EVALUATION OF PLATELET FUNCTION IN CHILDREN AND ADOLESCENTS WITH KIDNEY DISEASES AND HYPERTENSION

Ph.D. Thesis

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***In extenso* publications in topic of the thesis**

I. Túri S., **Bereczki Cs.**, Torday Cs., Havass Z., Németh M.: The effects of bicarbonate and acetate haemodialysis on platelet cyclic AMP concentration, thromboxane B_2 release and aggregation.
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II. **Bereczki Cs.**, Túri S., Sallai É., Torday Cs., Mohácsi G., Sonkodi S.: Increased platelet thromboxane release in focal segmental glomerulosclerosis.
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III. Túri S., **Bereczki Cs.**, Torday Cs., Havass Z.: Laser-aggregometer studies, ATP release and thromboxane B_2 release and cAMP concentration of the platelets in nephrotic syndrome.
Prostaglandins, Leukotrienes and Essential Fatty Acids **(1994)**, 51: 69-73.

IV. Túri S., Soós J., Torday Cs., **Bereczki Cs.**, Havass Z.: The effect of erythropoietin on platelet function in uraemic children on haemodialysis.
Pediatric Nephrology **(1994)**, 8: 727-732.

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VI. Túri S., Soós J., Torday Cs., **Bereczki Cs.**, Havass Z.: Effect of r-HuEPO on platelet function in uraemic children.
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VII. **Bereczki Cs.**, Túri S., Németh I., Sallai É., Torday Cs., Nagy E., Haszon I., Papp F.: The roles of platelet function, thromboxane, blood lipids and nitric oxide in hypertension of children and adolescents.
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European Dialysis and Transplant Association Meeting, Amsterdam, 1996
Nephrology Dialysis and Transplantation 1996, 11: A56

IV. **Bereczki Cs.**, Túri S., Sághy L., Mohácsi G., Ábrahám G, Torday Cs., Sonkodi S.: Platelet function in focal segmental glomerulosclerosis and minimal change nephrotic syndrome.
American National Kidney Foundation Meeting Dallas 1997
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V. Túri S., **Bereczki Cs.,** Sághy L., Mohácsi G., Ábrahám Gy., Sonkodi S., Torday Cs.: The role of platelets in the pathomechanism of focal segmental glomerulosclerosis and minimal change nephrotic syndrome
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VI. Túri S., **Bereczki Cs.,** Karg E., Sallai É., Torday Cs., Nagy E., Haszon I.: The role of platelet function, blood lipids and nitric oxide in hypertension of children
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ABBREVIATIONS

ABPM	ambulatory blood pressure monitoring
AC	adenile cyclase
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ANS	8-aniline-1-naphthalene sulphate
ATP	adenosine triphosphate
BP	blood pressure
cAMP	cyclic adenosine monophosphate
COX	cyclooxygenase
ESRF	end stage renal failure
ET-1	endothelin-1
FSGS	focal segmental glomerulosclerosis
GDP	guanosine diphosphate
GP	glycoprotein
GTP	guanosine triphosphate
Hct	haematocrit
HD	haemodialysis
HDL	high density lipoprotein
IgAN	IgA nephropathy
JEHT	juvenile essential hypertension
LDL	low density lipoprotein
MCNS	minimal change nephrotic syndrome
MGN	membranous nephropathy
MPGN	membranoproliferative glomerulonephritis
NO	nitric oxide
NO_x	nitric oxide end products (NO_2/NO_3)
NS	nephrotic syndrome
PAF	platelet activating factor
PD	peritoneal dialysis
PDGF	platelet-derived growth factor
PGE_1	prostaglandin E_1
PGI_2	prostacyclin
PPP	platelet-poor plasma
PRP	platelet-rich plasma
PTH	parathyroid hormone
RAS	renin-angiotensin system
RBC	red blood cell
rHu-EPO	recombinant human erythropoietin
SLE	systemic lupus erythemathosus
TxA_2	thromboxane A_2
TxB_2	thromboxane B_2
VLDL	very low density lipoprotein
vWF	von Willebrand factor

SUMMARY

This thesis focuses on platelet functional disturbances in three groups of paediatric patients: children with nephrotic syndrome, children and adolescents with end stage-renal failure in a chronic haemodialysis programme and hypertensive children and adolescents.

Our study did not reveal any significant difference in the results of platelet aggregation, ATP release, TxB2 release, or platelet cAMP concentration between steroid-treated and non-treated patients. The plasma cholesterol and triglyceride levels were significantly higher in relapse than in remission; nevertheless, no differences in platelet function were seen between these groups. In conclusion oral prednisone therapy on alternate days and the high cholesterol and triglyceride level in the plasma cannot be responsible for the hyperaggregation of platelets in nephrotic children. The increased platelet aggregability and TxB_2 release suggesting that abnormalities may be involved in the pathogenesis of both MCNS and FSGS. The platelets TxB_2 is significantly higher in FSGS than in MCNS, which may be have an impact in the progression of glomerular damage by an increased vasoconstriction and intraglomerular pressure. Our results demonstrate that platelets from dialysed or non-dialysed ESRF patients display a significantly higher cAMP, a lower TxB_2 and a lower aggregability, than those of the controls. Our observations seem to confirm that there is no correlation between the serum PTH level and the degree of disturbances of the biochemistry and aggregation of platelets of ESRF patients. The platelet cAMP level decreased more markedly during bicarbonate HD, than during acetate HD, and greater increases in platelet aggregation and TxB_2 formation were observed following bicarbonate HD. As the normalisation of the bleeding time following 1 year of rHu-EPO therapy was not associated with the normalisation of the platelet aggregability and biochemistry, we can not exclude the role of the increased Hct in the shortening of the bleeding time. However, the increased RBC count did not improve the platelet aggregation. Although rHu-EPO itself did not improve the platelet aggregation directly during 1 year of rHu-EPO therapy, a number of platelet functional changes were observed, including increased aggregability, and ATP and TxB_2, release and a decrease in cAMP concentration. These contribute to the improvement of the bleeding time. Therefore we consider that rHu-EPO therapy can improve the platelet function through biochemical changes and has a combined effect on the haemostasis of ESRF patients. Increased platelet aggregation and TxB_2 are characteristic feature of juvenile essential hypertension and ESRF associated hypertension, contributing the blood pressure elevation, while hyperlipidaemia is more common in ESRF, regardless blood pressure and does not have a role in the pathogenesis of JEHT at an early stage. In addition JEHT patients exhibits a compensatory increases in plasma NO level.

1. INTRODUCTION

1.1. Platelets

The main role in the maintenance of haemostasis is played by the platelets. These fulfil a number key functions, these being highly dependent not only on their number but, also on their integral physiology. Resting, unstimulated platelets circulate without adhesing to the vessel wall and without aggregating, but they respond rapidly to exposure to subendothelial matrix proteins, such as collagen, von Willebrand factor (vWF), fibronectin, or laminin by disruption of the endothelial layer. Thus, the endothelium serves as physical barrier to prevent platelet activation by contact with subendothelial matrix proteins and additionally exerts antithrombotic effects by the production of prostacyclin (PGI_2) and nitric oxide (NO) productions. [5,15,97]

The platelet activation processes are initiated by the platelets coming into contact with subendothelial matrix proteins and circulating soluble pro-aggregatory agents (e.g.endothelin-1 (ET-1) and thromboxane A_2 (TxA_2)). These processes result in adhesiveness and the subsequent activation and recruitment of more platelets (platelet-platelet interaction), leading to an occlusive haemostatic plug. This is the process of primary haemostasis [5] . (Fig.1)

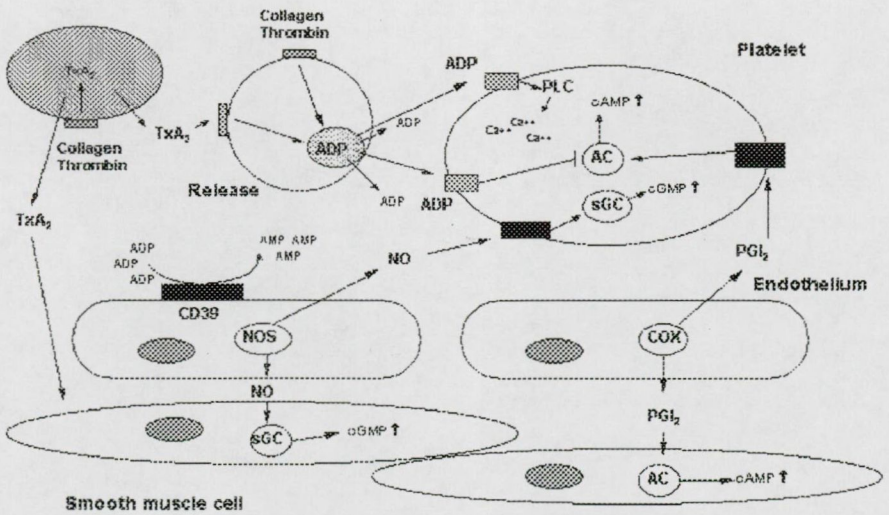

Fig 1. Platelet-vessel wall and platelet-platelet interaction

The activation of platelets is associated with marked morphological changes: the discoid form of resting platelets is changed to a more spherical appearance of stimulated platelets (shape change), which is accompanied by a complex cytoskeletal reorganisation [69]. The transformed platelets have an increased membrane reservoir and an increased potential to attach irreversibly to the altered vascular surface by a process called adhesion. Adhesion is mediated by the platelet membrane glycoproteins GPIa-IIa, GP-Ib-V-IX and GPIc-IIa, which serve as receptors for collagen, vWF and fibronectin, respectively [37].

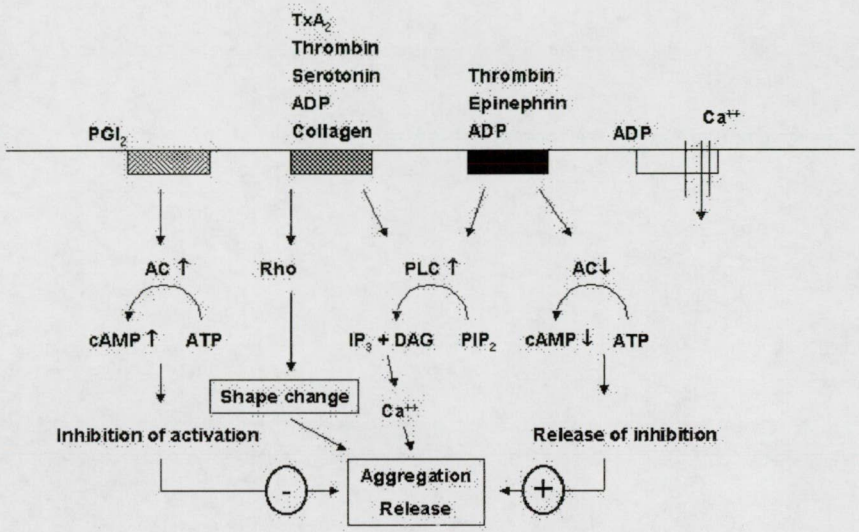

Fig.2. The platelet activation and inhibition of activation
(IP_3: inositol 1,4,5-triphosphate, DAG: diacylglycerol, PIP_2:phosphatidylinositol-4,5 biphosphate)

Adhesion is followed by subsequent platelet-platelet interactions called aggregation. This process is mainly mediated by the interaction between the fibrinogen and the platelet-specific GPIIb-IIIa complex. Additionally, the platelets release their secretory granules (α-granules and dense bodies). The platelet-derived growth factor (PDGF), β-thromboglobulin, platelet factor 4 and other coagulation factors are localised in α-granules. The principal constituents of dense granules are non-metabolic pools of adenine nucleotides (adenosine triphosphate and diphosphate: ATP and ADP), PPi, Ca^{2+} and Mg^{2+}, P-selectin and serotonin (5-hydroxytriptamine). The adenine nucleotides are synthesised and segregated by megakariocytes. In addition the dense bodies contain guanosine triphosphate and diphosphate (GTP and GDP), which are the targets of the anti-platelet effects of endothelial NO, elevating the cyclic guanosine monophosphate (cGMP) level in the platelets. Both ADP and ATP produce adenosine

monophosphate (AMP); this is dephosphorylated to adenosine, which ultimately forms cyclic AMP (cAMP), which inhibits the platelet stimulatory response [8,69]. (Fig.2)

The agonists elevate the cytosolic Ca^{2+} concentration in human platelets via a receptor-operated mechanism, involving both Ca^{2+} release from intracellular stores and subsequent Ca^{2+} entry, which can be inhibited by platelet inhibitors such as prostaglandin E1 (PGE_1) and NO donors which, elevate the cAMP and cGMP levels, respectively. The secreted ADP and TxA_2 activate more platelets; this process is called the release reaction [5,8]. (Fig.1)

Furthermore the platelets are closely linked to plasmatic coagulation system and contribute to thrombin and subsequent fibrin generation. Coagulation factors such as factor V, factor XI and others are stored in the platelet α-granules, secreted upon platelet stimulation and expressed at the cellular membrane, which serves as a surface for various enzymatic reactions of the coagulation cascade [5,37,69].

Table 1. Testing of platelet function

Bleeding time:	
	Screening test for quantitative platelet disorders
	(Platelet activation, adhesion and aggregation)
Aggregation test:	
	Measurement of the formation platelet clumps in platelet-rich plasma (PRP) following stimulation with ADP, collagen, epinephrine, arachidonic acid, ristocetin, thrombin and platelet activating factor (PAF)
Release reaction analysis:	
	Platelet-specific α-granules proteins:
	Platelet factor 4, β-thromboglobulin, PDGF
	and soluble P-selectin (CD62P)
	Platelet dense granules:
	ATP, ADP, serotonine and CD63
	CD63 and CD62P analysed by flow cytometry
Platelet adhesion test:	
	Monoclonal antibodies against GPIb-V-IX and β1-integrins
Platelet procoagulant activities:	
	Platelet factor 3
	Platelet prothrombinase activity

Platelet function analysis in children and adolescents may involve the studies bleeding time, platelet aggregation, secretion (release reactions) and measurements of platelet procoagulant activity. Analysis of platelet membrane glycoproteins (GPs) on resting and stimulated platelets by flow cytometry has become available (Table 1.).

This thesis will focus on platelet functional disturbances in three groups of paediatric patients:

- Children with nephrotic syndrome (NS)
- Children and adolescents with end stage-renal failure in a chronic haemodialysis programme
- Hypertensive children and adolescents

1.2. Nephrotic syndrome and platelet function

The term nephrotic syndrome is applicable to a condition characterised by heavy proteinuria, hypoproteinaemia, hyperlipidaemia and oedema. (Table 2.)

Table 2. Definitions of nephrotic syndrome [4]

Nephrotic syndrome: Oedema, plasma albumin < 25 g/l, proteinuria > 40mg/m2/hr or protein/creatinine ratio > 200mg/mmol
Remission: Urinary protein excretion < 40mg/m2/hr or Albustix = 0/trace for 3 consecutive days
Relapse: Urinary protein excretion > 40mg/m2/hr or Albustix ++ or more for 3 consecutive days having previously been in remission

It is a disorder of glomerular permselectivity and charge selectivity that may be primary or secondary to an overt systemic disease (e.g. SLE). In children, the nephrotic syndrome is an uncommon disorder (cumulative prevalence 15.7 per 100 000 children). The International Study of Kidney Disease in Children (ISKDC) reported that 77% of nephrotic children had minimal change nephrotic syndrome (MCNS); focal segmental glomerulosclerosis (FSGS) was present in 10%, membranoproliferative glomerulonephritis (MPGN) in 5%, diffuse mesangial proliferation in 3%, crescentic glomerulonephritis in 3% and membranous nephropathy (MGN) in 2%. It is unclear whether MCNS and FSGS should be considered to be separate entities or different ends of a single spectrum of disease. Current opinion tends to group the two under the rubric of idiopathic nephrotic syndrome (INS), as there are examples of MCNS evolving into FSGS.

However, in their typical expression, the two disorders differ not only in glomerular histology and response to corticosteroid therapy, but most importantly in the tendency for FSGS to progress to end stage renal failure (ESRF), which hardly ever happen in MCNS [4].

In current clinical practice, most centres accept a high dose prednisolone (60 mg/m^2/day) for the induction of remission. After remission has been achieved, the dose of prednisolone is reduced to 40 mg/m^2/ alternate day for the next 4 weeks [4].

The platelet vessel-wall interaction has an important role in the pathogenesis of glomerular diseases. We have presented data, that there is a reduced level of plasma factors influencing PGI_2-like activities and platelet aggregation in patients with IgA nephropathy and Hennoch-Schönlein purpura [101]. Various abnormalities in both coagulation and the fibrinolytic system have been observed in many nephrotic syndrome patients [1,11,65]. The plasma from nephrotic patients is hypercoagulable with high levels of fibrinogen, factor VIII:RAg and α2-macroglobulin and with reductions in both functional and immunologic antithrombin III. Antithrombin III concentration is reduced due to the urinary loss and the plasma is resistant to anticoagulation with heparin [95] . Because of the hypovolaemia, the microcirculation is sluggish, while the blood is viscous due to the haemoconcentration. Ueda et al. [102] postulate that steroid treatment acts as a thrombogenetic factor by accelerating thrombocytosis and hyperlipidaemia and by reducing plasma fibrinolysis in children with MCNS. The lipoproteins have been shown experimentally to be harmful for damaged and sclerotic glomeruli. The clinical implication of these changes is not clear, although they result in a hypercoagulable state with increased thromboembolic problems and in the progression of glomerulosclerosis [9,50,51]. Bang et al. [3] found that the degree of platelet dysfunction was correlated with the amount of proteinuria and the level of hypoalbuminaemia. The platelet counts within the glomeruli rise in several renal diseases, but not in MCNS. The spontaneous aggregation of the platelets and aggregation in response to collagen and ADP are enhanced [1,3]. The platelets adhere to the damaged endothelial surface and release their granular contents consisting of PDGF, serotonin and thromboxane A_2 (TxA_2). In glomerular disease, the increased platelet aggregation is frequently associated with an elevated production of PAF, and therapy with specific PAF antagonists has prevented or reduced proteinuria [78] . In MGN, a significant positive correlation has been detected between the urinary excretion of PAF and proteinuria [74]. The platelet deposition in glomerulosclerosis and the release of PDGF, PAF and TxA_2 could cause further increases in platelet aggregation and local vasoconstriction, and result in the progression of glomerular damage.

1.3. Platelet function in ESRF patients

Patients with uncontrolled renal failure exhibit a haemorrhagic tendency [57]. A number of early studies have reported a prolonged bleeding time, a decreased platelet count [27,58], and a reduced platelet factor 3 availability [86,109]. *In vitro* platelet aggregation studies have led to conflicting results.

Table 3. Causes of the haemostatic defect in ESRF [76]

Platelet abnormalities
Subnormal dense granule content
Reductions in intracellular ADP and serotonin
Impaired release of platelet α-granule protein and β-thromboglobulin
Enhanced intracellular cAMP
Abnormal mobilisation of platelet Ca^{2+}
Abnormal platelet arachidonic acid metabolism
Abnormal ex vivo platelet aggregation in response to different stimuli
Defective cyclooxygenase activity
Abnormal GP IIa-IIIa binding
Uremic toxins
Abnormal platelet-vessel wall interaction
Abnormal platelet adhesion
Increased formation of vascular PGI_2
Altered vWF
Anaemia
Altered blood rheology
Erythropoietin deficiency
Abnormal NO production
Drug treatment (non-steroid antiinflammatory drug, antihypertensive, etc.)

A reduced aggregation, in response to ADP, epinephrine, collagen and thrombin, has been reported by some investigators [46,62,84], but others have found an enhanced aggregability [27,113]. Several biochemical abnormalities have been observed in platelets from uraemic subjects. TxB_2 production in response to exogenous arachidonic acid was normal, whereas it was decreased by 40-50% in response to collagen and thrombin [19], which reflects a decreased

release of arachidonic acid from platelet phospholipids. On the other hand, there are reductions in ATP secretion in response to thrombin, collagen and arachidonic acid, associated with a reduced ADP content of the dense granules, suggesting a storage pool defect [19]. An elevation in the cAMP level [108] in ESRF patients could contribute to a defective aggregation response by reducing the availability of intracytoplasmatic Ca^{2+}. There are conflicting results on the role of parathyroid hormone (PTH) in uremic thrombocytopathy [20,63]. Noris and Remuzzi[76] recently published data indicating that monomethyl-L-arginin (L-NMMA), and NO inhibitor, normalised the platelet dysfunction and bleeding time in experimental uraemia, suggesting the crucial role of NO. The pathogenesis of uremic bleeding is considered to be multifactorial (Table 3.), but the platelet-platelet and platelet-vessel wall interaction appear to be of crucial importance [76]. Modern dialysis techniques and the use of recombinant human erythropoietin (rHu-EPO) reduce the frequency of bleeding in ESRF.

Erythropoietin is the primary regulator of red blood cell (RBC) production and furnishes a major advantage in the treatment of anaemia in the ESRF [30,45,110]. rHu-EPO enhances the growth-promoting action of thrombopoietin on the megakariocytes and therefore elevates the platelet production. The side effects of rHu-EPO are considered to be minimal; hypertension and thrombotic events have been reported in haemodialysis (HD) patients during clinical trials [12,33,95,106,107]. It has also been stated to shorten the skin capillary bleeding time, indicating a correction of the uraemic bleeding tendency. The decrease in the bleeding time after treatment with rHu-EPO could be due to elevation of the haematocrit (Hct), with an increased blood viscosity, or to changes in the platelet function or both. A number of studies have examined, how rHu-EPO could improve the primary haemostasis. Gorge et al. [39] found that the shortening of the prolonged bleeding time following rHu-EPO treatment was due to an increase in the circulating RBCs. Fabris et al. [33] observed that correction of the anaemia with rHu-EPO was followed by an improved platelet aggregation and the serum TxB_2 levels rose to the normal range. They concluded that treatment with rHu-EPO improved the haemostasis not only by correcting the anaemia, but also by increasing the platelet count and function. In contrast with the platelets, which are clearly affected, rHu-EPO does not appear to have a significant effect on the blood coagulation and fibrinolytic factors.

In addition to these observations, increasing evidence has accumulated on the role of surface negative charge on the platelets, preventing their aggregation and adhesion to the endothelial cell. In most blood cells the resultant membrane surface charge is negative, but may be altered by factors such as pH, proteins and phospholipids [32,52,67].

1.3 Hypertension in children and adolescents

Hypertension is an important cause of vascular morbidity and mortality in adults, and in the majority it is essential and multifactorial in origin. In childhood it is less common and if severe, is usually secondary with 90-95% of the cases due to renal disease. In the patients with ESRF the high blood pressure (BP) remains a major problem and it is probably responsible for a sizeable portion of the morbidity imposed by cardiovascular disease in dialysis patients [16]. The endothelium emerged as an important participant in a number of human diseases, because it controls the vasomotor tone by releasing number of substances. Some of these have vasoconstrictive effect (ET-1, TxA_2), others cause vasodilatation (NO, PGI_2, bradykinin) [72].
NO, which was identified in 1980 as the endothelial dependent relaxing factor (EDRF), is an important mediator of the vascular tone. NO mediates vasodilatation, inhibits the platelet aggregation and expression of adhesion molecules for monocytes and neutrophils, and impairs the growth of vascular smooth muscle cells [71]. Hypertension is one of a number of cardiovascular risk factors [83] (including diabetes mellitus and hypercholesterolaemia) for the development of atherosclerosis, in which endothelial, NO and platelet dysfunctions are known to play an important part [20,66]. The role of NO in childhood hypertension is less well defined. Goonasekera et al. [38] found evidence of an increased NO activity in the plasma of hypertensive children. An increased oxidative stress, decreasing the bioavailability of NO, is mainly responsible for a blunted endothelium-dependent vasoreactivity [105]. Whereas ET-1 has a relatively small influence on the basal regulation of the blood pressure, NO appears to play a central role under normal conditions [66]. On the other hand, in pathological states, a balance between NO and local ET-1 production may be central to changes in blood vessel reactivity, smooth muscle proliferation and blood coagulability. The opposing actions of NO and angiotensin II (Ang II) in vascular contraction, vascular smooth muscle cell proliferation and apoptosis are well documented [108]. Various experimental approaches have demonstrated that NO negatively modulates the renin-angiotensin system (RAS) by inhibiting the angiotensin converting enzyme (ACE) activity and down-regulating the AT-1 receptors. NO also inhibits the proliferation and migration of vascular smooth muscle cells induced by Ang II. NO has emerged as a factor regulating the RAS at different levels, while Ang II seems to have a regulatory influence on NO generation [23,72].

Under physiological conditions, there is equilibrium between pro-aggregating and anti-aggregating factors. The adult patients with hypertension display a state of platelet hyperaggregability, even if there is no other risk factor for cardiovascular disease. The platelet

aggregation, the P-selectin (CD62) expression on platelet surface, the serum levels of IL-1β and IL-6, and plasma levels of soluble P-selectin and ET-1 are reduced with the normalisation of blood pressure [91,92]. Dockrell et al. [22] demonstrated that enhanced platelet sensitivity to ET-1 in young men with high blood pressure appears to be a feature of familial predisposition to hypertension.

Diabetes mellitus, cigarette smoking, hypercholesterolaemia and an increased body mass index (BMI) are well known risk factors in adults. Experimental and clinical data suggest an important interaction between hyperlipidaemia and hypertension. Not only do they frequently coexist, but also hypertension dramatically exaggerates hyperlipidaemic injury and substances, which maintain the vascular tone [49,83]. The occurrence and role of this metabolic state have not been investigated in children.

2. AIMS OF THE STUDY

The following questions were considered during investigation of platelet function in children and adolescents with kidney diseases and hypertension.

2.1. Platelet aggregation studies in nephrotic syndrome

2.1.1. How is the platelet function changed in children with nephrotic syndrome?

2.1.2. Do steroid treatment and hyperlipidaemia influence the platelet function in nephrotic syndrome?

2.1.3. Are there differences in platelet function in the patients with MCNS as compared to FSGS?

2.2. Effect of haemodialysis and rHu-EPO on platelet aggregation

2.2.1. How is the platelet function changed in children with ESRF?

2.2.2. Can bicarbonate and acetate HD improve the platelet function in ESRF?

2.2.3. How does the platelet surface charge change during HD in ESRF?

2.2.4. Can the one-year rHu-EPO treatment improve the platelet function in ESRF?

2.3 The role of platelet function, thromboxane and nitric oxide in hypertension of children and adolescents

2.3.1. Does high blood pressure influences the platelet function in children and adolescents?

2.3.2. Is there any difference in platelet function in essential and secondary, ESRF-associated hypertension in childhood?

2.3.3. Is there any role of hyperlipidaemia in the pathogenesis of essential hypertension in childhood?

2.3.4. Can the NO regulate the platelet function in hypertension?

3. PATIENTS

None of the patients received non-steroid anti-inflammatory drugs, albumin or blood transfusion in the 2-week period prior to the studies. Neither the patients, nor controls received any medication known to affect the platelet function.

The following groups of patients were investigated:

3.1. Platelet aggregation studies in nephrotic syndrome

3.1.1 Platelet aggregation in nephrotic syndrome

57 patients with nephrotic syndrome (28 boys and 29 girls, aged 4-17 years) and 18 age- and sex-matched controls were studied. 34 patients were in relapse (urinary protein >3 g/24 h, plasma albumin 20±3 g/l (±SEM) and 23 were in early (< 6 month) remission (urinary protein =0 g/24h, plasma albumin 26±2g/l). Histological examination of kidney biopsy specimens indicated that 25 patients had MCNS, 9 had FSGS, 6 had MPGN, 6 had IgA nephropathy (IgAN), 7 had SLE and 4 had MGN. Only one patient had unilateral renal vein thrombosis 8 months before the study and received 60mg/m2/day prednisolone treatment.

None of the other patients had renal or extrarenal thrombotic complication. All the patients in relapse were treated with 0.5mg/kg/alternate day prednisolone as part of the immunosuppressive therapy with chlorambucil or azathioprine, or 1.0 mg/kg prednisolone monotherapy every other day in MCNS. 12 patients in remission were still on tapering dose of prednisolone, and 11 did not received treatment.

3.1.2. Comparison of platelet function in MCNS and FSGS

16 patients with FSGS (8 adults and 8 paediatric cases) 27 children with MCNS (12 in relapse and 15 remission) and 12 adult (aged 31±5.0 years) and 15 paediatric controls were investigated. None of the patients had renal or extrarenal thrombosis. The diagnostic criteria for

relapse are given in section 3.1.1. All MCNS patients in relapse were treated with 60mg/m2/day prednisolone and those with FSGS received 2.0mg/kg/day cyclophosphamide and 35mg/m2/alternate day prednisolone.

Table 4. Histological distribution of patients with NS

	NS in remission	NS in relapse
MCNS		
Prednisolone +	7	11
Prednisolone -	7	-
FSGS		
Prednisolone +	-	11
MPGN		
Prednisolone +	-	5
IgAN		
Prednisolone +	1	2
Prednisolone -	3	-
SLE		
Prednisolone +	4	3
MGN		
Prednisolone +	-	3
Prednisolone -	1	-
	23	33

3.2. Platelet aggregation studies in ESRF

3.2.1. Effects of bicarbonate and acetate haemodialysis on platelet aggregation

6 patients with ESRF (3 boys and 3 girls, aged 7-16 years) who had been on HD treatment for 27.4 months (10–68 months) (group 1), 8 patients with ESRF (5 boys and 3 girls, aged 4–15 years) on conservative treatment (group 2) and 10 age- and sex-matched controls were investigated. The primary diagnosies in group 1 and group 2 were 5 cases of chronic glomerulonephritis, 3 of hydronephrosis, 4 of renal agenesia with contralateral hypoplasia, 1 of nephrosclerosis and 1 of polycystic kidney disease. The patients from group 1. and 2. had a history of bleeding tendency (easy bruising, and nose and gum bleeding). The patients in group 1 had a pre-dialysis Ivy bleeding time of 9.5±0.7 min., while that in the controls was 4.7±0.9 min. The patients in group 1. were each treated with 5 acetate and 5 bicarbonate HD. (cuprophan dialyser, blood flow 200 ml/min, duration 210min.) Heparin was administered in an initial dose of 2000 IU and which was followed by a 1200 IU/hr constant infusion. The pre-dialysis Hct was 0.24±0.02, the platelet count 183±31 G/l, the serum Na^+: 139.3±4.6 mM, K^+: 5.4±0.4 mM, BUN: 36.9±6.8 mM, creatinine: 929±112 μM.

3.2.2. Effect of rHu-EPO on the platelet function in ESRF children on HD

8 patients with ESRF (4 boys and 4 girls, aged 6–17 years) who had been on bicarbonate HD for 31.8 months (10–68 months) and 8 age- and sex-matched controls were studied. Platelets from ESRF patients were investigated before and after 12 months of rHu-EPO therapy. rHu-EPO was administered sc.. In the first 12 weeks of the study, the dose of rHu-EPO was the same in all 8 patients: weeks 1-4: 50 U/kg; weeks 5-8: 75 U/kg; weeks 9-12: 100 U/kg three times a week following HD. After 12 weeks the dose of rHu-EPO was individually modified to maintain a target Hct 0.35. The primary diagnosies in the ESRF group was 3 cases of chronic glomerulonephritis, 2 of hydronephrosis, 2 of renal agenesia with contralateral hypoplasia, and 1 of polycystic kidney disease. The patients had a history of bleeding tendency (easy bruising and nose and gum bleeding). The ESRF patients were treated with bicarbonate HD (cuprophan dialyser, blood flow: 200 ml/min, duration: 210min.). Heparin was administered in an initial dose of 2000 IU, followed by an 800 IU/hr constant infusion. The pre-dialysis Hct was 0.21±0.01 before and 0.36±0.01 after the 1-year rHu-EPO treatment. The serum concentrations were: Na^+: 138.2±3.6 mM, K^+: 5.3±0.3 mM, BUN: 35.8±7.1 mM, and creatinine: 917±121μM. There was no significant difference in the biochemical values following the 1-year rHu-EPO therapy.

3.3. Platelet function, thromboxane and nitric oxide in hypertension of children and adolescents

Examinations were performed on 14 patients with ESRF on chronic bicarbonate HD; 3 times a week, 8 of them with hypertension (4 boys and 4 girls, aged 9-19 years) and 6 with normal blood pressure (3 boys and 3 girls, aged 8-18 years), on 12 patients with juvenile essential hypertension (JEHT) (6 boys 6 girls aged 12.7±4.1 years) and on 10 age- and sex-matched controls. The patients with ESRF had an original nephrological diagnosies of obstructive uropathy (5), renal agenesia with hypoplastic kidney on the contralateral side (4), polycystic kidney disease (2), acute tubulointerstitial nephritis, with tubular necrosis (1), MPGN (1), or FSGS (1). In both hypertensive groups BP was investigated according to the WHO-ISH 1999 Guideline [15] and Update on the 1987 Task Force Report on High Blood Pressure in Children and Adolescents. BP also was measured in all groups, with 24 hours ambulatory blood pressure monitoring (ABPM) (Meditech, APBM-04) [95]. In the JEHT group, all secondary causes of hypertension were excluded; in this group the renal function and ultrasonographic findings were normal. The ESRF-associated hypertensive patients were treated with an ACE

inhibitor or calcium channel blockers, while the patients with JEHT were examined before the start of any treatment.

3.4. Ethical issues

The University Ethical Committee approved these studies.

4. METHODS

Blood samples (9 ml) from a peripheral vein were collected in plastic tubes containing 1 ml 3.8% trisodium citrate. Citrated blood was centrifuged at 500 g for 10 min. at room temperature to obtain platelet-rich plasma (PRP). Platelet-poor plasma (PPP) was obtained by centrifugation of PRP at 4000g for 10 min.. The PRP platelet count was adjusted to 300±10 G/l.

Plasma triglyceride, cholesterol, LDL-cholesterol, HDL-cholesterol, and VLDL-cholesterol were determined by standard laboratory methods.

4.1. Platelet aggregation

Platelet aggregation was carried out with a laser-rheoaggregometer (Servobio, Meudon, France)[48], using collagen stimulation (Hormon Chemie, Munich, Germany) at a final concentration of 2 μg/ml.

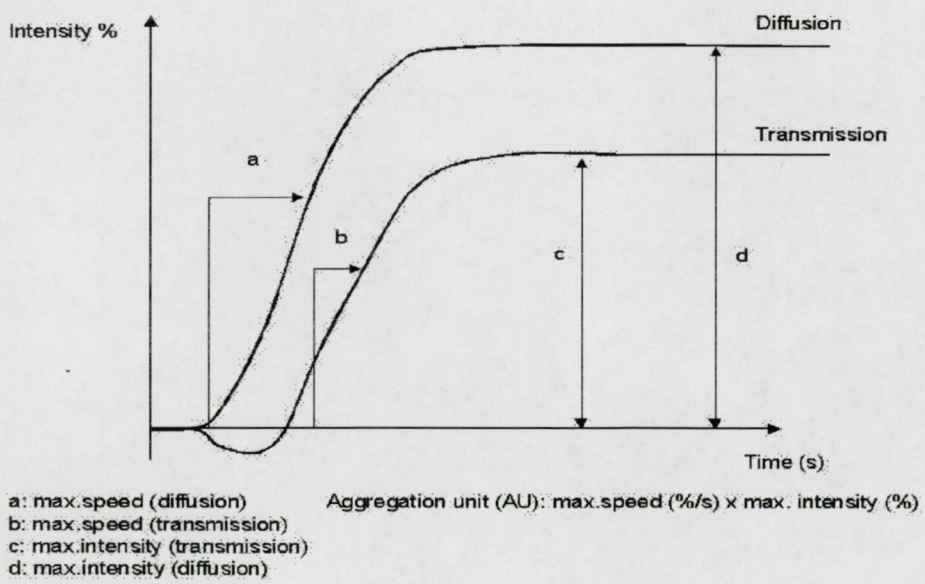

Fig.3. Platelet aggregation curves (transmission and diffusion) induced by collagen.

The aggregation of platelets from ESRF patients was induced with collagen in two concentrations: 1 or 3 μg/ml PRP. The apparatus was adjusted so that the PRP and PPP produced 10% and 90% light transmittance, respectively. During aggregation, the changes in the transmittance of PRP were measured using a control of PRP obtained from the same patient. With this method, the disturbing effects of various plasma constituents (e.g. lipids) were avoided. Aggregating agent was added to PRP that had been stirred at 1000 rpm at 37 °C for 1 min. The speed and the intensity of the platelet aggregation curves obtained with transmitted light and diffused light simultaneously were estimated by computer analysis. The results of platelet aggregation were expressed in aggregation units. (Fig.3)

4.2. Platelet adenosine triphosphate release

ATP release from platelets during aggregation was studied with a Chronolog Lumi-aggregometer. A parallel PRP sample (350 μl) was investigated by using luciferin as substrate and luciferase as enzyme (30μl) (Sigma, Poole, UK) [34] The amount of ATP released from platelets during aggregation stimulated by collagen was estimated from comparison of the peak of the curve obtained by the addition of 20 ng ATP (Sigma, Poole, UK) to a mixture of 350 μl 0.9% NaCl and 30 μl luciferase-luciferin. The ATP release was expressed in ng/ml. The ATP sensitivity of this method is 0.5 ng/ml.

4.3. Cyclic adenosine monophosphate concentration in platelets

The concentration of cAMP in the platelets was measured following aggregation [36]. Platelets were lysed and cAMP was released by cytolysis. During preparation, the platelet cAMP was protected from cAMP-dependent phosphodiesterase (PDE), with diluting buffer containing (in final concentrations) 0.5 mM 3-isobutyl-1-methylxanthine (Sigma, Poole, UK), a specific PDE inhibitor, 4 mM EDTA (Sigma, Poole, UK) and 50 mM TRIS-HCl buffer (pH 7.5), and the mixture was centrifuged at 800g for 10 min. at 4 °C. The sediment was lysed in a hypotonic solution containing 4 mM EDTA, 8 mM theophylline and 20 mM TRIS-HCl (pH 7.5). For more complete exposure of the platelets, boiling this mixture for 2 min. in a hot-water bath completed cytolysis [36]. The protein free supernatant was obtained by centrifugation for 30 min. at 6000 g in a refrigerated centrifuge. cAMP was determined in 50 μl of the supernatant with a cAMP binding assay kit (Amersham TRK 432,Amersham, UK) The buffer used for binding assay was

the same as that used for cytolysis. Radioactive samples were measured in a Triton-toulene cocktail with a tritium programme with a Beckman LS 100C liquid scintillation counter.

4.4. Platelet Thromboxane B_2 release

TxB_2 (the stabile metabolite of TxA_2) levels were measured by radioimmunoassay (RIA) [40]. This was carried out on aliquots of the supernatant after the measurement of platelet aggregation. The sensitivity of the method was 3 ng/ml.

4.5. Platelet surface positive charge

Platelet surface positive charge was measured by the method of Wojtczak and Nalecz [111] The binding of 8-aniline-1-naphthalene sulphate (ANS) to the platelet surface was measured with a Hitachi F2000 fluorimeter at 366 nm and 460 nm (excitation and emission wavelengths, respectively). 3µl of 5 mM alcoholic ANS solution was added to 3 ml PRP and 3 ml PPP. Following each ANS challenge, the fluorescence intensity was measured and the value obtained before addition of ANS was subtracted from each post-ANS result and then related to the platelet number in the PRP and expressed in arbitrary units per platelets.

4.6. Direct effect of rHu-EPO on platelet aggregation

The direct effect of rHu-EPO on platelet aggregation was investigated *in vivo* and *in vitro*. In the *in vivo* study, platelet aggregation was determined prior to and 30 min. following intravenously administration of rHu-EPO. In the in vitro study, blood samples from uraemic patients were treated with rHu-EPO in vitro (10 U rHu-EPO in 10ml citrated blood at 37 °C for 30 min.) and the platelet aggregation was measured prior and following incubation. The method was the same as described in session 4.1.

4.7. Direct effects of red blood cell count on platelet aggregation

The direct effects of the RBC count on platelet aggregation were investigated by preparing a low-Hct (0.23±.0.02) and a high-Hct (0.38±0.02) sample in vitro using autologous RBCs. Following a 60-min. incubation at room temperature PRP was prepared from each samples and platelet aggregation was examined with a laser-rheoaggregometer as described in session 4.1.

4.8. Nitric oxide end-products in plasma (NO_x)

Nitrite and nitrate were simultaneously determined by an anion-exchange chromatographic HPLC method (1). The Pharmacia LKB HPLC system was used with a Variable Wavelength Detector (at 210 nm). [68]

4.9. Statistical analysis

All platelet aggregations, ATP, cAMP, TxB_2, nitrite and nitrite measurements and platelet surface charge studies were carried out in duplicate. Statistical analysis was performed with the paired t-test and the rank correlation test with SigmaStat 1.0 statistical software.
The results for the groups are given as means plus or minus standard errors (±SEM).

5.RESULTS

5.1. Platelet aggregation studies in nephrotic syndrome

5.1.1. Platelet aggregation

In vitro laser-rheoaggregometer studies revealed significantly higher diffusion and transmission results for platelets from nephrotic patients than for those from the controls ($p<0.05$). There was no significant difference between the groups in remission and in relapse. (Fig.4). The platelet aggregation results demonstrated a significantly higher aggregation for all the different histological subgroups than for the controls ($p<0.01$). The number of patients with MGN was too low for statistical analysis, although these patients displayed the highest platelet aggregability amongst the nephrotic children.

The transmission values were significantly higher for the platelets from the patients with IgAN and SLE than for those from MCNS patients in early remission ($p<0.05$). Similarly, the diffusion values were higher in IgAN and SLE than in MCNS, although the differences were not significant (Table 5.). A significant positive correlation was observed between the diffusion and transmission in the nephrotic groups ($r=0.85$, $p<0.05$).

Table 5. Laser-rheoaggregometer studies with platelets from nephrotic patients

Diagnosis	n	Platelet Aggregation (AU) Diffusion	Transmission
MCNS in remission	14	27185±3705*	11722±1006*
in relapse	11	36913±3146*	12456±1503*
FSGS	9	31409±5886*	12916±1012*
MPGN	5	29325±4148	12572±2116
SLE	7	31873±3194*	15657±2420*
MGN	4	40574±3070	16419±954
Controls	18	19898±3206	9769±918

*p <0.05

5.1.2. Platelet cAMP concentration

The platelet cAMP concentration was significantly lower in the nephrotic patients than in controls ($p<0.05$). There was no significant difference between remission and relapse or between steroid-treated and non-treated patients in remission. (Table 6.) A significant negative correlation was detected between the platelet cAMP concentration and the diffusion results obtained with platelet aggregation ($r= -0.64$, $p<0.05$)

Table 6. Platelet cAMP concentration, ATP release and TxB_2 release in nephrotic syndrome

	n	ATP release (ng/ml)	cAMP (pmol/10^9 platelets)	TxB_2 (ng/ml)
Nephrotic syndrome				
In relapse	33	100.9±28.4*	12.1±3.4*	13.2±1.9**
In remission	22	92.2±18.9*	11.9±2.9*	13.0±2.1**
Prednisolone positive	12	84.4±12.6*	12.2±2.7*	12.1±1.8**
Prednisolone negative	11	97.5±11.0*	11.7±2.8*	14.2±2.2**
Controls	18	34.2±10.4	25.5±2.1	6.9±1.8

*p<0.05, **p<0.01

5.1.3. Platelet ATP release

Platelets from nephrotic patients either in relapse or in remission released significantly more ATP than did those from the controls ($p<0.01$). A higher ATP release was observed in relapse than in the remission, and in remission group without prednisolone than in the group taking prednisolone, but the difference was not significant. There was a positive correlation between the ATP release and the diffusion ($r=0.68$, $p<0.05$), and the ATP release and transmission values ($r=0.65$, $p<0.05$) (Table 6.)

5.1.4. Platelet TxB_2 release

TxB_2 formation by platelets in response to collagen was significantly higher in the nephrotic patients than in the controls ($p<0.01$). No difference was observed between the results for the subgroups of nephrotic patients. (Table 6.) The correlation coefficients for the TxB_2 release and the diffusion and the transmission values were 0.68 and 0.67 respectively ($p<0.05$). A significant negative correlation was detected between the platelet cAMP concentration and TxB_2 release ($r=-0.63$, $p<0.05$).

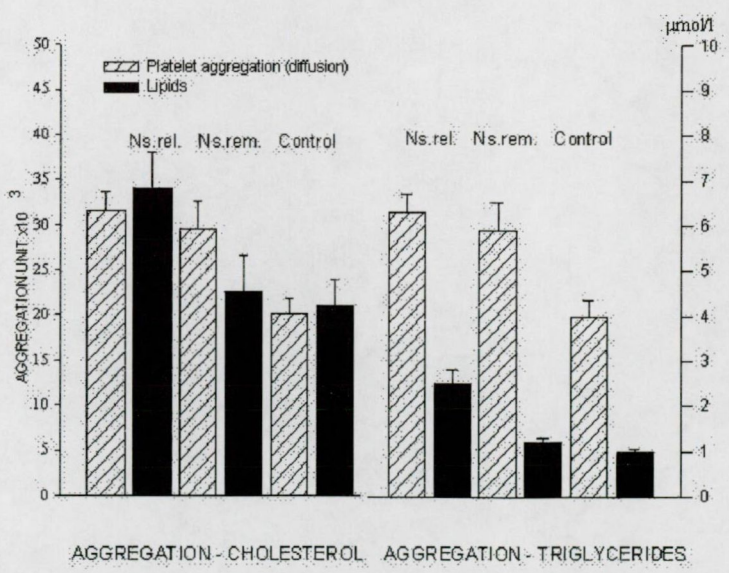

Fig.4.

Comparison of changes in platelet aggregation and plasma lipids (cholesterol and triglyceride) in nephrotic patients in relapse or in early remission.

Cholesterol and TGs: NS rel. (nephrotic syndrome in relapse) – controls and NS rel. (nephrotic syndrome in remission): $p<0,05$.

5.1.5. Effect of lipids on platelet aggregation

The platelet aggregability was similar in the nephrotic patients in relapse, or in early remission. The plasma cholesterol and triglyceride were significantly higher in relapse than in remission ($p<0.05$) or in the controls. (Fig.4) No correlation was observed between the platelet aggregation and serum cholesterol and triglyceride values.

5.2. *Comparison of platelet function in MCNS and FSGS*

5.2.1. Platelet aggregation

The platelet aggregation was significantly higher in all the patient groups than in the age-matched controls. FSGS and MCNS patients in relapse showed more elevated platelet aggregability than in MCNS patients in remission; the differences were not significant ($p<0.05$ and $p<0.01$ respectively) (Table 7.).

5.2.2. Platelet cAMP concentration

The platelet cAMP concentration was significantly lower in all the investigated groups as compared to the controls ($p<0.01$). (Table 7.) A significant negative correlation was observed between the platelet aggregation and the platelet cAMP concentration ($r=-0.62$, $p<0.05$).

Table 7. Platelet function in FSGS and in MCNS patients in relapse and in remission

	FSGS (A+P)	MCNS relapse	MCNS remission	Control adult	Control Paediatric
Number of pts.	16	12	15	12	15
Platelet aggregation (AU)	287±381**	26954±3016**	25537±3165*	19898±3206	18648±3165
TxB_2 release (ng/ml)	17.1±2.2**	12.1±3.4*	11.9±2.9*	7.1±1.6	6.9±1.8
cAMP (pmol/10^9 platelets)	14.1±2.9*	12.1±3.4*	11.7±2.7*	26.8±4.8	25.5±2.1

A: adult, P: paediatric, ** $p<0.01$, * $p<0.005$

5.2.3. Platelet TxB_2 release

The platelet TxB_2 release was higher in all the patients than in the controls (FSGS: $p<0.01$, MCNS in relapse and MCNS in remission: $p<0.05$) (Table 7.). A significant positive correlation was detected between platelet TxB_2 release and the diffusion obtained with platelet aggregation ($r=0.63$, $p<0.05$). The correlation coefficient for the platelet TxB_2 release and platelet cAMP concentration was significantly negative ($r=-0.60$, $p<0.05$). There was no significant difference, between the adult and paediatric controls in any of the investigated platelet function parameters.

5.3 Effects of bicarbonate and acetate haemodialysis on the platelet aggregation

5.3.1. Platelet aggregation

The PRP platelet counts were slightly, but significantly reduced in the ESRF patients: 350±5.1 G/l in normal subjects, 290±6.1 G/l in ESRF patients. Platelets from ESRF patients, either dialysed or treated conservatively, displayed a significantly lower aggregability than those from controls ($p<0.001$). In response to 1 or 3 μg/ml collagen a significantly greater increase in platelet aggregation was observed following bicarbonate HD than following acetate HD ($p<0.05$). (Table 8.) We observed a significant difference in platelet aggregation between the conservatively treated and dialysed ESRF patients before and after acetate HD ($p<0.05$).

Table 8. Platelet aggregation in ESRF

	n	Platelet aggregation induced by collagen (AU)	
		1 μg/ml	3 μg/ml
ESRF before acetate HD	6	905±457**	910±350**
after acetate HD		954±450**	721±254**
ESRF before bicarbonate HD	6	1390±454**	1363±542**
after bicarbonate HD		1750±460**	2150±832**
ESRF on conservative therapy	8	1520±330**	1704±380**
Control	10	3150±580	4050±761

** $p< 0.001$

There was no significant correlation between the serum PTH values and the platelet aggregability in the non-dialysed and dialysed ESRF patients

5.3.2 Platelet cAMP concentration

The platelet cAMP concentration was significantly higher in the ESRF patients (on conservative treatment, before bicarbonate and acetate HD) than in the controls. In the non-aggregated platelets the cAMP concentration was decreased significantly following acetate and bicarbonate HD ($p<0.05$). The tendency was similar during bicarbonate HD in the aggregated platelets (aggregation was induced with either 1 or 3 mg/ml collagen, $p<0.05$). (Table 9.)
A significant negative correlation was observed between the platelet aggregation value and the platelet cAMP concentration in all groups ($r=-0.7$, $p<0.05$). The intact PTH levels did not correlate with the platelet cAMP values.

Table 9. Platelet cAMP concentration and TxB_2 release in ESRF

	n	TxB_2 ng/ml	cAMP pmol/10^9 platelets before	1μg/ml Collagen	3 μg/ml Collagen
ESRF before acetate HD	6	38.7±18.2**	100.6±28.4*	75.1±20.8*	90.7±20.6*
after acetate HD		43.4±27.6**	44.5±7.41	56.7±18.7	86.5±20.7*
ESRF before bicarbonate HD	6	56.5±28.9*	80.2±21.9*	88.9±22.1*	80.7±22.4*
after bicarbonate HD		72.4±28.2*	25.2±16.1	42.7±18.9	43.1±20.4
ESRF on conservative therapy	8	-	72.5±28.9*	101.9±37.3*	115.2±37.5*
Control	10	112.1±29.1	35.5±6.5	38.8±5.1	54.0±8.8

* $p<0,05$, ** $p<0,01$

5.3.3. Platelet TxB_2 release

The TxB_2 formation by the platelets in response to 3 μg/ml collagen was measured in the dialysed (acetate HD and bicarbonate HD) and control groups. There was a significant difference between the values for the dialysed ESRF patients and the controls ($p<0.01$). The level of TxB_2

released from the aggregating platelets was increased more after bicarbonate HD than after acetate HD. The correlation coefficient for the platelet aggregability and the TxB_2 level was 0.67 ($p<0.05$). A negative correlation was observed between the platelet cAMP and TxB_2 levels ($r=0.60$, $p<0.05$). There was no significant correlation between the serum PTH and the platelet TxB_2.

5.4 Effect of rHu-EPO on platelet function in ESRF children on HD

5.4.1. Bleeding time and platelet count

Before rHu-EPO treatment, the pre-dialysis bleeding time was 9.4±0.6 min. which decreased to 5.7±0.6 min. following the 1-year rHu-EPO therapy ($p<0.05$). In the controls, it was 4.6±0.8 min. There was no significant difference in the platelet count before (178±32 G/l) and after (187±26.5 G/l) 1 year of rHu-EPO treatment and in controls (241±12 G/l). The *in vitro* and *in vivo* studies did not reveal any direct effect of rHu-EPO on the platelet aggregability. The increased Hct *in vitro* did not influence the platelet aggregation (Table 10.).

Table 10. Effect of rHu-EPO on the platelet aggregability in ESRF

	Platelet aggregation (AU)
In vivo study	
Before rHu-EPO	10140±2300
After rHu-EPO	9464±3429
In vitro study	
Before rHu-EPO	9308±1382
After rHu-EPO	8950±1080
Hct 0.23±0.02	9415±1329
Hct 0.38±0.02	9239±1290
Controls	21305±1763

5.4.2. Platelet aggregation

Platelets from the ESRF patients, either treated with rHu-EPO or non-treated, displayed a significantly lower aggregabilty *in vitro,* than those from the controls ($p<0.001$). One year of rHu-EPO therapy resulted in a significant increase in platelet aggregability ($p<0.05$). There was also an improvement following bicarbonate HD, either with or without rHu-EPO treatment ($p<0.05$, $p<0.01$ respectively) (Fig. 5A.).

5.4.3. ATP release from the platelets

A significantly lower ATP release was found from the ESRF platelets during aggregation (with or without rHu-EPO treatment) than from the controls ($p<0.001$). After 1 year of rHu-EPO therapy, the ATP release was significantly higher from the aggregated platelet than prior to rHu-EPO treatment ($p<0.05$). Bicarbonate HD itself caused a significant increase in the ATP release with or without rHu-EPO therapy ($p<0.01$, Fig. 5B). A significant positive correlation was observed in both groups between platelet aggregation and ATP release ($r = 0.78$, $p<0.05$)

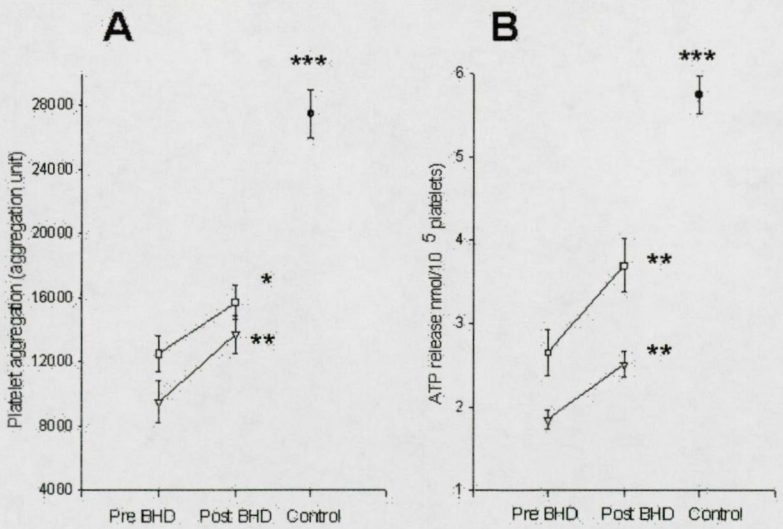

Fig. 5.

Changes in platelet aggregability (A) and ATP release from the platelet (B) prior to and following 1 year of rHu-EPO therapy (±SEM) in the patients before and after bicarbonate HD. (▽—▽) BHD before rHu-EPO, (□ — □) after 1 year of rHu-EPO therapy. Patients with or without rHu-EPO therapy (either pre- or post-bicarbonate HD), $p<0.05$, patients before and after bicarbonate HD (with or without rHu-EPO), *$p<0.05$, **$p<0.01$, patients and controls ***$p<0,001$.

5.4.4. Platelet cAMP concentration

The platelet cAMP concentration was significantly higher before bicarbonate HD than in the controls, either with or without rHu-EPO therapy ($p<0.05$, $p<0.01$ respectively). Nevertheless, 1 year of rHu-EPO treatment resulted in a significant decrease in the platelet cAMP concentration ($p<0.01$). During bicarbonate HD, a significant decrease was observed in the values, regardless of the performance of rHu-EPO therapy ($p<0.01$, Fig.6B). There was significant negative correlation in both groups of patients between platelet aggregation and the platelet cAMP concentration.($r=-0.75$, $p<0.05$)

5.4.5. Platelet TxB_2 release

The TxB_2 formation by the platelets in response to collagen was significantly lower in the ESRF patients (either with or without rHu-EPO) than in the controls ($p<0.001$). Following 1 year of rHu-EPO therapy, a significant increase was observed in the values ($p<0.01$).

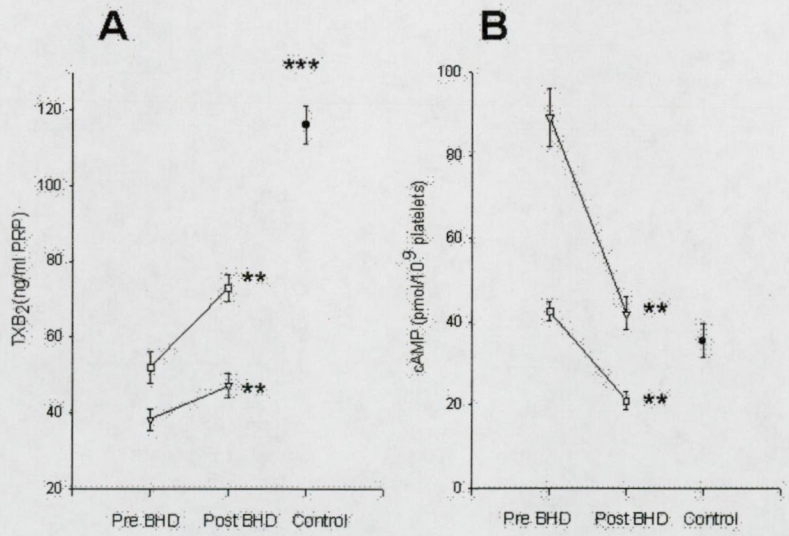

Fig. 6.

TxB_2 production (A) and cAMP concentration (B) in platelets during aggregation.

(∇ — ∇) BHD before rHu-EPO, ($\square$ — $\square$) after 1 year of rHu-EPO therapy. cAMP and TxB_2, with or without rHu-EPO (either pre- or post-bicarbonate HD) $p<0.01$; cAMP, patients (pre- dialysis with or without rHu-EPO) and controls, $p<0.05$ and $p<0.01$ respectively; patients (post- dialysis without rHu-EPO) and controls, $p<0.05$; no difference between patients (post-dialysis with rHu-EPO) and controls; patients before and after bicarbonate HD (with or without rHu-EPO), $p<0.01$; TxB_2, patients with or without rHu-EPO, before and after bicarbonate HD and controls, $p<0.01$, pre- and post-bicarbonate HD $p<0.01$.

**$p<0.01$, ***$p<0.001$, PRP: platelet-rich plasma

Bicarbonate HD (with or without rHu-EPO) resulted in an elevation in the TxB_2 release ($p<0.01$, Fig. 6A). The correlation coefficient for the platelet aggregability and the TxB_2 level was 0.68 ($p<0.05$). A negative correlation was observed between the platelet cAMP and TxB_2 levels ($r= -0.62$, $p<0.05$)

5.4.6. Platelet surface positive charge

The platelet positive surface charge (measured by ANS titration) was significantly lower in the ESRF patients than in the controls. rHu-EPO treatment did not influence the values significantly. Nevertheless bicarbonate HD resulted in a significant increase in the platelet surface positive charge ($p<0.05$), though it was still much lower than in the controls (Fig.7). A significant positive correlation was observed in both groups between the platelet aggregability and the surface positive charge ($r= 0.76$, $p<0.05$).

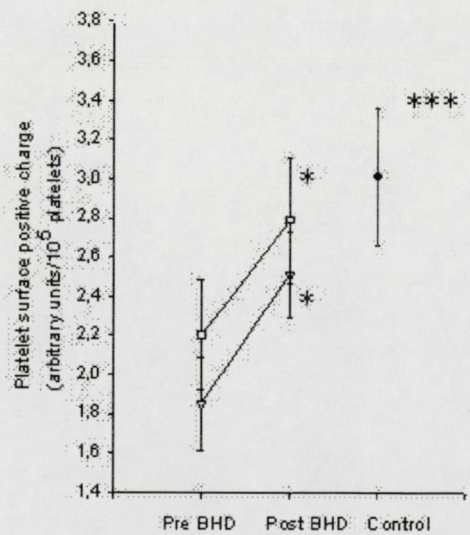

Fig.7.

Changes in platelet positive surface charge after bicarbonate HD (±SEM) (▽ —▽) BHD before rHu-EPO, (□ — □) after 1 year of rHu-EPO therapy. Pre- and post-bicarbonate HD, *$p<0.05$; patients (with or without rHu-EPO, before and after bicarbonate HD) and controls ***$p<0.01$.

5.5 *Role of platelet function, thromboxane and nitric oxide in hypertension of children and adolescents*

5.5.1. Blood pressure

The systolic and diastolic blood pressure was significantly elevated in JEHT ($p<0.001$) and in ESRF-associated hypertension (ESRFH) before and after bicarbonate HD ($p<0.001$). The post-dialysis values were significantly higher than those prior to HD ($p<0.05$). There were no significant differences between the ESRF patients (before and after bicarbonate HD) and the controls. (Table 11.)

5.5.2. Platelet aggregation

The platelet aggregation was significantly higher in the JEHT and ESRFH groups following bicarbonate HD ($p<0.01$). A significantly reduced platelet aggregation as compared with the controls ($p<0.01$) was demonstrated in the ESRF group both before and after bicarbonate HD. There was a positive correlation between either the systolic or the diastolic blood pressure and the platelet aggregation ($r=0.59$ and $r=0.56$, respectively, $p<0.05$).

Table 11. Systolic or the diastolic blood pressure and the platelet aggregation

Diagnosis	n	Platelet aggregation (AU)	Systolic BP (mm Hg)	Diastolic BP (mm Hg)
JEHT	33	33904±2869***	174.1±14.9***	106.3±10.4***
ESRFH before BHD	8	21599±3742**	157.1±13.6***	99.2±8.9***
ESRFH after BHD	8	30629±4303***	187.1±12.4***	117.5±3.9***
ESRF before BHD	8	13846±1923*	114.4±9.1	80.2±6.2
ESRF after BHD	8	13306±1923*	112.3±11.5	72.3±13.5
Control	10	16893±1258	104.4±6.9	74.2±5.3

JEHT: juvenile essential hypertension, ESRFH: ESRF-associated hypertension, BHD: bicarbonate haemodialysis. Statistical analysis – comparison with control values: $p<0.05$, **$p<0.01$, ***$p<0.001$

5.5.3. Platelet cAMP concentration

The platelet cAMP concentration was significantly lower in the JEHT group ($p<0.01$) and in the ESRFH group after bicarbonate HD ($p<0.001$) as compared with the controls. In the ESRF group (before and after bicarbonate HD), the results did not reveal any significant differences from the control data. A negative correlation was found between the platelet aggregation and cAMP levels ($r= -0.42$, $p<0.05$) (Table 12.)

5.3.4. Platelet TxB_2 release

TxB_2 release was significantly higher during platelet aggregation in the JEHT group ($p<0.001$) and in the ESRFH group ($p<0.01$ and $p<0.001$, respectively, before and after bicarbonate HD). The correlation was significantly positive between the platelet aggregation and TxB_2 release data ($r=0.47$, $p<0.05$) No differences were observed in the platelet TxB_2 results between the ESRF group and the controls.

Table 12. Platelet cAMP concentration, TxB_2 release and plasma NO_x

Diagnosis	n	Platelet TxB_2 (ng/ml PRP)	cAMP (pmol/10^9 platelets)	Plasma NO_x (μM)
JEHT	33	392±69***	24.3±5.0***	120±3.9**
ESRFH before BHD	8	273±34**	29.1±4.0*	85.8±10.4
ESRFH after BHD	8	388±29***	15.4±7.3***	59.7±6.8**
ESRF before BHD	8	156.4±28.2	32.5±5.6	78.5±7.2
ESRF after BHD	8	165.3±17.4	43.2±7.6	48.5±5.8**
Control	10	178±16	39.1±9.6	89.0±21.0

JEHT: juvenile essential hypertension, ESRFH: ESRF-associated hypertension, BHD: bicarbonate haemodialysis. Statistical analysis – comparison with control values: *$p<0.05$, **$p<0.01$, ***$p<0.001$

5.5.4. Nitric oxide end-products (NO_x) in plasma

The plasma NO_x concentration was significantly higher in the JEHT group ($p<0.01$) and lower in the ESRF and ESRFH groups as compared with the controls ($p<0.01$). There was a weak negative correlation between the plasma NO_x level and platelet aggregation ($r=0.42$, $p<0.05$).

5.5.5. Plasma lipoproteins

The plasma total cholesterol and triglyceride levels were significantly higher in each ESRF group as compared with the controls (before and after bicarbonate HD, $p<0.05$, ESRFH after bicarbonate HD, $p<0.01$). The LDL cholesterol level was significantly higher ($p<0.001$) and the HDL cholesterol level was lower ($p<0.001$) in the ESRF and ESRFH groups (before and after bicarbonate HD) as compared with the controls. All these lipoprotein values were normal in the JEHT group. There was no significant difference in plasma VLDL concentration between any patients group and the controls. No correlation was found between the plasma lipid values and the BP or the platelet aggregation.

Table 13. Plasma lipoproteins

Diagnosis	n	Cholesterol (mmol/l)	Triglyceride (mmol/l)	LDL cholesterol (%)	HDL cholesterol (%)	VLDL cholesterol (%)
JEHT	12	4.5±0.8	1.4±0.9	44.2±4.5	28.2±5.9	25.9±6.2
ESRFH before HD	8	5.9±1.1*	2.1±1.2*	64.4±5.5***	15.2±0.8***	19.2±3.9
ESRFH after HD	8	5.6±0.5*	3.6±1.1**	62.7±6.2***	15.4±0.5***	21.2±3.4
ESRF before HD	6	5.8±0.7*	2.3±1.1*	58.3±5.3***	15.6±0.4***	24.1±2.2
ESRF after HD	6	5.9±0.8*	2.7±1.2*	53.6±6.1***	15.8±0.5***	25.3±6.4
Controls	10	4.5±0.7	1.2±0.6	43.6±8.8**	28.6±6.1	27.7±8.1

JEHT: juvenile essential hypertension, ESRFH: ESRF-associated hypertension, HD: haemodialysis. Statistical analysis – comparison with control values: *$p<0.05$, **$p<0.01$, ***$p<0.001$

6. DISCUSSION

6.1. Nephrotic syndrome and platelet function

A number of haemostatic parameters change in nephrotic syndrome. Increases have been found in coagulation factors (I, II, VIII, X and XII), due to the increased hepatic synthesis accompanying the increased syntheses of albumin and lipoproteins [1,65,74,79]. The plasma fibrinogen level is elevated [1,65], as are the plasma concentrations of protein C and S. Antithrombin III plays a major role in preventing the intravascular deposition of fibrin, but its level is decreased in nephrotic syndrome, because of the urinary loss.

There is substantial evidence of an impaired platelet function during glomerular injury, primarily based on the detection of elevated plasma concentrations of platelet-derived substances, suggesting an enhanced platelet activity in children with nephrotic syndrome. Important differences may exist depending on the type of glomerular lesion. MGN is the most frequent glomerulonephritis in adults, and it is also the lesion in which thrombosis most common [74]. Conversely, MCNS is the lesion least often complicated by thrombosis. Cameron [11] found no cases of renal venous thrombosis in MCNS, whereas Llach [64] reported an occurrence of 40% in MGN. In a survey of 155 nephrotic patients with renal venous thrombosis, 57% had MCN and only 6% had MCNS [65]. Diffuse mesangiocapillary glomerulonephritis is also associated with thrombosis, but it is only one-third as frequent as MGN. Like patients with MCNS, those with FSGS have this complication only rarely. Of the secondary causes of nephrotic syndrome, thrombosis is often seen in SLE, diabetes mellitus and amyloidosis [74].

Intraglomerular fibrin deposition and thrombosis may play important roles in the onset of glomerular sclerosis [9,50]. The importance of glomerular fibrin formation is emphasised by the beneficial effects of anticoagulant or anti-platelet drugs, which in some studies markedly reduced the severity of the lesions and functional impairment in treated animals [81,82].

In our laser-rheoaggregometer study, both transmission and diffusion methods revealed an increased *in vitro* aggregability in nephrotic patients. The transmission data showed that patients with IgA and SLE nephropathies have significantly higher platelet aggregability than those with MCNS in early remission, while no difference in platelet aggregation could be detected between MCNS patients in remission or in relapse. The highest level of platelet aggregation was observed in MGN patients, one of who had unilateral renal venous thrombosis. Abnormalities in the prostaglandin system may contribute to the platelet aggregation. Albumin is

involved in the transport of arachidonic acid. With hypoalbuminaemia, freer arachidonate is available for platelet cyclooxygenase, permitting an increased synthesis of thromboxanes, thereby fostering the aggregation of platelets [11]. An increased platelet aggregation in response to arachidonic acid infusion has been demonstrated in platelets from nephrotic syndrome patients; this is normalised by the addition of albumin [1].

Our study demonstrated an increased TxB_2 release from the platelets of nephrotic patients either in relapse or in early remission, when no proteinuria was detected and the serum albumin level was undergoing normalisation. Our patients did not receive an albumin infusion. This means that in the early phase of remission the increased TxB_2 production continues until the enhanced serum albumin and other regulatory mechanisms reduce it. The increased TxB_2 level in remission was compatible with the increased aggregation results and the increased ATP release during aggregation. Ueda et al. [102] observed the hyperaggregation of platelets in response to ADP in nephrotic children, with no relationship to the serum albumin concentration. On the other hand, the cAMP concentration of the platelets from our patients with nephrotic syndrome was reduced. This may imply that, apart from the serum albumin level, intraplatelet mechanisms could play a role in the behaviour of platelets in nephrotic syndrome allowing Ca^{2+} release from the cytosol, which results in platelet activation. The effects of hyperlipidaemia and steroid therapy on the coagulation system and platelet function are not completely clear. The fluctuation in the antithrombin III level in childhood nephrotic syndrome is determined by the response to steroids [28]. In remission induced by prednisone, the platelet count and cholesterol, antithrombin III and protein C levels were still increased. This suggests that the steroid therapy could induce hyperlipidaemia and thrombosis.

The change in platelet function of nephrotic patients is part of the changes in the haemostatic system. The extent of the hyperfunction may vary with the type of glomerular lesion, and in some forms, e.g. MGN, thrombosis could develop, which is determined by the collective change in the haemostatic system. The serum albumin level is not the only factor influencing the platelet function, and the intraplatelet cAMP could play an important role in the extended hyperaggregation of platelets from nephrotic patients in remission. Low-dose oral steroid therapy and hyperlipidaemia *per se* do not increase platelet aggregation. On the other hand, nephrotic patients in early remission still have hyperaggregable platelets producing vasoconstrictor and proaggregatory factors, and more time is needed for normalisation.

The question as to whether MCNS and FSGS, the two most common cause of nephrotic syndrome in childhood, are different entities or merely different manifestations of one disease remains unanswered. There are significant dissimilarities in the course of the disease and in the

effectiveness of the treatment of MCNS and FSGS. The increased platelet aggregability and TxB_2 release suggesting that abnormalities may be involved in the pathogenesis of both MCNS and FSGS. The platelets TxB_2 is significantly higher in FSGS than in MCNS, which may be have an impact in the progression of glomerular damage by an increased vasoconstriction and intraglomerular pressure. The intraplatelet cAMP is a regulator of platelet activity by inhibiting Ca^{2+} release from the cytosol [107]. An increased production of cAMP results in a diminished adhesion and aggregation ability in the platelets. In the investigated FSGS and MCNS patients, the inhibitory effect of cAMP on the platelet activation is decreased by reduction of the platelet concentration. Interestingly, the patients with MCNS in remission still had platelet disorders. Koyama et al.[54] reported a vascular permeability factor, a postulated lymphokine, which induces nephrotic syndrome by weakening the GBM anionic charge. This factor impairs not only the GBM permeability, but also that of other cell membranes, as presumed by Levin et al. [60] and Boulton-Jones et al.[7]. If this factor impairs the platelet function and still remains functional shortly after the proteinuria remission obtained with corticosteroid, this could explain these findings.

6.2. Platelet function in ESRF patients

The dysfunction of the platelets is one of the major contributing factors in the haemorrhagic tendency of patients with ESRF. The platelet aggregation is reduced in response to a variety of single agents [13,31]. These abnormalities are partially and transiently corrected in some cases after HD and peritoneal dialysis (PD)[86,98]. Therefore, it has been assumed that a low molecular weight inhibitor is present in these patients. Individual studies suggested that the inhibitor might be guanidinosuccinic acid [46,76], phenolic acid [85], urea [26] or a combination of these [89]. di Minno et al.[19] found that uremic platelets have a defective aggregation, and they appear to release arachidonic acid from phospholipids abnormally after stimulation with some agents. However, the metabolism of exogenous arachidonic acid and the response to its metabolites are normal. There is also a modest reduction in the storage pool of adenine nucleotides. Dialysis resulted in partial correction of the abnormalities of aggregation and the arachidonic acid metabolism. However, it did not alter the storage pool defect.

In this study, the platelets from dialysed or non-dialysed patients with ESRF showed a significantly higher cAMP, a lower TxB_2 and a lower aggregability than, those of the platelets from the controls. These results are consistent with observations that in ESRF patients the platelet cAMP is increased [107]. This elevation may contribute to the defective aggregation

response. The lower level of platelet TxB_2 also observed by di Minno et al. [19], suggests a decreased release of arachidonic acid from platelet phospholipids in ESRF patients. Although the stimulation of platelets by aggregating agents is usually accompanied by a decrease in cAMP [90], in our patients (following 1 or 3 μg/ml collagen stimulation) the pre-dialysis (acetate HD) samples exhibited only slightly lower cAMP contents than before the addition of collagen. The platelet cAMP decreased more markedly after bicarbonate HD than after acetate HD, and greater increases in platelet aggregation and TxB_2 formation were observed after bicarbonate HD than after acetate HD. One possible reason for the different results may be that acetate is a weak acid, and in protonated form it is lipid-soluble. It behaves as an uncoupling agent, prevents the phosphorylation of ADP to ATP, and possibly decreases the electrochemical potential on the internal mitochondria [59]. In the platelets, experimental control is required.

Lindsay at al. [62] demonstrated that platelets from ESRF patients aggregate normally in response to ADP as long as the serum creatinine level is less than 530 μM. The platelet aggregation was found to be impaired in patients with a more severe renal insufficiency. We have demonstrated that the platelet aggregability might be seriously impaired at a lower level of serum creatinine (average 480 μM) and the cAMP could be as high as in the dialysed patients with a higher level of serum creatinine. This might reflect the multifactorial origin of uraemic thrombocytopathy. In this respect, the possible role of concomitant hyperlipidaemia seen in dialysed ESRF patients should be taken into account [2,53,83]. The picture is more complicated as regards the possible platelet activation induced by the dialysis membrane [6] or heparin [113]. At the time of this study our patients had secondary hyperparathyroidism. It has been found that PTH inhibits the platelet aggregation induced by ADP, arachidonic acid and Ca^{2+} ionophore [90]. It was hypothesised that PTH interferes with the platelet function by altering the platelet cAMP and/or Ca^{2+} level via the stimulation of adenylcyclase and the augmentation of intracellular cAMP. However a recent study did not find a correlation between the platelet function and the degree of hyperparathyroidism in ESRF patients. Moreover the platelet aggregation was more altered in the ESRF patients on conservative treatment than in the patients on chronic HD, in spite of the higher blood PTH level in the latter. In the patients of Docci et al. [21] treatment with 1,25-$(OH)_2$ Vitamin D_3, which should normalise the elevated intraplatelet Ca^{2+} level, did not ameliorate the platelet dysfunction, thereby indicating that PTH can aggravate uremic thrombocytopathy, but is probably not the main factor which determines it. In our study, the ESRF patients on HD had much higher levels of PTH as compared to the ESRF patients on conservative treatment. The platelet cAMP and aggregability, particularly before and after bicarbonate HD, did not differ significantly from the results on the ESRF patients on

conservative treatment. These observations seem to confirm that there is no correlation between the serum PTH level and the degree of disturbances of the biochemistry and aggregation of uremic platelets.

Many studies have investigated the effect of rHu-EPO on thrombotic complications in patients with ESRF who undergo HD or PD treatment with rHu-EPO; the results are controversial and only a slightly higher incidence of vascular access failure due to thrombosis has been reported [39,70,87]. In our study, the bleeding time of the ESRF children was markedly shortened following 1 year of rHu-EPO treatment, as has been reported by other authors [39,70]. The platelet aggregation increased in accordance with the results of Fabris et al. [33]. Taylor et al [100] reported an improvement in spontaneous platelet aggregation in ESRF patients on HD and rHu-EPO therapy. Although the bleeding time normalised in our patients and the platelet aggregation increased, it was still lower than in the controls. Therefore we could not exclude the beneficial effect of the increased Hct on the bleeding time. Nevertheless, our *in vitro* study indicated that the increased Hct did not increase the platelet aggregation. Accordingly, an increased RBC count does not influence the platelet aggregability. rHu-EPO itself did not have a direct effect on aggregation, in either the *in vitro,* or the *in vivo* experiment. Thrombotic events did not develop in our patients during the treatment time. Horina et al. [45] demonstrated an increased platelet count during rHu-EPO therapy, which was not confirmed in our study. However, we observed a number of changes in the platelet biochemistry during rHu-EPO treatment. There were significant increases in platelet ATP release and TxB_2 production, which further increased during bicarbonate HD, as did the platelet aggregability. Van Geet et al. [103] and Fabris et al. [33] also observed increased TxB_2 production during rHu-EPO therapy. At the same time the platelet cAMP concentration decreased significantly. Vasiri et al. [104] recently reported that the observed improvement of the platelet function with rHu-EPO therapy is due to a correction of the defective Ca^{2+} signalling in the platelets.

The presence of the platelet surface positive charge was demonstrated by ANS titration. These results revealed a significant correlation with the platelet aggregability. In most biological membranes, the resultant charge is negative [43]. The surface of the platelets [67] and other blood cells [32] is negatively charged, largely due to the presence of sialic acid residues in the GPs and glycolipids of the cell membranes. Factors decreasing the proportion of surface positive charge on the platelets make the membrane more negative and probably reduce the adherence of cells to the endothelium. The nature of these substances is not yet known. Cationic surfactants decrease, whereas anionic surfactants increase the negative surface charge. The beneficial effect of

bicarbonate HD suggests the role of uraemic toxins and pH, which change during dialysis sessions, but not on rHu-EPO therapy.

6.3. Hypertension in children and adolescents

An important interaction occurs between hyperlipidaemia and hypertension. Hypertension exaggerates a hyperlipidaemic injury, and hyperlipidaemia alters the systemic and glomerulo-vascular production of vasoactive substances, which maintain the basal vascular tone [51,66]. Hayakawa and Raij [42] revealed that lipid oxidation products might reduce the NO bioactivity without affecting the endothelial nitric oxide synthase (NOS) activity. A decreased NO bioavailability does not necessarily result in systemic hypertension, but it may enhance the sensitivity of the hypertensiogenic effect of dietary salt. In our patients with JEHT, the total cholesterol and LDL cholesterol levels were normal, which is very uncommon in adult patients with essential hypertension. The role of dyslipidaemia in JEHT cannot be supported. Other risk factors, such as smoking, diabetes and alcohol consumption, can also be excluded in these patients with JEHT. The absence of dyslipidaemia in JEHT and the presence of ESRF with or without hypertension may exclude its pathogenetic role in JEHT, and it seems to be a consequence of ESRF rather than the cause of hypertension. Nevertheless, hyperlipidaemia and dyslipidaemia may increase the progression of vascular damage in ESRF-associated hypertension.

In vitro platelet aggregability and TxB_2 release were significantly higher in the JEHT and ESRFH groups. Although ESRF itself is associated with low platelet aggregability, this becomes increased in hypertension. The platelet aggregation was independent of the plasma lipid concentration, but there was a significant positive correlation between the platelet aggregation and TxB_2 release. The lower level of platelet cAMP resulted in a reduced control in platelet activation in both hypertensive groups. In these patients, the significantly higher TxB_2 release may contribute to a generalised vasoconstriction and a further increase in platelet aggregation. Our patients with JEHT and ESRFH presented a significant positive correlation between the platelet aggregation and the blood pressure. In the study by Riondino et al. [92], significant correlations were observed between all platelet function parameters and blood pressure values. An increased platelet aggregation in JEHT may therefore play a role in the progression of hypertension before the development of atherosclerosis. Hypertension and platelet hyperaggregability might have a common aetiological factor. As demonstrated by Riondino et al. [92] in a population of older hypertensive patients with no other risk factor or atherogenic disease,

normalisation of the blood pressure resulted in a significant reduction in platelet hyperactivity. In ESRFH, the platelets are over activated, probably by the same factors as those responsible for the hypertension. The increased platelet aggregation with hyperlipidaemia and dyslipidaemia (a high level of LDL cholesterol and a low level of HDL cholesterol) may increase the progression of atherosclerosis and hypertension in ESRFH.

AT II may stimulate endothelial NOS activity and NO release, which could further inhibit the ACE activity and down-regulate the AT-1 receptor via a negative feedback [108]. An increase in NO generation would be balanced by an activation of the RAS and AT I synthesis, which might compete with NO for the active centre of ACE. A lack of NO, results in an increased action of AT II with severe pathophysiological consequences. Our patients with ESRFH displayed significantly decreased level of plasma NO_x by the end of HD in parallel with a markedly increased blood pressure. This may correspond to the ACE and AT-1 receptor modelling role of NO. In JEHT patients, the observed elevation in plasma NO_x level might be a result of AT II stimulation or The explanation of the missing vasodilatator effect of NO could be an increased degradation, probably caused by superoxide anion released from the dysfunctional vascular endothelium [105]. Endothelin-1 produced locally could strengthen the effects of other vasoconstrictors. Besides its vasoactive properties, NO inhibits the platelet aggregation *in vitro* and platelet adhesion to cultured endothelial cells [38,66]. The anti-platelet properties of NO *in vivo* may not be so efficient in the presence in the presence of NO inhibitors (e.g. renal failure, pre-eclampsia, JEHT), a mechanism that may provide an explanation for increased risk of vascular thrombotic events in this cases, including hypertensive children and adolescents [38].

7. CONCLUSIONS

7.1. Our study did not reveal any significant difference in the results of platelet aggregation, ATP release, TxB_2 release, or platelet cAMP concentration between steroid-treated and non-treated patients. The plasma cholesterol and triglyceride levels were significantly higher in relapse than in remission; nevertheless, no differences in platelet function were seen between these groups. In conclusion oral prednisone therapy on alternate days and the high cholesterol and triglyceride level in the plasma cannot be responsible for the hyperaggregation of platelets in nephrotic children.

7.2. The increased platelet aggregability and TxB_2 release suggesting that abnormalities may be involved in the pathogenesis of both MCNS and FSGS. The platelets TxB_2 is significantly

higher in FSGS than in MCNS, which may be have an impact in the progression of glomerular damage by an increased vasoconstriction and intraglomerular pressure.

7.3. Our results demonstrate that platelets from dialysed or non-dialysed ESRF patients display a significantly higher cAMP, a lower TxB_2 and a lower aggregability, than those of the controls.

7.4. Role of PTH, in spite of its significant increase, seems to be less important. Our observations seem to confirm that there is no correlation between the serum PTH level and the degree of disturbances of the biochemistry and aggregation of platelets of ESRF patients.

7.5. Platelet cAMP level decreased more markedly during bicarbonate HD, than during acetate HD, and greater increases in platelet aggregation and TxB_2 formation were observed following bicarbonate HD.

7.6. Normalisation of the bleeding time following 1 year of rHu-EPO therapy was not associated with the normalisation of the platelet aggregability and biochemistry, we can not exclude the role of the increased Hct in the shortening of the bleeding time. However, the increased RBC count did not improve the platelet aggregation. Although rHu-EPO itself did not improve the platelet aggregation directly during 1 year of rHu-EPO therapy, a number of platelet functional changes were observed, including increased aggregability, and ATP and TxB_2, release and a decrease in cAMP concentration. These contribute to the improvement of the bleeding time. Therefore we consider that rHu-EPO therapy can improve the platelet function through biochemical changes and has a combined effect on the haemostasis of ESRF patients.

7.7. Increased platelet aggregation and TxB_2 are characteristic feature of juvenile essential hypertension and ESRF associated hypertension, contributing the blood pressure elevation, while hyperlipidaemia is more common in ESRF, regardless blood pressure and does not have a role in the pathogenesis of JEHT at an early stage. In addition JEHT patients exhibits a compensatory increases in plasma NO level. The anti-platelet properties of NO may not be so efficient in the presence of NO inhibitors, a mechanism that may provide an explanation for increased platelet aggregability in these cases.

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