosis of the disease. The retrospective nature and the rather small number of patients were the main limitations of Study I. Also, the cohort is historical. It included patients who were diagnosed between 1996 and 2005 and primarily given CYC and GC as an induction treatment and AZA with GC as a maintenance treatment. In addition, the fact that Study I included only patients in whom a renal biopsy had been performed may bias the results. The oldest and most severely ill patients likely never underwent renal biopsy, due to their condition or premises of being treated with potentially toxic medication. Likewise, not all AAV patients with minor signs of renal disease may have undergone renal biopsy, either. Thus, the conclusions from Study I may be less valid regarding the aforementioned patients. In addition, the retrospective set-up of Study I permits the possibility that minor recurrent disease activity was never recorded and the data on relapses were thus underestimated. Furthermore, we only analysed the effect of baseline parameters on survival. However, the disease course is a dynamic process, and the longer the follow-up, the more factors may interfere with the endpoints.

The strength of Study II was the combination of two AAV cohorts comprised of patients in various disease states. Furthermore, the S. aureus nasal cultures were obtained at short intervals. Even though the planned monthly collection of nasal swaps was not feasible in every patient, the median number of S. aureus nasal cultures per patient was 12. Hence, the number of swaps enabled the reliable segregation of patients into different nasal carrier status subgroups. The limitation of Study II was that the potential use of antibiotics prior to AAV

diagnosis, which is quite common among AAV patients presenting with flu-like symptoms, was not recorded, and this may have had an impact on the frequency of CNSAC. Furthermore, for the diagnosis of an ENT relapse (which constituted 48% of all relapses), a biopsy was not required, and the clinical separation of nasal infection from nasal AAV activity may be difficult.

The strength of Study III was the use of a novel method of defining peak value for PR3-ANCA, which permitted the identification and comparison of the highest PR3-ANCA values for both relapsing and non-relapsing patients. In addition, the monthly ANCA measurements obtained during the RCT under a pre-scheduled and controlled treatment scheme made it possible to observe the effects of medication on PR3-ANCA levels. However, even though the peak value could identify relapse in practically all relapsing patients, the usefulness of that method is limited in a real clinical context because the peak can only be defined retrospectively. The limitation of Study III is the small number of patients, which was due to the lack of sufficient amount of sera collected according to the protocol. In addition, the study population had early systemic GPA without overt renal disease, and the results may be less valid for a more severe disease.

The strength of Study IV was that it included a wide range of renal AAV patients in various disease states, although the total number of AAV patients was limited. The use of control groups with other renal diseases made it possible to examine the effect of renal impairment on coagulation activity. Even though the group of AAV patients in Study IV was heterogeneous, with factors and complications interfering with the delicate system of haemostasis, the prospective nature of the study allowed us to assess many contributors to coagulation.

7 CONCLUSIONS

The conclusions of the present series of studies are as follows:

1. In the long-term follow-up of renal AAV patients, improved patient and renal survival were confirmed. Moreover, survival data for up to 20 years could be presented. Both patient and renal survival were negatively predicted by MPO-ANCA. ESRD was more common in men. As anticipated, younger age was associated with favourable patient survival and better renal function at baseline, as well as with better renal survival. The risk of ESRD was the lowest in patients with focal AAGN and the highest in patients with sclerotic AAGN. The relapse risk proved to be high in this renal cohort, and patients with GPA were more prone to relapse than MPA patients. (Study I)

2. Among patients with AAV, the frequency of CNSAC was lower than that reported previously. In GPA patients, however, CNSAC was higher than in non-GPA patients and comparable to the general population. In patients with generalised GPA, CNSAC was associated with relapse, and in patients with early systemic GPA using immunosuppressive treatment, a similar trend for this association was found. Thus, patients with CNSAC represent a GPA subgroup with an increased risk of relapse. (Study II)

3. In patients with early systemic GPA, the PR3-ANCA peak coincided with relapse. In non-relapsing patients, such a peak in PR3-ANCA could also be identified, reflecting the tapering of the immunosuppressive medication. Thus, PR3-ANCA levels were reflected not only by the disease activity but also by the level of immunosuppressive medication. The large overlaps in PR3-ANCA values at relapse or peak (in non-relapsing patients) made it difficult to differentiate relapsing patients from non-relapsing patients. (Study III)

4. Patients with active renal AAV exhibited a state of enhanced coagulation and fibrinolysis. During remission, thrombin formation and fibrin turnover were largely reduced, whereas FVIII activity remained high, indicating ongoing endothelial involvement. Markers of coagulation and fibrinolysis were comparable between AAV patients in remission and other renal patients with at least moderate renal impairment (Study IV).

8 ACKNOWLEDGEMENTS

The present study was carried out at Helsinki University Hospital, Abdominal Center, Nephrology (formerly: Department of Internal Medicine, Division of Nephrology) and Vaasa Central Hospital, Department of Internal Medicine during the years 2006–2018.

I wish to express my deepest gratitude to my supervisor, Docent Agneta Ekstrand. The initial idea for this work came from her, and her guidance both to the subject and to research itself has been excellent. Her European contacts enabled valuable co-operation with the EUVAS colleagues. We have had research meetings not only in the office but many times also at her lovely home as well as her summer house in Ostrobothnia, where I moved in the middle of this project. Innumerable are also the discussions we have had over the phone during these years. This long-distance arrangement and prolonged project has required great flexibility and never-ending patience on her part.

I am most grateful to the reviewers of this study, Professor Emerita Marjatta Leirisalo-Repo and Docent Satu Mäkelä, for their constructive and thorough comments which certainly improved the final version of this manuscript. The English language editing by Anna Vuolteenaho, MA, was excellent.

My sincere thanks are due to my co-authors. I owe my gratitude to the EUVAS colleagues participating in these studies. In particular, I want to thank Professor Niels Rasmussen who co-wrote two articles with me. The valuable discussions and comments on manuscripts from Professor Jan-Willem Cohen-Tervaert and Professor David Jayne are highly appreciated. I'm so grateful to have had the opportunity to cowork with and learn from the real gurus in the ANCA field. Professor Riitta Lassila and Docent Lotta Joutsi-Korhonen are warmly thanked for elegantly introducing me to the complicated world of blood coagulation. Renal pathologists, Docent Anne Räisänen-Sokolowski and Docent Tom Törnroth are both thanked for their contribution. Renal histology was once opened up to me in the renal meetings led by Docent Tom Törnroth, and I was so happy to work with him again after many years. Statistician Tuija Poussa, MSc, is one of the key persons of this work. I wish to thank her for all the help and guidance with biostatistics.

Apart from the co-authors there are many important persons who contributed to this work. My colleague Sari Aaltonen is warmly thanked for her help in recruiting the study patients. Tiina Vesterinen, MSc, is thanked for preparing immunohistochemistry of renal biopsies. Mrs Kristina Saloranta is thanked for her assistance with schedules of the participating patients. Mrs Tarja Porkka, Mrs Marja Lemponen and Mrs Ellen Saarela are all thanked for their help with laboratory analyses.

I have been fortunate to have fine superiors. I owe my warmest thanks to Docent Eero Honkanen for creating an encouraging and inspiring atmosphere and providing possibilities to start and maintain research work alongside the clinical duties.

Another previous superior, Alpo Hirvioja, is deeply acknowledged for his kind and human-centric attitude towards his crew, including me. He never denied my applications for research leave and encouraged me endlessly. Docent Auvo Rauhala is thanked for emphasizing the importance of academic work and facilitating the possibilities for that in Vaasa Central Hospital. I'm grateful to my current superior Christian Kantola, for his open and straightforward leadership and providing me the possibility to complete this research project.

I warmly thank my former and current colleagues in Helsinki University Hospital and Vaasa Central Hospital for their support and for sharing their mutual professionalism every day. My heartfelt thanks go to the renal team in the nephrology outpatient unit and to the personnel in the dialysis unit of Vaasa Central Hospital: they have patiently believed in me and tolerated my research leaves during all these years. I'm very grateful to my nearest colleague Katariina Kiili; her loyal devotion to work and great flexibility made my absences from the clinics possible. The latest member of our renal team, Professor Risto Tertti, has made a great impact on the last steps of this work by stabilising our team and giving me the faith that this research is just a project among others and possible to accomplish.

I am blessed with many dear friends: thank you for your good company and many happy moments together. Even though my career is not important in our relationships, you have encouraged me patiently. I am already looking forward to seeing you more often in the future.

Many thanks to my family, in many generations, for always being there. Due to this work, I may have been absent(minded) or sometimes moody, but in times to come I will meet and treat you in an appropriate manner and frequency.

Special thanks to dear Kari for so many things and qualities. Your understanding and support gave me the strength to finalise this work. It is a pleasant coincidence that our PhD projects are being completed simultaneously, hand in hand.

My warm sympathy goes to the patients suffering from ANCA-vasculitis. I thank all the patients who participated in the studies. I have the heart of a clinician and I hope that the time taken by this scientific work has given me knowledge and tools to help them in their struggle against this disease.

Financial support from the Competitive State Research Financing of the Expert Responsibility Area of Tampere University Hospital and Vaasa Central Hospital, Medical Society of Finland (Finska Läkaresällskapet), Kidney Foundation in Finland (Munuaissäätiö), Medical Foundation of Vaasa (Medicinska stiftelsen i Vasa – Vaasan lääketieteellinen säätiö rs) and Jussi Lall's and Eeva Meripori-Lall's Foundation is gratefully acknowledged.

Vaasa, early November 2018
Anna Salmela

9 REFERENCES

1. Berden AE, Ferrario F, Hagen EC, *et al*. Histopathologic classification of ANCA-associated glomerulonephritis. J Am Soc Nephrol 2010; 21(10):1628-36.
2. Pinching AJ, Rees AJ, Pussell BA, Lockwood CM, Mitchison RS, Peters DK. Relapses in Wegener's granulomatosis: the role of infection. Br Med J 1980; 281(6244):836-8.
3. Fauci AS, Haynes BF, Katz P, Wolff SM. Wegener's granulomatosis: prospective clinical and therapeutic experience with 85 patients for 21 years. Ann Intern Med 1983; 98(1):76-85.
4. Stegeman CA, Tervaert JW, Sluiter WJ, Manson WL, de Jong PE, Kallenberg CG. Association of chronic nasal carriage of Staphylococcus aureus and higher relapse rates in Wegener granulomatosis. Ann Intern Med 1994; 120(1):12-7.
5. von Scheven E, Lu TT, Emery HM, Elder ME, Wara DW. Thrombosis and pediatric Wegener's granulomatosis: acquired and genetic risk factors for hypercoagulability. Arthritis Rheum 2003; 49(6):862-5.
6. Merkel PA, Lo GH, Holbrook JT, *et al*. Brief communication: high incidence of venous thrombotic events among patients with Wegener granulomatosis: the Wegener's Clinical Occurrence of Thrombosis (WeCLOT) Study. Ann Intern Med 2005; 142(8):620-6.
7. Kussmaul A, Maier R. Über eine bisher nicht beschriebene eigenthümliche Arterienerkrankung (Periarteritis nodosa), die mit Morbus Brightii und rapid fortschreitender, allgemeiner Muskellähmung einhergeht. Deutsches Archiv für klinische Medicin 1866; 1:484-518.
8. Wegener F. Uber eine eigenartige rhinogene Granulomatose mit besonderer Beteiligung des Arteriensystems und der Nieren. Beitr Path Anat 1939; 102:36-68.
9. Klinger H. Grenzformen der Periarteriitis nodosa. Frankfurt Z Pathol 1931; 42:455.
10. Churg J, Strauss L. Allergic granulomatosis, allergic angiitis, and periarteritis nodosa. Am J Pathol 1951; 27(2):277-301.
11. van der Woude FJ, Rasmussen N, Lobatto S, *et al*. Autoantibodies against neutrophils and monocytes: tool for diagnosis and marker of disease activity in Wegener's granulomatosis. Lancet 1985; 1(8426):425-9.
12. Hunder GG, Arend WP, Bloch DA, *et al*. The American College of Rheumatology 1990 criteria for the classification of vasculitis. Introduction. Arthritis Rheum 1990; 33(8):1065-7.
13. Jennette JC, Falk RJ, Andrassy K, *et al*. Nomenclature of systemic vasculitides. Proposal of an international consensus conference. Arthritis Rheum 1994; 37(2):187-92.
14. Watts R, Lane S, Hanslik T, *et al*. Development and validation of a consensus methodology for the classification of the ANCA-associated vasculitides and polyarteritis nodosa for epidemiological studies. Ann Rheum Dis 2007; 66(2):222-7.

15. Jennette JC, Falk RJ, Bacon PA, *et al*. 2012 revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides. Arthritis Rheum 2013; 65(1):1-11.
16. Craven A, Robson J, Ponte C, *et al*. ACR/EULAR-endorsed study to develop Diagnostic and Classification Criteria for Vasculitis (DCVAS). Clin Exp Nephrol 2013; 17(5):619-21.
17. Koldingsnes W, Nossent H. Epidemiology of Wegener's granulomatosis in northern Norway. Arthritis Rheum 2000; 43(11):2481-7.
18. Watts RA, Lane SE, Bentham G, Scott DG. Epidemiology of systemic vasculitis: a ten-year study in the United Kingdom. Arthritis Rheum 2000; 43(2):414-9.
19. Takala JH, Kautiainen H, Malmberg H, Leirisalo-Repo M. Incidence of Wegener's granulomatosis in Finland 1981-2000. Clin Exp Rheumatol 2008; 26(3 Suppl 49):81-5.
20. Reinhold-Keller E, Herlyn K, Wagner-Bastmeyer R, Gross WL. Stable incidence of primary systemic vasculitides over five years: results from the German vasculitis register. Arthritis Rheum 2005; 53(1):93-9.
21. Mohammad AJ, Jacobsson LT, Westman KW, Sturfelt G, Segelmark M. Incidence and survival rates in Wegener's granulomatosis, microscopic polyangiitis, Churg-Strauss syndrome and polyarteritis nodosa. Rheumatology (Oxford) 2009; 48(12):1560-5.
22. Watts RA, Gonzalez-Gay MA, Lane SE, Garcia-Porrua C, Bentham G, Scott DG. Geoepidemiology of systemic vasculitis: comparison of the incidence in two regions of Europe. Ann Rheum Dis 2001; 60(2):170-2.
23. Gonzalez-Gay MA, Garcia-Porrua C, Guerrero J, Rodriguez-Ledo P, Llorca J. The epidemiology of the primary systemic vasculitides in northwest Spain: implications of the Chapel Hill Consensus Conference definitions. Arthritis Rheum 2003; 49(3):388-93.
24. Panagiotakis SH, Perysinakis GS, Kritikos H, *et al*. The epidemiology of primary systemic vasculitides involving small vessels in Crete (southern Greece): a comparison of older versus younger adult patients. Clin Exp Rheumatol 2009; 27(3):409-15.
25. Mahr A, Guillevin L, Poissonnet M, Ayme S. Prevalences of polyarteritis nodosa, microscopic polyangiitis, Wegener's granulomatosis, and Churg-Strauss syndrome in a French urban multiethnic population in 2000: a capture-recapture estimate. Arthritis Rheum 2004; 51(1):92-9.
26. Mohammad AJ, Jacobsson LT, Mahr AD, Sturfelt G, Segelmark M. Prevalence of Wegener's granulomatosis, microscopic polyangiitis, polyarteritis nodosa and Churg-Strauss syndrome within a defined population in southern Sweden. Rheumatology (Oxford) 2007; 46(8):1329-37.
27. Watts RA, Mooney J, Skinner J, Scott DG, Macgregor AJ. The contrasting epidemiology of granulomatosis with polyangiitis (Wegener's) and microscopic polyangiitis. Rheumatology (Oxford) 2012; 51(5):926-31.

28. Calabresi P, Edwards EA, Schilling RF. Fluorescent antiglobulin studies in leukopenic and related disorders. J Clin Invest 1959; 38:2091-100.
29. Davies DJ, Moran JE, Niall JF, Ryan GB. Segmental necrotising glomerulonephritis with antineutrophil antibody: possible arbovirus aetiology? Br Med J (Clin Res Ed) 1982; 285(6342):606.
30. Cohen Tervaert J, Huitema M, Van der Giessen, Goldschmeding R, Van der Woude F, Kallenbeg C. Wegener's Granulomatosisandanti-cytoplasmicantibodies: the Groningenexperience. APMIS 1989; 97(Suppl 6):36.
31. Falk R, Jennette J. Immunofluorescence and ELISA determination of ANCA with description of a sub-class with anti-myeloperoxidase activity. APMIS 1989; 97(Suppl 6):45.
32. Wiik A. Rational use of ANCA in the diagnosis of vasculitis. Rheumatology (Oxford) 2002; 41(5):481-3.
33. Cui Z, Zhao MH, Segelmark M, Hellmark T. Natural autoantibodies to myeloperoxidase, proteinase 3, and the glomerular basement membrane are present in normal individuals. Kidney Int 2010; 78(6):590-7.
34. Roth AJ, Ooi JD, Hess JJ, *et al*. Epitope specificity determines pathogenicity and detectability in ANCA-associated vasculitis. J Clin Invest 2013; 123(4):1773-83.
35. Baslund B, Segelmark M, Wiik A, Szpirt W, Petersen J, Wieslander J. Screening for anti-neutrophil cytoplasmic antibodies (ANCA): is indirect immunofluorescence the method of choice? Clin Exp Immunol 1995; 99(3):486-92.
36. Westman KW, Selga D, Bygren P, *et al*. Clinical evaluation of a capture ELISA for detection of proteinase-3 antineutrophil cytoplasmic antibody. Kidney Int 1998; 53(5):1230-6.
37. Csernok E, Holle J, Hellmich B, *et al*. Evaluation of capture ELISA for detection of antineutrophil cytoplasmic antibodies directed against proteinase 3 in Wegener's granulomatosis: first results from a multicentre study. Rheumatology (Oxford) 2004; 43(2):174-80.
38. Hellmich B, Csernok E, Fredenhagen G, Gross WL. A novel high sensitivity ELISA for detection of antineutrophil cytoplasm antibodies against proteinase-3. Clin Exp Rheumatol 2007; 25(1 Suppl 44):1-5.
39. Holle JU, Herrmann K, Gross WL, Csernok E. Comparative analysis of different commercial ELISA systems for the detection of anti-neutrophil cytoplasm antibodies in ANCA-associated vasculitides. Clin Exp Rheumatol 2012; 30(1 Suppl 70):66-9.
40. Radice A, Bianchi L, Maggiore U, Vaglio A, Sinico RA. Comparison of PR3-ANCA specific assay performance for the diagnosis of granulomatosis with polyangiitis (Wegener's). Clin Chem Lab Med 2013; 51(11):2141-9.
41. Boomsma MM, Stegeman CA, Oost-Kort WW, *et al*. Native and recombinant proteins to analyze auto-antibodies to myeloperoxidase in pauci-immune crescentic glomerulonephritis. J Immunol Methods 2001; 254(1-2):47-58.

42. Csernok E, Moosig F. Current and emerging techniques for ANCA detection in vasculitis. Nat Rev Rheumatol 2014; 10(8):494-501.
43. Savige J, Gillis D, Benson E, *et al*. International Consensus Statement on Testing and Reporting of Antineutrophil Cytoplasmic Antibodies (ANCA). Am J Clin Pathol 1999; 111(4):507-13.
44. Damoiseaux J, Csernok E, Rasmussen N, *et al*. Detection of antineutrophil cytoplasmic antibodies (ANCAs): a multicentre European Vasculitis Study Group (EUVAS) evaluation of the value of indirect immunofluorescence (IIF) versus antigen-specific immunoassays. Ann Rheum Dis 2017; 76(4):647-53.
45. Bossuyt X, Cohen Tervaert JW, Arimura Y, *et al*. Position paper: Revised 2017 international consensus on testing of ANCAs in granulomatosis with polyangiitis and microscopic polyangiitis. Nat Rev Rheumatol 2017; 13(11):683-92.
46. Knight A, Sandin S, Askling J. Risks and relative risks of Wegener's granulomatosis among close relatives of patients with the disease. Arthritis Rheum 2008; 58(1):302-7.
47. Nowack R, Lehmann H, Flores-Suarez LF, Nanhou A, van der Woude FJ. Familial occurrence of systemic vasculitis and rapidly progressive glomerulonephritis. Am J Kidney Dis 1999; 34(2):364-73.
48. Cao Y, Schmitz JL, Yang J, *et al*. DRB1*15 allele is a risk factor for PR3-ANCA disease in African Americans. J Am Soc Nephrol 2011; 22(6):1161-7.
49. Lyons PA, Rayner TF, Trivedi S, *et al*. Genetically distinct subsets within ANCA-associated vasculitis. N Engl J Med 2012; 367(3):214-23.
50. Xie G, Roshandel D, Sherva R, *et al*. Association of granulomatosis with polyangiitis (Wegener's) with HLA-DPB1*04 and SEMA6A gene variants: evidence from genome-wide analysis. Arthritis Rheum 2013; 65(9):2457-68.
51. Zycinska K, Wardyn KA, Zielonka TM, Demkow U, Traburzynski MS. Chronic crusting, nasal carriage of Staphylococcus aureus and relapse rate in pulmonary Wegener's granulomatosis. J Physiol Pharmacol 2008; 59(Suppl 6):825-31.
52. Laudien M, Gadola SD, Podschun R, *et al*. Nasal carriage of Staphylococcus aureus and endonasal activity in Wegener s granulomatosis as compared to rheumatoid arthritis and chronic Rhinosinusitis with nasal polyps. Clin Exp Rheumatol 2010; 28(1 Suppl 57):51-5.
53. Kuehnert MJ, Kruszon-Moran D, Hill HA, *et al*. Prevalence of Staphylococcus aureus nasal colonization in the United States, 2001-2002. J Infect Dis 2006; 193(2):172-9.
54. Wertheim HF, Melles DC, Vos MC, *et al*. The role of nasal carriage in Staphylococcus aureus infections. Lancet Infect Dis 2005; 5(12):751-62.
55. Besada E, Koldingsnes W, Nossent JC. Staphylococcus Aureus carriage and long-term Rituximab treatment for Granulomatosis with polyangiitis. PeerJ 2015; 3:e1051.
56. Hui Y, Wohlers J, Podschun R, *et al*. Antimicrobial peptides in nasal secretion and mucosa with respect to S. aureus colonisation in Wegener s granulomatosis. Clin Exp Rheumatol 2011; 29(1 Suppl 64):49-56.

57. Wohlers J, Breucker K, Podschun R, *et al*. Aberrant cytokine pattern of the nasal mucosa in granulomatosis with polyangiitis. Arthritis Res Ther 2012; 14(5):R203.
58. Fraser JD, Proft T. The bacterial superantigen and superantigen-like proteins. Immunol Rev 2008; 225:226-43.
59. Popa ER, Stegeman CA, Abdulahad WH, *et al*. Staphylococcal toxic-shock-syndrome-toxin-1 as a risk factor for disease relapse in Wegener's granulomatosis. Rheumatology (Oxford) 2007; 46(6):1029-33.
60. Glasner C, van Timmeren MM, Stobernack T, *et al*. Low anti-staphylococcal IgG responses in granulomatosis with polyangiitis patients despite long-term Staphylococcus aureus exposure. Sci Rep 2015; 5:8188.
61. Glasner C, de Goffau MC, van Timmeren MM, *et al*. Genetic loci of Staphylococcus aureus associated with anti-neutrophil cytoplasmic autoantibody (ANCA)-associated vasculitides. Sci Rep 2017; 7:12211.
62. Pendergraft WF,3rd, Preston GA, Shah RR, *et al*. Autoimmunity is triggered by cPR-3(105-201), a protein complementary to human autoantigen proteinase-3. Nat Med 2004; 10(1):72-9.
63. Wilde B, van Paassen P, Witzke O, Tervaert JW. New pathophysiological insights and treatment of ANCA-associated vasculitis. Kidney Int 2011; 79(6):599-612.
64. Tadema H, Kallenberg CG, Stegeman CA, Heeringa P. Reactivity against complementary proteinase-3 is not increased in patients with PR3-ANCA-associated vasculitis. PLoS One 2011; 6(3):e17972.
65. Kain R, Exner M, Brandes R, *et al*. Molecular mimicry in pauci-immune focal necrotizing glomerulonephritis. Nat Med 2008; 14(10):1088-96.
66. Roth AJ, Brown MC, Smith RN, *et al*. Anti-LAMP-2 antibodies are not prevalent in patients with antineutrophil cytoplasmic autoantibody glomerulonephritis. J Am Soc Nephrol 2012; 23(3):545-55.
67. Gregorini G, Ferioli A, Donato F, *et al*. Association between silica exposure and necrotizing crescentic glomerulonephritis with p-ANCA and anti-MPO antibodies: a hospital-based case-control study. Adv Exp Med Biol 1993; 336:435-40.
68. Hogan SL, Satterly KK, Dooley MA, *et al*. Silica exposure in anti-neutrophil cytoplasmic autoantibody-associated glomerulonephritis and lupus nephritis. J Am Soc Nephrol 2001; 12(1):134-42.
69. Lane SE, Watts RA, Bentham G, Innes NJ, Scott DG. Are environmental factors important in primary systemic vasculitis? A case-control study. Arthritis Rheum 2003; 48(3):814-23.
70. Gomez-Puerta JA, Gedmintas L, Costenbader KH. The association between silica exposure and development of ANCA-associated vasculitis: systematic review and meta-analysis. Autoimmun Rev 2013; 12(12):1129-35.
71. Takeuchi Y, Saito A, Ojima Y, *et al*. The influence of the Great East Japan earthquake on microscopic polyangiitis: A retrospective observational study. PLoS One 2017; 12(5):e0177482.
72. Knight A, Sandin S, Askling J. Occupational risk factors for Wegener's granulomatosis- a case control study. Ann Rheum Dis 2010; 69(4):737-49.

73. Choi HK, Slot MC, Pan G, Weissbach CA, Niles JL, Merkel PA. Evaluation of antineutrophil cytoplasmic antibody seroconversion induced by minocycline, sulfasalazine, or penicillamine. Arthritis Rheum 2000; 43(11):2488-92.
74. Gao Y, Zhao MH. Review article: Drug-induced anti-neutrophil cytoplasmic antibody-associated vasculitis. Nephrology (Carlton) 2009; 14(1):33-41.
75. Falk RJ, Terrell RS, Charles LA, Jennette JC. Anti-neutrophil cytoplasmic autoantibodies induce neutrophils to degranulate and produce oxygen radicals in vitro. Proc Natl Acad Sci U S A 1990; 87(11):4115-9.
76. Huugen D, Xiao H, van Esch A, *et al*. Aggravation of anti-myeloperoxidase antibody-induced glomerulonephritis by bacterial lipopolysaccharide: role of tumor necrosis factor-alpha. Am J Pathol 2005; 167(1):47-58.
77. Hewins P, Morgan MD, Holden N, *et al*. IL-18 is upregulated in the kidney and primes neutrophil responsiveness in ANCA-associated vasculitis. Kidney Int 2006; 69(3):605-15.
78. Schreiber A, Xiao H, Jennette JC, Schneider W, Luft FC, Kettritz R. C5a receptor mediates neutrophil activation and ANCA-induced glomerulonephritis. J Am Soc Nephrol 2009; 20(2):289-98.
79. Jennette JC, Falk RJ. Pathogenesis of antineutrophil cytoplasmic autoantibody-mediated disease. Nat Rev Rheumatol 2014; 10(8):463-73.
80. Porges AJ, Redecha PB, Kimberly WT, Csernok E, Gross WL, Kimberly RP. Anti-neutrophil cytoplasmic antibodies engage and activate human neutrophils via Fc gamma RIIa. J Immunol 1994; 153(3):1271-80.
81. Kocher M, Siegel ME, Edberg JC, Kimberly RP. Cross-linking of Fc gamma receptor IIa and Fc gamma receptor IIIb induces different proadhesive phenotypes on human neutrophils. J Immunol 1997; 159(8):3940-8.
82. Williams JM, Ben-Smith A, Hewins P, *et al*. Activation of the G(i) heterotrimeric G protein by ANCA IgG F(ab')2 fragments is necessary but not sufficient to stimulate the recruitment of those downstream mediators used by intact ANCA IgG. J Am Soc Nephrol 2003; 14(3):661-9.
83. Xiao H, Hu P, Falk RJ, Jennette JC. Overview of the Pathogenesis of ANCA-Associated Vasculitis. Kidney Dis (Basel) 2016; 1(4):205-15.
84. Jarrot PA, Kaplanski G. Pathogenesis of ANCA-associated vasculitis: An update. Autoimmun Rev 2016; 15(7):704-13.
85. Halbwachs L, Lesarve P. Endothelium-Neutrophil Interactions in ANCA-associated Diseases. J Am Soc Nephrol 2012; 23(9):1449-61.
86. Xiao H, Schreiber A, Heeringa P, Falk RJ, Jennette JC. Alternative complement pathway in the pathogenesis of disease mediated by anti-neutrophil cytoplasmic autoantibodies. Am J Pathol 2007; 170(1):52-64.
87. Xing GQ, Chen M, Liu G, *et al*. Complement activation is involved in renal damage in human antineutrophil cytoplasmic autoantibody associated pauci-immune vasculitis. J Clin Immunol 2009; 29(3):282-91.

88. Gou SJ, Yuan J, Chen M, Yu F, Zhao MH. Circulating complement activation in patients with anti-neutrophil cytoplasmic antibody-associated vasculitis. Kidney Int 2013; 83(1):129-37.
89. Xiao H, Dairaghi DJ, Powers JP, *et al*. C5a receptor (CD88) blockade protects against MPO-ANCA GN. J Am Soc Nephrol 2014; 25(2):225-31.
90. Brinkmann V, Reichard U, Goosmann C, *et al*. Neutrophil extracellular traps kill bacteria. Science 2004; 303(5663):1532-5.
91. Kessenbrock K, Krumbholz M, Schonermarck U, *et al*. Netting neutrophils in autoimmune small-vessel vasculitis. Nat Med 2009; 15(6):623-5.
92. Nakazawa D, Tomaru U, Yamamoto C, Jodo S, Ishizu A. Abundant neutrophil extracellular traps in thrombus of patient with microscopic polyangiitis. Front Immunol 2012; 3:333.
93. Kambas K, Chrysanthopoulou A, Vassilopoulos D, *et al*. Tissue factor expression in neutrophil extracellular traps and neutrophil derived microparticles in antineutrophil cytoplasmic antibody associated vasculitis may promote thromboinflammation and the thrombophilic state associated with the disease. Ann Rheum Dis 2014; 73(10):1854-63.
94. Hong Y, Eleftheriou D, Hussain AA, *et al*. Anti-neutrophil cytoplasmic antibodies stimulate release of neutrophil microparticles. J Am Soc Nephrol 2012; 23(1):49-62.
95. Eleftheriou D, Hong Y, Klein NJ, Brogan PA. Thromboembolic disease in systemic vasculitis is associated with enhanced microparticle-mediated thrombin generation. J Thromb Haemost 2011; 9(9):1864-7.
96. Luqmani RA, Bacon PA, Moots RJ, *et al*. Birmingham Vasculitis Activity Score (BVAS) in systemic necrotizing vasculitis. QJM 1994; 87(11):671-8.
97. Mahr A, Katsahian S, Varet H, *et al*. Revisiting the classification of clinical phenotypes of anti-neutrophil cytoplasmic antibody-associated vasculitis: a cluster analysis. Ann Rheum Dis 2013; 72(6):1003-10.
98. Solans-Laque R, Fraile G, Rodriguez-Carballeira M, *et al*. Clinical characteristics and outcome of Spanish patients with ANCA-associated vasculitides: Impact of the vasculitis type, ANCA specificity, and treatment on mortality and morbidity. Medicine (Baltimore) 2017; 96(8):e6083.
99. Lionaki S, Boletis JN. The Prevalence and Management of Pauci-Immune Glomerulonephritis and Vasculitis in Western Countries. Kidney Dis (Basel) 2016; 1(4):224-34.
100. Bajema IM, Hagen EC, van der Woude FJ, Bruijn JA. Wegener's granulomatosis: a meta-analysis of 349 literary case reports. J Lab Clin Med 1997; 129(1):17-22.
101. Hauer HA, Bajema IM, Van Houwelingen HC, *et al*. Determinants of outcome in ANCA-associated glomerulonephritis: a prospective clinico-histopathological analysis of 96 patients. Kidney Int 2002; 62(5):1732-42.
102. Hellmich B, Flossman O, Gross WL, *et al.* EULAR recommendations for conducting clinical studies and/or clinical trials in systemic vasculitis: focus on

anti-neutrophil cytoplasm antibody-associated vasculitis. Ann Rheum Dis 2007; 66(5):605-617.
103. Kemna MJ, Tervaert JW. Does one size fit all? J Rheumatol 2013; 40(11):1781-4.
104. Fauci AS, Wolff SM. Wegener's granulomatosis: studies in eighteen patients and a review of the literature. Medicine (Baltimore) 1973; 52(6):535-61.
105. Jayne D, Rasmussen N, Andrassy K, *et al*. A randomized trial of maintenance therapy for vasculitis associated with antineutrophil cytoplasmic autoantibodies. N Engl J Med 2003; 349(1):36-44.
106. de Groot K, Harper L, Jayne DR, *et al*. Pulse versus daily oral cyclophosphamide for induction of remission in antineutrophil cytoplasmic antibody-associated vasculitis: a randomized trial. Ann Intern Med 2009; 150(10):670-80.
107. Harper L, Morgan MD, Walsh M, *et al*. Pulse versus daily oral cyclophosphamide for induction of remission in ANCA-associated vasculitis: long-term follow-up. Ann Rheum Dis 2012; 71(6):955-60.
108. Yates M, Watts RA, Bajema IM, *et al*. EULAR/ERA-EDTA recommendations for the management of ANCA-associated vasculitis. Ann Rheum Dis 2016; 75(9):1583-94.
109. Stone JH, Merkel PA, Spiera R, *et al*. Rituximab versus cyclophosphamide for ANCA-associated vasculitis. N Engl J Med 2010; 363(3):221-32.
110. Jones RB, Tervaert JW, Hauser T, *et al*. Rituximab versus cyclophosphamide in ANCA-associated renal vasculitis. N Engl J Med 2010; 363(3):211-20.
111. Geetha D, Hruskova Z, Segelmark M, *et al*. Rituximab for treatment of severe renal disease in ANCA associated vasculitis. J Nephrol 2016; 29(2):195-201.
112. Unizony S, Villarreal M, Miloslavsky EM, *et al*. Clinical outcomes of treatment of anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis based on ANCA type. Ann Rheum Dis 2016; 75(6):1166-9.
113. Jayne DR, Gaskin G, Rasmussen N, *et al*. Randomized trial of plasma exchange or high-dosage methylprednisolone as adjunctive therapy for severe renal vasculitis. J Am Soc Nephrol 2007; 18(7):2180-8.
114. Walsh M, Merkel PA, Peh CA, *et al.* Plasma exchange and glucocorticoid dosing in the treatment of anti-neutrophil cytoplasm antibody associated vasculitis (PEXIVAS) protocol for a randomized controlled trial. Trials 2013; 14:73.
115. De Groot K, Rasmussen N, Bacon PA, *et al*. Randomized trial of cyclophosphamide versus methotrexate for induction of remission in early systemic antineutrophil cytoplasmic antibody-associated vasculitis. Arthritis Rheum 2005; 52(8):2461-9.
116. Faurschou M, Westman K, Rasmussen N, *et al*. Brief Report: long-term outcome of a randomized clinical trial comparing methotrexate to cyclophosphamide for remission induction in early systemic antineutrophil cytoplasmic antibody-associated vasculitis. Arthritis Rheum 2012; 64(10):3472-7.
117. Hu W, Liu C, Xie H, Chen H, Liu Z, Li L. Mycophenolate mofetil versus cyclophosphamide for inducing remission of ANCA vasculitis with moderate renal involvement. Nephrol Dial Transplant 2008; 23(4):1307-12.

118. Han F, Liu G, Zhang X, *et al*. Effects of mycophenolate mofetil combined with corticosteroids for induction therapy of microscopic polyangiitis. Am J Nephrol 2011; 33(2):185-92.
119. Lee T, Gasim A, Derebail VK, *et al*. Predictors of treatment outcomes in ANCA-associated vasculitis with severe kidney failure. Clin J Am Soc Nephrol 2014; 9(5):905-13.
120. Guillevin L, Pagnoux C, Karras A, *et al*. Rituximab versus azathioprine for maintenance in ANCA-associated vasculitis. N Engl J Med 2014; 371(19):1771-80.
121. Terrier B, Pagnoux C, Perrodeau, *et al*. Long-term efficacy of remission-maintenance regimens in ANCA-associated vasculitides. Ann Rheum Dis 2018; 77:1150-1156.
122. Pagnoux C, Mahr A, Hamidou MA, *et al*. Azathioprine or methotrexate maintenance for ANCA-associated vasculitis. N Engl J Med 2008; 359(26):2790-803.
123. Metzler C, Miehle N, Manger K, *et al*. Elevated relapse rate under oral methotrexate versus leflunomide for maintenance of remission in Wegener's granulomatosis. Rheumatology (Oxford) 2007; 46(7):1087-91.
124. Maritati F, Alberici F, Oliva E, *et al*. Methotrexate versus cyclophosphamide for remission maintenance in ANCA-associated vasculitis: A randomised trial. PLoS One 2017; 12(10):e0185880.
125. Hiemstra TF, Walsh M, Mahr A, *et al*. Mycophenolate mofetil vs azathioprine for remission maintenance in antineutrophil cytoplasmic antibody-associated vasculitis: a randomized controlled trial. JAMA 2010; 304(21):2381-8.
126. Stone JH, Holbrook JT, Marriott MA, *et al*. Solid malignancies among patients in the Wegener's Granulomatosis Etanercept Trial. Arthritis Rheum 2006; 54(5):1608-18.
127. Stegeman CA, Tervaert JW, de Jong PE, Kallenberg CG. Trimethoprim-sulfamethoxazole (co-trimoxazole) for the prevention of relapses of Wegener's granulomatosis. Dutch Co-Trimoxazole Wegener Study Group. N Engl J Med 1996; 335(1):16-20.
128. Ntatsaki E, Carruthers D, Chakravarty K, *et al*. BSR and BHPR guideline for the management of adults with ANCA-associated vasculitis. Rheumatology (Oxford) 2014; 53(12):2306-9.
129. Jennette JC, Nachman PH. ANCA Glomerulonephritis and Vasculitis. Clin J Am Soc Nephrol 2017; 12(10):1680-91.
130. de Joode AA, Sanders JS, Rutgers A, Stegeman CA. Maintenance therapy in antineutrophil cytoplasmic antibody-associated vasculitis: who needs what and for how long? Nephrol Dial Transplant 2015; (30 Suppl 1):150-8.
131. Walsh M, Merkel PA, Mahr A, Jayne D. Effects of duration of glucocorticoid therapy on relapse rate in antineutrophil cytoplasmic antibody-associated vasculitis: A meta-analysis. Arthritis Care Res (Hoboken) 2010; 62(8):1166-73.
132. Sanders JS, de Joode AA, DeSevaux RG, *et al*. Extended versus standard azathioprine maintenance therapy in newly diagnosed proteinase-3 anti-neutrophil

cytoplasmic antibody-associated vasculitis patients who remain cytoplasmic anti-neutrophil cytoplasmic antibody-positive after induction of remission: a randomized clinical trial. Nephrol Dial Transplant 2016; 31(9):1453-9.
133. de Joode AAE, Sanders JSF, Puechal X, *et al*. Long-term azathioprine maintenance therapy in ANCA-associated vasculitis: combined results of long-term follow-up data. Rheumatology (Oxford) 2017; 56(11):1894-901.
134. Flossmann O, Baslund B, Bruchfeld A, *et al*. Deoxyspergualin in relapsing and refractory Wegener's granulomatosis. Ann Rheum Dis 2009; 68(7):1125-30.
135. Jayne DR, Chapel H, Adu D, *et al*. Intravenous immunoglobulin for ANCA-associated systemic vasculitis with persistent disease activity. QJM 2000; 93(7):433-9.
136. Tomasson G, Grayson PC, Mahr AD, Lavalley M, Merkel PA. Value of ANCA measurements during remission to predict a relapse of ANCA-associated vasculitis--a meta-analysis. Rheumatology (Oxford) 2012; 51(1):100-9.
137. Thai LH, Charles P, Resche-Rigon M, Desseaux K, Guillevin L. Are anti-proteinase-3 ANCA a useful marker of granulomatosis with polyangiitis (Wegener's) relapses? Results of a retrospective study on 126 patients. Autoimmun Rev 2014; 13(3):313-8.
138. Verstockt B, Bossuyt X, Vanderschueren S, Blockmans D. There is no benefit in routinely monitoring ANCA titres in patients with granulomatosis with polyangiitis. Clin Exp Rheumatol 2015; 33(2 Suppl 89):72-6.
139. Finkielman JD, Lee AS, Hummel AM, *et al*. ANCA are detectable in nearly all patients with active severe Wegener's granulomatosis. Am J Med 2007; 120(7):643.e9-14.
140. Kemna MJ, Damoiseaux J, Austen J, *et al*. ANCA as a predictor of relapse: useful in patients with renal involvement but not in patients with nonrenal disease. J Am Soc Nephrol 2015; 26(3):537-42.
141. Fussner LA, Hummel AM, Schroeder DR, *et al*. Factors Determining the Clinical Utility of Serial Measurements of Antineutrophil Cytoplasmic Antibodies Targeting Proteinase 3. Arthritis Rheumatol 2016; 68(7):1700-10.
142. Charles P, Terrier B, Perrodeau E, *et al*. Comparison of individually tailored versus fixed-schedule rituximab regimen to maintain ANCA-associated vasculitis remission: results of a multicentre, randomised controlled, phase III trial (MAINRITSAN2). Ann Rheum Dis 2018; 77(8):1143-1149.
143. Walton EW. Giant-cell granuloma of the respiratory tract (Wegener's granulomatosis). Br Med J 1958; 2(5091):265-70.
144. Holle JU, Gross WL, Latza U, *et al*. Improved outcome in 445 patients with Wegener's granulomatosis in a German vasculitis center over four decades. Arthritis Rheum 2011; 63(1):257-66.
145. Hilhorst M, Wilde B, van Paassen P, *et al*. Improved outcome in anti-neutrophil cytoplasmic antibody (ANCA)-associated glomerulonephritis: a 30-year follow-up study. Nephrol Dial Transplant 2013; 28(2):373-9.

146. Rhee RL, Hogan SL, Poulton CJ, *et al*. Trends in Long-Term Outcomes Among Patients With Antineutrophil Cytoplasmic Antibody-Associated Vasculitis With Renal Disease. Arthritis Rheumatol 2016; 68(7):1711-20.
147. Flossmann O, Berden A, de Groot K, *et al*. Long-term patient survival in ANCA-associated vasculitis. Ann Rheum Dis 2011; 70(3):488-94.
148. de Joode AA, Sanders JS, Stegeman CA. Renal survival in proteinase 3 and myeloperoxidase ANCA-associated systemic vasculitis. Clin J Am Soc Nephrol 2013; 8(10):1709-17.
149. Moroni G, Binda V, Leoni A, *et al*. Predictors of renal survival in ANCA-associated vasculitis. Validation of a histopatological classification schema and review of the literature. Clin Exp Rheumatol 2015; 33(2 Suppl 89):56-63.
149. Puechal X, Pagnoux C, Perrodeau E, *et al*. Long-Term Outcomes Among Participants in the WEGENT Trial of Remission-Maintenance Therapy for Granulomatosis with Polyangiitis (Wegener's) or Microscopic Polyangiitis. Arthritis Rheumatol 2016; 68(3):690-701.
151. Tan JA, Dehghan N, Chen W, Xie H, Esdaile JM, Avina-Zubieta JA. Mortality in ANCA-associated vasculitis: ameta-analysis of observational studies. Ann Rheum Dis 2017; 76(9):1566-74.
152. Sriskandarajah S, Aasarod K, Skrede S, Knoop T, Reisaeter AV, Bjorneklett R. Improved prognosis in Norwegian patients with glomerulonephritis associated with anti-neutrophil cytoplasmic antibodies. Nephrol Dial Transplant 2015; 30(Suppl 1):67-75.
153. Mukhtyar C, Flossmann O, Hellmich B, *et al*. Outcomes from studies of Antineutrophil Cytoplasm Antibody Associated Vasculitis: a systematic review by the EULAR Systemic Vasculitis Task Force. Ann Rheum Dis 2007; 67(7):1004-10.
154. Mahr A, Katsahian S, Varet H, *et al*. Revisiting the classification of clinical phenotypes of anti-neutrophil cytoplasmic antibody-associated vasculitis: a cluster analysis. Ann Rheum Dis 2013; 72(6):1003-10.
155. Romeu M, Couchoud C, Delaroziere JC, *et al*. Survival of patients with ANCA-associated vasculitis on chronic dialysis: data from the French REIN registry from 2002 to 2011. QJM 2014; 107(7):545-55.
156. Bjorneklett R, Sriskandarajah S, Bostad L. Prognostic Value of Histologic Classification of ANCA-Associated Glomerulonephritis. Clin J Am Soc Nephrol 2016; 11(12):2159-67.
157. Cordova-Sanchez BM, Mejia-Vilet JM, Morales-Buenrostro LE, Loyola-Rodriguez G, Uribe-Uribe NO, Correa-Rotter R. Clinical presentation and outcome prediction of clinical, serological, and histopathological classification schemes in ANCA-associated vasculitis with renal involvement. Clin Rheumatol 2016; 35(7):1805-16.
158. Chang DY, Wu LH, Liu G, Chen M, Kallenberg CG, Zhao MH. Re-evaluation of the histopathologic classification of ANCA-associated glomerulonephritis: a study of 121 patients in a single center. Nephrol Dial Transplant 2012; 27(6):2343-9.

159. Hilhorst M, Wilde B, van Breda Vriesman P, van Paassen P, Cohen Tervaert JW, Limburg Renal Registry. Estimating renal survival using the ANCA-associated GN classification. J Am Soc Nephrol 2013; 24(9):1371-5.
160. Ellis CL, Manno RL, Havill JP, Racusen LC, Geetha D. Validation of the new classification of pauci-immune glomerulonephritis in a United States cohort and its correlation with renal outcome. BMC Nephrol 2013; 14:210.
161. Iwakiri T, Fujimoto S, Kitagawa K, *et al*. Validation of a newly proposed histopathological classification in Japanese patients with anti-neutrophil cytoplasmic antibody-associated glomerulonephritis. BMC Nephrol 2013; 14:125.
162. Ford SL, Polkinghorne KR, Longano A, *et al*. Histopathologic and clinical predictors of kidney outcomes in ANCA-associated vasculitis. Am J Kidney Dis 2014; 63(2):227-35.
163. Quintana LF, Perez NS, De Sousa E, *et al*. ANCA serotype and histopathological classification for the prediction of renal outcome in ANCA-associated glomerulonephritis. Nephrol Dial Transplant 2014; 29(9):1764-9.
164. Tanna A, Guarino L, Tam FW, *et al*. Long-term outcome of anti-neutrophil cytoplasm antibody-associated glomerulonephritis: evaluation of the international histological classification and other prognostic factors. Nephrol Dial Transplant 2015; 30(7):1185-92.
165. Kristensen T, Gregersen JW, Krag SR, Ivarsen P. The relation between histopathological classification and renal outcome, ANCA subtype and treatment regimens in ANCA-associated vasculitis. Clin Exp Rheumatol 2016; 34(3 Suppl 97):105-10.
166. Booth AD, Almond MK, Burns A, *et al*. Outcome of ANCA-associated renal vasculitis: a 5-year retrospective study. Am J Kidney Dis 2003; 41(4):776-84.
167. Hogan SL, Falk RJ, Chin H, *et al*. Predictors of relapse and treatment resistance in antineutrophil cytoplasmic antibody-associated small-vessel vasculitis. Ann Intern Med 2005; 143(9):621-31.
168. Pierrot-Deseilligny Despujol C, Pouchot J, Pagnoux C, Coste J, Guillevin L. Predictors at diagnosis of a first Wegener's granulomatosis relapse after obtaining complete remission. Rheumatology (Oxford) 2010; 49(11):2181-90.
169. Walsh M, Flossmann O, Berden A, *et al*. Risk factors for relapse of antineutrophil cytoplasmic antibody-associated vasculitis. Arthritis Rheum 2012; 64(2):542-8.
170. Slot MC, Tervaert JW, Boomsma MM, Stegeman CA. Positive classic antineutrophil cytoplasmic antibody (C-ANCA) titer at switch to azathioprine therapy associated with relapse in proteinase 3-related vasculitis. Arthritis Rheum 2004; 51(2):269-73.
171. Hilhorst M, Arndt F, Joseph Kemna M, *et al*. HLA-DPB1 as a Risk Factor for Relapse in Antineutrophil Cytoplasmic Antibody-Associated Vasculitis: A Cohort Study. Arthritis Rheumatol 2016; 68(7):1721-30.
172. Goceroglu A, Berden AE, Fiocco M, *et al*. ANCA-Associated Glomerulonephritis: Risk Factors for Renal Relapse. PLoS One 2016; 11(12):e0165402.

173. Weidner S, Hafezi-Rachti S, Rupprecht HD. Thromboembolic events as a complication of antineutrophil cytoplasmic antibody-associated vasculitis. Arthritis Rheum 2006; 55(1):146-9.
174. Stassen PM, Derks RP, Kallenberg CG, Stegeman CA. Venous thromboembolism in ANCA-associated vasculitis--incidence and risk factors. Rheumatology (Oxford) 2008; 47(4):530-4.
175. Allenbach Y, Seror R, Pagnoux C, *et al*. High frequency of venous thromboembolic events in Churg-Strauss syndrome, Wegener's granulomatosis and microscopic polyangiitis but not polyarteritis nodosa: a systematic retrospective study on 1130 patients. Ann Rheum Dis 2009; 68(4):564-7.
176. Kronbichler A, Leierer J, Leierer G, *et al*. Clinical associations with venous thromboembolism in anti-neutrophil cytoplasm antibody-associated vasculitides. Rheumatology (Oxford) 2017; 56(5):704-8.
177. Faurschou M, Obel N, Baslund B. High risk of pulmonary embolism and deep venous thrombosis but not of stroke in granulomatosis with polyangiitis (Wegener's). Arthritis Care Res (Hoboken) 2014; 66(12):1910-4.
178. Sebastian JK, Voetsch B, Stone JH, *et al*. The frequency of anticardiolipin antibodies and genetic mutations associated with hypercoagulability among patients with Wegener's granulomatosis with and without history of a thrombotic event. J Rheumatol 2007; 34(12):2446-50.
179. Rees JD, Lanca S, Marques PV, *et al*. Prevalence of the antiphospholipid syndrome in primary systemic vasculitis. Ann Rheum Dis 2006; 65(1):109-11.
180. Sebastian JK, Mahr AD, Ahmed SS, *et al*. Antiendothelial cell antibodies in patients with Wegener's granulomatosis: prevalence and correlation with disease activity and manifestations. J Rheumatol 2007; 34(5):1027-31.
181. Hergesell O, Andrassy K, Nawroth P. Elevated levels of markers of endothelial cell damage and markers of activated coagulation in patients with systemic necrotizing vasculitis. Thromb Haemost 1996; 75(6):892-8.
182. Koster T, Blann AD, Briet E, Vandenbroucke JP, Rosendaal FR. Role of clotting factor VIII in effect of von Willebrand factor on occurrence of deep-vein thrombosis. Lancet 1995; 345(8943):152-5.
183. Kraaijenhagen RA, in't Anker PS, Koopman MM, *et al*. High plasma concentration of factor VIIIc is a major risk factor for venous thromboembolism. Thromb Haemost 2000; 83(1):5-9.
184. Berden AE, Nolan SL, Morris HL, *et al*. Anti-Plasminogen Antibodies Compromise Fibrinolysis and Associate with Renal Histology in ANCA-Associated Vasculitis. J Am Soc Nephrol 2010; 21(12):2169-79.
185. Wattanakit K, Cushman M, Stehman-Breen C, Heckbert SR, Folsom AR. Chronic kidney disease increases risk for venous thromboembolism. J Am Soc Nephrol 2008; 19(1):135-40.
186. Wannamethee SG, Shaper AG, Lowe GD, Lennon L, Rumley A, Whincup PH. Renal function and cardiovascular mortality in elderly men: the role of

inflammatory, procoagulant, and endothelial biomarkers. Eur Heart J 2006; 27(24):2975-81.
187. Jalal DI, Chonchol M, Targher G. Disorders of hemostasis associated with chronic kidney disease. Semin Thromb Hemost 2010; 36(1):34-40.
188. Shashar M, Francis J, Chitalia V. Thrombosis in the uremic milieu--emerging role of "thrombolome". Semin Dial 2015; 28(2):198-205.
189. Johannesdottir SA, Horvath-Puho E, Dekkers OM, *et al*. Use of Glucocorticoids and Risk of Venous Thromboembolism: A Nationwide Population-Based Case-Control Study. JAMA Intern Med 2013; 173(9):743-52.
190. Hilhorst M, Winckers K, Wilde B, van Oerle R, ten Cate H, Tervaert JW. Patients with antineutrophil cytoplasmic antibodies associated vasculitis in remission are hypercoagulable. J Rheumatol 2013; 40(12):2042-6.
191. Little MA, Nightingale P, Verburgh CA, *et al*. Early mortality in systemic vasculitis: relative contribution of adverse events and active vasculitis. Ann Rheum Dis 2010; 69(6):1036-43.
192. Charlier C, Henegar C, Launay O, *et al*. Risk factors for major infections in Wegener granulomatosis: analysis of 113 patients. Ann Rheum Dis 2009; 68(5):658-63.
193. Hoffman GS, Kerr GS, Leavitt RY, *et al*. Wegener granulomatosis: an analysis of 158 patients. Ann Intern Med 1992; 116(6):488-98.
194. Knight A, Askling J, Ekbom A. Cancer incidence in a population-based cohort of patients with Wegener's granulomatosis. Int J Cancer 2002; 100(1):82-5.
195. Faurschou M, Sorensen IJ, Mellemkjaer L, *et al*. Malignancies in Wegener's granulomatosis: incidence and relation to cyclophosphamide therapy in a cohort of 293 patients. J Rheumatol 2008; 35(1):100-5.
196. Heijl C, Harper L, Flossmann O, *et al*. Incidence of malignancy in patients treated for antineutrophil cytoplasm antibody-associated vasculitis: follow-up data from European Vasculitis Study Group clinical trials. Ann Rheum Dis 2011; 70(8):1415-21.
197. van Daalen EE, Rizzo R, Kronbichler A, *et al*. Effect of rituximab on malignancy risk in patients with ANCA-associated vasculitis. Ann Rheum Dis 2017; 76(6):1064-9.
198. Faurschou M, Mellemkjaer L, Sorensen IJ, Svalgaard Thomsen B, Dreyer L, Baslund B. Increased morbidity from ischemic heart disease in patients with Wegener's granulomatosis. Arthritis Rheum 2009; 60(4):1187-92.
199. Suppiah R, Judge A, Batra R, *et al*. A model to predict cardiovascular events in patients with newly diagnosed Wegener's granulomatosis and microscopic polyangiitis. Arthritis Care Res (Hoboken) 2011; 63(4):588-96.
200. Bramlage CP, Kroplin J, Wallbach M, *et al*. Management of cardiovascular risk factors in patients with ANCA-associated vasculitis. J Eval Clin Pract 2017; 23(4):747-54.
201. Berti A, Matteson EL, Crowson CS, Specks U, Cornec D. Risk of Cardiovascular Disease and Venous Thromboembolism Among Patients With Incident ANCA-

Associated Vasculitis: A 20-Year Population-Based Cohort Study. Mayo Clin Proc 2018; 93(5):597-606.
202. Levey AS, Stevens LA, Schmid CH, *et al*. A new equation to estimate glomerular filtration rate. Ann Intern Med 2009; 150(9):604-12.
203. Takala JH, Kautiainen H, Leirisalo-Repo M. Survival of patients with Wegener's granulomatosis diagnosed in Finland in 1981-2000. Scand J Rheumatol 2010; 39(1):71-6.
204. Mohammad AJ, Segelmark M. A population-based study showing better renal prognosis for proteinase 3 antineutrophil cytoplasmic antibody (ANCA)-associated nephritis versus myeloperoxidase ANCA-associated nephritis. J Rheumatol 2014; 41(7):1366-73.
205. Kramer A, Pippias M, Noordzij M, *et al*. The European Renal Association - European Dialysis and Transplant Association (ERA-EDTA) Registry Annual Report 2015: a summary. Clin Kidney J 2018; 11(1):108-22.
206. Nouwen JL, Ott A, Kluytmans-Vandenbergh MF, *et al*. Predicting the Staphylococcus aureus nasal carrier state: derivation and validation of a "culture rule". Clin Infect Dis 2004; 39(6):806-11.
207. Ma TT, Huang YM, Wang C, Zhao MH, Chen M. Coagulation and fibrinolysis index profile in patients with ANCA-associated vasculitis. PLoS One 2014; 9(5):e97843.