

Institutionen för Klinisk Vetenskap, Barnens sjukhus,
Huddinge Universitetssjukhus, Karolinska Institutet,
Stockholm

NEPHROTIC SYNDROME IN CHILDREN

Functional, Morphological and Therapeutical
Aspects

Eva Löwenborg

Leg.läkare



Stockholm 2003

Cover: An artist view of foot processes of the podocyte

All previously published papers were reproduced with permission from the publisher.

Lay-out with permission from Erik Wassberg

Cover is painted by Göran Rucker

Published and printed by Karolinska University Press

Box 200, SE-171 77 Stockholm, Sweden

© Eva Löwenborg, 2003

ISBN 91-7349-360-0

To Johanna och Andreas

ABSTRACT

Although cure rates of the idiopathic nephrotic syndrome in children have improved because of advances in immunosuppressive therapy, the pathogenesis and course of the disease remain controversial. We have followed children with nephrotic syndrome to study renal function and morphological changes in kidney biopsies and in an experimental study. Immunosuppressive treatment with cyclosporine in severe nephrotic syndrome has been shown to be effective, but nephrotoxicity is a major concern.

Some studies have suggested that glomerular hypertrophy precedes the development into FSGS, which comprises focal and segmental scarring of the glomerulus, a frequent cause of renal failure. In this syndrome the podocyte and its foot processes show foot process effacement, usually reversible. However, if the disease becomes worse, it leads to detachment of the foot processes from the glomerular basement membrane and initiates renal scarring of the glomerulus. DMP and FSGS are regarded as more severe types of the nephrotic syndrome, clinically and histopathologically, than MCNS.

In 58 children with MCNS (40), DMP (4) and FSGS (14) we investigated retrospectively the glomerular volume. GFR and ERPF were used to determine renal function. The patients were BSA or age matched. The glomerular volumes in DMP and FSGS were significantly larger than in those with MCNS ($P < 0.005$, $P < 0.002$). We also found direct correlation between glomerular volume and GFR and ERPF in all patients. In conclusion these results show that renal hemodynamics may contribute to glomerular hypertrophy.

Moreover, in an experimental study in rats with PAN-induced nephrotic syndrome, mimics the nephrotic syndrome in children, we found that the severity of the foot process effacement and the increasing length of the slit pores and fractional albumin excretion were related to a reduction in glomerular filtration. In an early stage of the disease the glomerular volume was enhanced.

To investigate GFR and ERPF in relation to oncotic pressure (s-albumin concentration) we evaluated 119 children who had various types of the nephrotic syndrome. The renal function was determined at additional times according to the clinical course of the nephrotic stage, the recovery stage and in remission. In the nephrotic stage the GFR was significantly lower than in remission (and compared to controls), while ERPF was higher in the nephrotic stage, especially in the types with histological lesions. Thus, the filtration fraction was much lower in the nephrotic stage. The low GFR was therefore caused not by hypoperfusion, but by on a very low ultrafiltration coefficient.

In therapy-resistant MCNS and FSGS, cyclosporine is used as immunosuppressive therapy. In a prospective study, which started in 1987, 22 children were investigated as regards to GFR and ERPF every sixth month and repeated kidney biopsies were taken to evaluate cyclosporine nephrotoxicity. We found very mild morphological changes in the MCNS patients. Most of the FSGS children showed a decline in proteinuria, which may delay or induce a sustained remission. In both groups, renal function declined from the first to the last investigation, as assessed by GFR

(from 110 to 93 and from 96 to 77 ml/min per 1.73m^2 in the MCNS and FSGS; respectively) and ERPF (from 521 to 468 and 581 to 398 ml/min per 1.73m^2 , respectively). In the MCNS patients, the GFR normalized after cessation of treatment in 4 patients, who have had renal function tests after CsA treatment. Both groups responded well to CsA. Therefore, CsA can be used as long-term treatment in selected cases.

Keywords: Children, glomerular volume, glomerular filtration rate, effective renal plasma flow, rats, nephrotic syndrome, cyclosporine, nephrotoxicity, rats, puromycin aminonucleoside, morphology

LIST OF PUBLICATIONS

This thesis is based on the following papers, referred to by their Roman numerals (I-IV)

- I.** Nyberg EKM., Bohman S.O., Berg UB.
Glomerular volume and renal function in children with different types of the nephrotic syndrome
Pediatr Nephrol (1994) 8:285-289
- II.** Löwenborg EKM., Berg UB.
Influence of serum albumin on renal function in nephrotic syndrome
Pediatr Nephrol (1999) 13:19-25
- III.** Löwenborg EKM., Jaremko G., Berg UB.
Glomerular function and morphology in puromycin aminonucleoside nephropathy in rats
Nephrol Dial Transplant (2000) 15:1547-1555
- IV.** Löwenborg EKM., Söderberg M., Bohlin A-B., Berg UB.
Renal function and morphology in children with the nephrotic syndrome during long-term treatment with cyclosporine
Submitted

CONTENTS

Contents

List of Abbreviations

List of Definitions

INTRODUCTION

1

NEPHROTIC SYNDROME IN CHILDHOOD

2

History

2

Incidence

3

The glomerulus

5

Underlying disorders

6

The minimal change nephrotic syndrome

Diffuse mesangial proliferation or mesangiolipid glomerulonephritis

Focal segmental glomerulosclerosis

Mesangiocapillary or membranoproliferative glomerulonephritis

Membranous nephropathy

The glomerulus and renal function

9

Glomerular filtration

10

The podocytes

10

Glomerular hypertrophy and progression of renal disease

13

Pathogenesis

15

Proteinuria

16

Treatment

16

Glucocorticosteroids

Alkylating agents

Cyclosporine

Nephrotoxicity

AIMS OF THE STUDY

20

PATIENTS AND CONTROLS	21
METHODS	
Renal function	24
Renal morphology	28
Animal study	29
Statistical methods	34
RESULTS AND DISCUSSION	35
CONCLUDING REMARKS AND FUTURE PERSPECTIVES	45
ACKNOWLEDGEMENTS	47
REFERENCES	49

LIST OF ABBREVIATIONS

BSA	Body surface area
BW	Body weight
CsA	Cyclosporine A
DMP	Diffus mesangial proliferation
EM	Electron microscopy
ERPF	Effective renal plasma flow
ESRF	End-stage renal failure
FF	Filtration fraction
FSGS	Focal segmental glomerulosclerosis
GBM	Glomerular basement membrane
GFR	Glomerular filtration rate
GA	Glomerular surface area
GV	Glomerular tuft volume
Kf	Ultrafiltration coefficient
LM	Light microscopy
MCGN	Mesangio-capillary glomerulonephritis
MCNS	Minimal change nephrotic syndrome
MN	Membranous nephropathy
NS	Nephrotic syndrome
PAH	Para-aminohippuric acid
PAN	Puromycin aminonucleoside
p_{UF}	Ultrafiltration pressure
RAS	Renin angiotensin system
SD	Steroid-dependent
SR	Steroid-resistant
SS	Steroid-sensitive

LIST OF DEFINITIONS

Idiopathic(primary)NS	NS of unknown origin
Congenital NS	NS presenting within the first 3 months of life
Infantile NS	NS presenting between 3 and 12 months of life
Familial NS	NS of the same histological type occurring in two or more family members
Syndromatic NS	NS occurring in a child with a congenital syndrome
Steroid-sensitive	Normalisation of proteinuria within 4 weeks after start of standard initial therapy with daily, oral corticosteroids
Steroid-resistant	NS unresponsive to corticosteroids during the first 8 weeks of treatment
Steroid-dependent	Frequently relapsing NS with two or more fast relapses per
Frequently relapsing NS	Two or more relapses per 6 months (or 4 or more per 12 months) following the initial therapy or a relapse therapy
Remission of NS	Proteinuria < 4mg/m ² /h for more than 3 days and serum albumin > 35 g/L
Partial remission	Serum albumin 25-35 g/L and/or non-nephrotic proteinuria
Urinary remission	Proteinuria < 4mg/m ² /h
Early non-responder	No normalization of proteinuria within 4 weeks of daily, oral corticosteroids
NS with primary steroid-resistance	Persistence of proteinuria after completion of standard initial corticosteroid therapy
Relapse of NS	Proteinuria > 40 mg/m ² /day on three consecutive days or during any three days in one week

INTRODUCTION

Since 1967, the International Study of Kidney Diseases in Children has collated most of our clinical knowledge about the idiopathic nephrotic syndrome (NS) in childhood (ISKDC, 1978; ISKDC, 1979; ISKDC, 1981) and has tried to standardize definitions concerning various aspects of this syndrome presented included in our list of definitions. The term nephrotic syndrome refers to clinical signs and symptoms, and is defined as severe proteinuria $> 40\text{mg/m}^2$ body surface area (BSA) per hour, hypoalbuminemia with a serum albumin concentration of $< 25\text{g/L}$, edema and hypercholesterolemia. The nephrotic range of proteinuria has also been defined as a urine albumin excretion $>50\text{ mg/kg}$ per 24 h. or $\text{U-alb/U-creat} >1000\text{ mg/mmol}$ (Barratt et al., 1970).

Our knowledge of the primary NS in children is still limited and the pathogenesis of the commonest form, minimal change nephrotic syndrome, remains largely unknown.

This thesis comprises two morphological studies of the glomeruli and their prognostic implications (Papers I and III), an experimental study on glomerular morphology and its significance for renal function (Paper III), a study on the relation between renal function and s-albumin concentration (Paper II) and one prospective study on renal function and morphology during long-term treatment with cyclosporine (Paper IV).

The aims were therefore to analyze further the relation between renal function and morphology and their predictive value for the progression of the disease. Another aim was to evaluate the effect of specific treatment with cyclosporine on renal function and morphology.

THE NEPHROTIC SYNDROME IN CHILDHOOD

History

In the medical studies of Hippocrates, dropsy (edema) in adults was a recognized condition, although its various causes could not be distinguished. Hippocrates noted that ‘when bubbles settle on the surface of the urine, it indicates disease of the kidneys and that the complaint will be protracted’ (Cameron, 1985).

In 1484, Cornelius Roelans in Belgium described ‘swelling of the whole body of the child’ without mentioning the kidneys. Two hundred and fifty years later, in 1722, the physical signs of the NS were described in *Paediatrica Practica* by Theodore Zwinger of Basel, who attributed the condition to the renal tubules. By 1830 Bright and others, had established that NS was caused by diseased kidneys leaking protein. This clinical entity was later called ‘Bright’s disease’. In 1846, Johnson described the fatty casts in patients urine and the fatty appearance of the kidneys. By 1905, a German pathologist had introduced the term ‘nephrosis’ to describe all ‘noninflammatory’ diseases of the kidney. Thereafter, this clinical term and its antithesis, ‘nephritis-nephrosis’, immediately became popular. The term ‘lipoid’ nephrosis was introduced by Munk about 1910 (Cameron, 1985; Cameron JS and Hicks J, 2002).

During the 1920s, Epstein suggested that ‘pure nephrosis’ was a systemic disorder, which was caused by a disturbance of protein synthesis, which might be the result of a thyroid disorder. Consequently many nephrotics were treated with thyroid extract for almost two decades (Epstein, 1927).

In the early 1920s, the proteinuria was thought to be tubular in origin. However, Bell and others suggested that the proteinuria was of glomerular origin, with secondary tubular changes. This view was based on improvements in histological staining techniques but the findings were ignored (Bell, 1929). It was not until 1948 that Bradley and Tyson reviewed the NS in the *New England Journal of Medicine* and gave a complete description of the nephrotic syndrome as a glomerular disease (Bradley SE and Tyson CJ, 1948).

In the early 1950s, Brun and Iversen in Denmark and Kark and Muerhke in USA introduced percutaneous needle renal biopsy, which made it possible to recognize its underlying histopathology (Iversen and Brun, 1951; Muehrcke et al., 1955; Kark et al., 1958) .

A complication of the NS-e.g., venous thrombosis was first described in 1840. The commonest complication in former times was infections, which, before the antibiotic era proved fatal in most of nephrotic children (Letter, 1931). Especially the susceptibility of encapsulated organisms, such as Pneumococci, was found. Another major problem was the rapid spread of cellulitis in the edematous tissues.

Until 1950, there was no effective treatment for the nephrotic syndrome, other than salt restriction and weak mercurial diuretics. Then, in 1949-1950, the first reports were published on the use of the newly synthesized corticosteroids and ACTH, for childhood nephrosis (Farnsworth, 1950). Immediate dramatic remissions were seen, some children did not respond to corticosteroids. Thus the terms steroid-responsive and steroid-resistant nephrotic syndrome came into use.

Incidence

The idiopathic nephrotic syndrome is relatively rare in children and adolescents.

Its incidence is about 2.2 per 100,000 children (Srivastava et al., 1999).

In a prospective study by ISKDC, including 521 newly diagnosed nephrotic children between 3 months and 16 years of age, all underwent a kidney biopsy in connection with onset of the disease. The distribution of the histopathological findings is shown in Figure 1 (ISKDC, 1978).

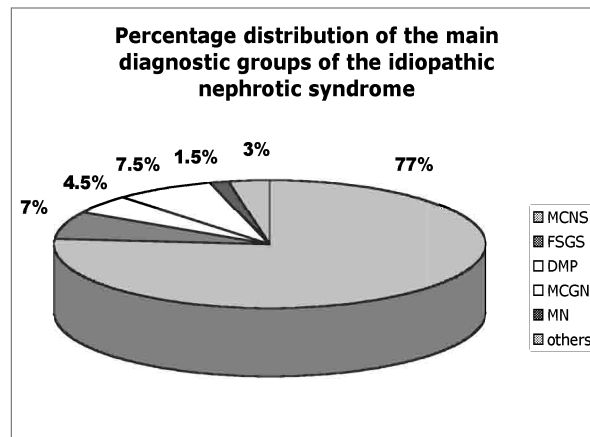
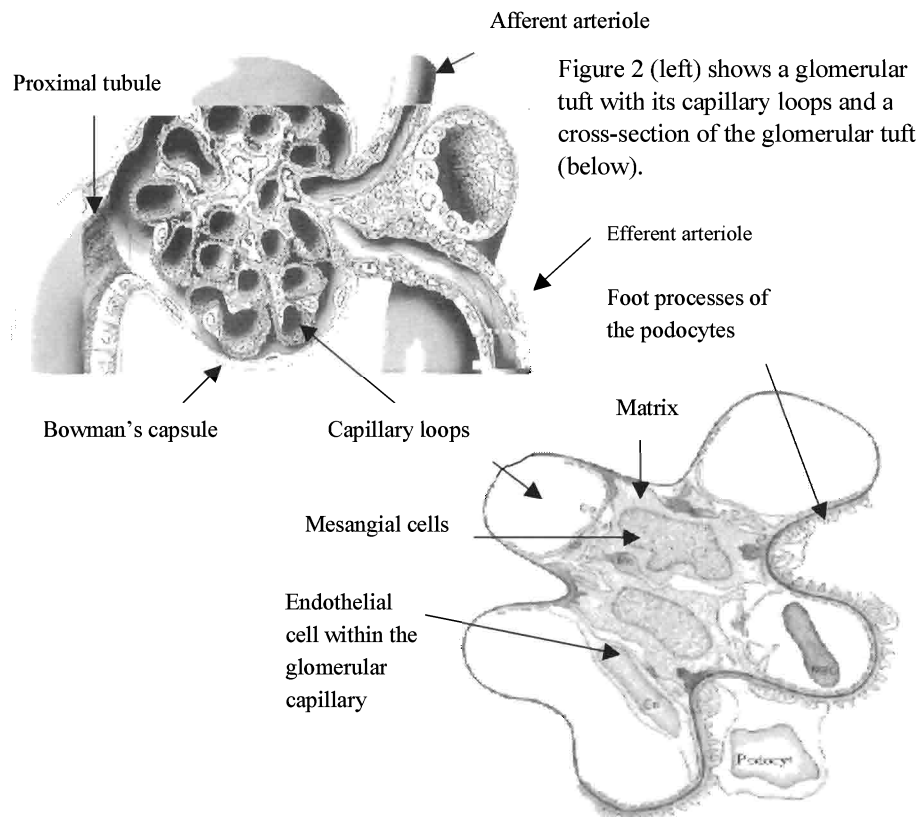


Figure 1 shows the distribution of 521 newly-diagnosed nephrotic children between 3 months and 16 years of age, undergoing a kidney biopsy, as regards the histopathological findings (After ISKDC 1978, Kidney Int.). MCNS (minimal change nephrotic syndrome), FSGS (focal segmental glomerulosclerosis), DMP (diffuse mesangial proliferation), MCGN (mesangio-capillary glomerulonephritis), MN (membranous nephropathy).

In a Swedish survey of all newly-diagnosed nephrotic children between 1995 and 1998, with a follow-up of 1-4 years, 105 children were found in 29 of 34 pediatric clinics in the country. Renal biopsies were taken in 32% of them (n=34); 41% of these (n=14) showed MCNS, 24% DMP (n=8), 9% FSGS (n=3), 12% MCGN (n= 4), 6% IgA (n=2) and 3% MN (n=1). If one assumes that all without a biopsy children had MCNS, its estimated frequency in Sweden would be 81% (L wenborg et al., 2001).

The glomerulus

The glomerulus consists of a tuft of capillaries interposed between the afferent and efferent arterioles. Each glomerulus is enclosed in an epithelial cell capsule (Bowman's



capsule) (Figure 2).

The mesangium of the glomerulus is a connective tissue tree arising at the vascular pole of the glomerulus and supporting the glomerular capillaries. The mesangium comprises of different types of cells, mesangial cells with contractile capacity and circulating macrophages and monocytes. The matrix surrounds the mesangial cell.

Underlying disorders

The minimal change nephrotic syndrome (MCNS)

The commonest type of idiopathic NS is MCNS (ISKDC, 1978).

Its peak incidence occurs between 2 and 4 years of age. Boys are usually affected more than girls (2:1) (Habib and Kleinknecht, 1971).

Light microscopy (LM) is normal or shows a slight increase in mesangial cell proliferation while immunofluorescence microscopy shows no evidence of immune complex deposits. On electron microscopy (EM), diffuse effacement of the epithelial cell foot processes (described later) is the most characteristic morphological finding, generally reversible (Habib and Kleinknecht, 1971).

Corticosteroids are the treatment of choice in MCNS (Churg J et al., 1970) and 93 % of the patients respond within 8 weeks (1981a). Three-fourths of the initial responders who remained in remission during the first 6 months after initial therapy, continue in remission or relapsed, though rarely, during a mean follow-up of 9.4 years. Thirty-one percent, however, of the initial responders relapsed frequently. At the 8-year follow-up, 80 % of the patients were in remission (Tarshish P et al., 1997).

MCNS children run risk of developing more severe types of the nephrotic syndrome-e.g., DMP or FSGS, which have less favorable prognoses (Habib R and Churg J, 1984).

Diffuse mesangial proliferation (DMP) or mesangioproliferative glomerulonephritis

This entity is characterized by the presence of diffuse mesangial hypercellularity with an increase in the mesangial matrix, but no other changes. Immunofluorescence microscopy shows no immune deposits or some IgM deposits mostly in the mesangial areas. The response to therapy is reported to be poor. The disease has a protracted course and worse outcome than MCNS (Waldherr R et al., 1978; 1981b; Garin EH et al., 1983). A morphological

transition from MCNS and DMP to FSGS has been reported (Waldherr R et al., 1978; Habib R and Churg J, 1984; Tejani A, 1985).

Focal segmental glomerulosclerosis (FSGS)

The most serious type of NS is FSGS. In most cases clinical picture can be distinguished from MCNS by hematuria, hypertension, renal insufficiency and a poorer response to corticosteroids (Habib, 1973). Persistence of nephrotic proteinuria is associated with progression to end-stage renal failure (ESRF), while patients who go into remission have a significantly better prognosis (Rydel et al., 1995; Korbet, 1999; Martinelli et al., 2001). Seventy percent of the children are steroid-resistant (1981a). FSGS accounts for 10% of all children requiring renal replacement therapy in Europe and North America (Mc Enery et al., 1993). Fifty percent of nephrotic FSGS children develop end-stage renal failure (ESRF) within 6-8 years (Korbet, 1999). Primary FSGS is a disease that seems to be increasing in frequency, especially in Afro-Americans (Bonilla-Felix et al., 1999; Srivastava et al., 1999).

FSGS is characterized by presence of sclerosis in some ('focal'), glomeruli. Only a part of the glomerular tuft, 'segmental', is affected by sclerosis (Rennke HG and Klein PS, 1989). The sclerotic process consists of glomerular capillary collapse with an increase of the matrix. The sclerotic changes occur first in juxtamedullary glomeruli and may therefore be missed in kidney biopsies. The various types of idiopathic FSGS consist mainly of hilar lesions, tip lesions, and a widespread collapse of the capillary loops (atypical collapsing FSGS) (Howie AJ, 1986; Yoshikawa N et al., 1986; Detwiler et al., 1994). Immunofluorescence microscopy usually shows no immune deposits, apart from nonspecific binding of IgM and/or C3 in sclerotic lesions.

Mesangio-capillary glomerulonephritis (MCGN) or membranoproliferative glomerulonephritis (MPGN)

About 8% of nephrotic children are classified as MCGN (1981a). Its peak age incidence is late childhood and adolescence and with a female preponderance.

Morphologically, there are three entities, types I-III. All types show thickening of GBM; hypercellularity due to proliferation of mesangial cells, and monocyte influx, which usually often leads to a lobular appearance of the glomerular tuft.

Type I is characterized by discrete immune deposits in the mesangium and subendothelial space. Idiopathic type I is rare. It may be secondary to hepatitis C, B and systemic lupus erythematosus (SLE). (Rennke, 1995). Types I and III differ from type II, which is characterized by immune dense deposits in the GBM, tubules and Bowman's capsule. Types I and III seem to be morphological variants, but in type III subepithelial deposits are more marked (Cameron et al., 1983). Type I is by far the commonest of the entities.

A nephritic onset can occur, and it may mimic poststreptococcal glomerulonephritis (Cameron et al., 1983). Hypertension and hematuria, also macroscopic, are common (Burgess E et al., 1990). About 1/3 of the patients have impaired renal function at onset. In 2/3 of the patients, especially those with type II, one finds persistent hypocomplementemia with reduced C₃. This is progressive, although the rates of decline in renal function vary.

Membranous nephropathy

Only few children with nephrotic syndrome have this immune complex-mediated disease. The immune complexes are deposited in the subepithelial space on the glomerular capillary wall. Seventy-four percent present with the nephrotic syndrome in children (which have a poorer prognosis) while the others initially have proteinuria and occasionally macroscopic hematuria. About 60% in the latter group go into remission. This entity is also associated with extrarenal diseases, as hepatitis B.

The glomerulus and renal function

The glomerular filtration barrier comprises three layers, through which the filtrate must pass: (1) a single cell layer of highly fenestrated capillary endothelial cells, (2) the glomerular basement membrane (GBM), and (3) a layer of glomerular epithelial cells, called as podocytes (Fig. 2).

The GBM is a fusion product of basement membrane material produced by glomerular epithelial and endothelial cells. Its basic structure is type IV collagen forming cords. The spaces between these cords are filled with substances including laminin, nidogen, and heparan sulfate proteoglycans (Groffen et al., 1999). The podocytes together with the mesangial cells form the glomerular tuft. One podocyte is connected to more than one capillary loop by its foot and primary processes, which hold adjacent capillaries together (Fig.3 a)(Kriz et al., 1996). The microfilaments in the foot processes are linked at the sole of the foot processes to integrins; and then anchor the foot processes to the GBM (Kriz et al., 1994b).

One of the main functions of the GBM and slit diaphragms between the foot processes (Fig.2) is to filter small solutes (such as sodium, potassium and urea) and water, while restricting the passage of larger molecules, size selectivity (Brenner et al., 1978; Kanwar et al., 1991). Solute up to the size of inulin (molecular weight 5,200) are freely filtered, but albumin (molecular weight 69,000) only to a slight extent. Another function of the GBM together with endothelial fenestrae is charge-selectivity. Anionic molecules such as albumin, are in part electrostatically repelled, because of the negative charge of heparan sulfate proteoglycans in the filtration barrier (Guasch et al., 1993). The endothelial cells regulate vasomotor tone by releasing prostacyclin, endothelin and nitric oxide (Savage, 1994).

Glomerular filtration

The formula used to determine glomerular filtration is:

$$GFR = k_f \times P_{UF}$$

where

k_f (ultrafiltration coefficient) = hydraulic permeability x surface area (available for filtration)

P_{UF} (ultrafiltration pressure) = is the sum of opposing hydraulic and colloid osmotic pressures acting across the capillary in the glomerulus.

The change in GFR depends on the balance between the hydraulic and oncotic pressures in the glomerular capillary and the gradient favors a filtration in the afferent arteriole, while the oncotic pressure in the efferent arteriole rises in normal conditions. This is called filtration equilibrium and it depends on renal plasma flow (RPF). Changes in the filtration coefficient (k_f), that is when a decrease occurs in filtration surface area - e.g., in MCNS tend to lower GFR. The podocyte presumably can regulate the filtration coefficient, by contraction and relaxation of the foot processes, which shortens, or in MCNS lengthens the foot processes (foot process effacement) and therefore increases the filtration area available for filtration (Drumond and Deen, 1994; Guasch and Myers, 1994).

One might assume that slight variations in hydraulic pressure in the glomerular capillary could cause large changes in GFR, but GFR and RPF remain roughly constant, due to s.c. autoregulation, which regulates arteriolar resistance and dilatation. Tubulo-glomerular feedback (TGF) also plays an important role in the regulation of GFR while changes in RPF affect the tubular flow rate in the macula densa, but the involvement of the RAS-system has not been entirely clarified.

The podocytes

The podocytes are highly specialized, terminally differentiated cells, with cell bodies, major processes, and finger-like small foot processes separated by narrow gaps (Mundel and Kriz, 1995). The soles of the foot processes are embedded in the GBM and separated by narrow gaps, the so-called filtration slits (Fig. 4).

The main function of the podocytes is to maintain the filtration barrier, which is essential for the glomerular filtration rate (GFR) (Drumond et al., 1994; Guasch and Myers, 1994). The total area of the filtration slit membranes is considered to constitute the effective filtration area of the glomerulus. (Drumond and Deen, 1994).



Figure 3 a shows a capillary loop from a normal rat glomerulus (Transmission electron microscopy). Original magnification x1400

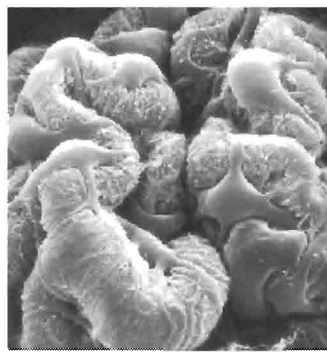


Figure 3 b shows normal podocytes outside the glomerular capillary loop with interdigitating foot processes. (Scanning electron micrograph x2125). (With permission from NEJM, Orth SR et al. 338:1202-1211,1998)
©2003Massachusetts Medical Society

In recent years, the molecular pathogenesis of some rare types of NS has been clarified. In 1998, the NPSH1, the gene coding for nephrin, was found, the mutation of which cause the Finnish type of congenital nephrotic syndrome (Kestilä et al., 1998). Nephrin is mainly located in the slit diaphragm, a still poorly understood structure, which connects adjacent foot processes (Routsalainen et al., 1999) (Fig.6). This underlines the role of nephrin in maintaining glomerular filtration and permselectivity. Recently, another gene has been described, NPSH2, that encodes for podocin and mutates for early onset steroid-resistant nephrotic syndrome (Boute N et al., 2000).

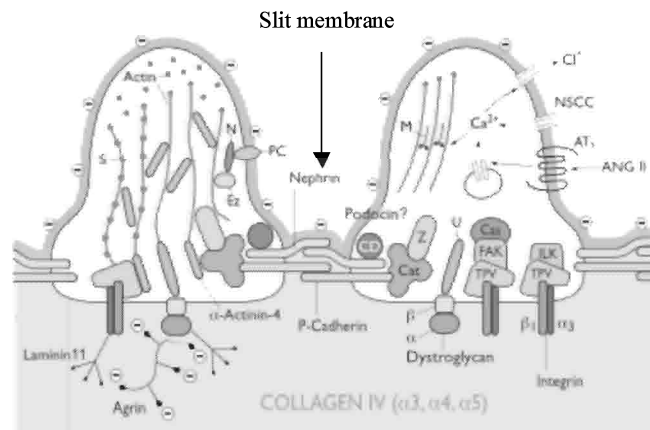


Figure 4 shows the complicated structure of the foot processes of the podocyte. The structure of the slit membrane is partly formed by nephrin and p-cadherin. (From Update in podocyte biology. Endlich K et al., Curr Opin Nephrol Hypertens 10:331-340, 2001).

In the nephrotic syndrome with foot process effacement, loss of the specialized slit membrane architecture is presumably due to loss of the negative charge on the foot process surface (Smoyer WE and Mundel P, 1998). Therefore, MCNS is regarded as a disease of the podocyte in the glomeruli, in which the essential morphological change is foot process effacement seen, which can be seen in EM (Figs. 5 a and b).

Figure 5 a shows a capillary loop from a PAN-treated rat with advanced foot process effacement. Arrows indicate slit pores. Original magnification x1400.

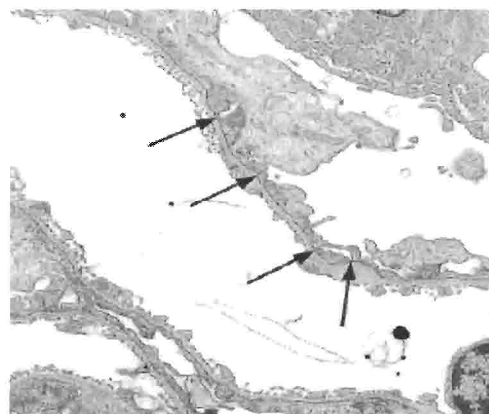
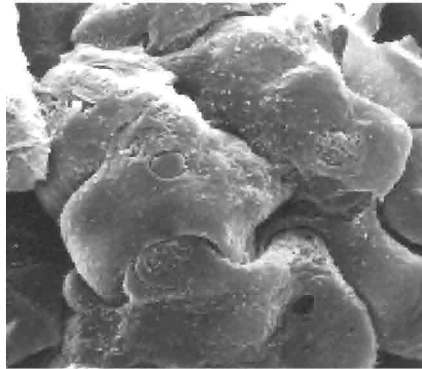


Figure 5 b shows the disorganized shape of the podocytes from a proteinuric rat, with effacement of the foot processes (Scanning electron micrograph x2125). (With permission from NEJM, Orth SR et al. 338:1202-1211, 1998 ©2003 Massachusetts Medical Society)



Glomerular hypertrophy and progression of renal disease

The glomerular capillaries are constantly exposed to high transmural hydrostatic pressure gradients. The GBM acts as a stabilizing framework together with the mesangium (Sakai and Kriz, 1987). This is an elastic structure characterized by considerable compliance of the glomerular tuft. The podocyte, which also has a contractile system, counteracts the elastic expansion of the GBM, by means of the soles of the foot processes, that are attached to the GBM (Kriz et al., 1994a). They are terminally differentiated and therefore can cope with substantial enlargement of the tuft surface only by hypertrophy of the podocyte cell (Kriz et al., 1994b). The development of glomerular hypertrophy occurs in various ways.

At present no data are available on the pathogenesis of the acute development of severe foot process fusion, as in nephrotic syndromes, and the development of glomerular hypertrophy. In PAN-treated rats, GFR declined because of a reduction in k_f (Ichikawa et al., 1983) with a loss of effective filtration area in each glomerulus. Thus, each glomerulus increased its size in an attempt to increase the filtration capacity. In MCNS, these changes are reversible, but in a few patients, MCNS becomes more severe with a worse prognosis. (Bohman et al., 1984; Fogo et al., 1988; Fogo et al., 1990; Fogo and Ichikawa, 1991; Drumond et al., 1994; Guasch and Myers, 1994; Fogo, 2001).

Most of the glomerular hypertrophy is compensatory and the podocyte layer initially remains intact, but the final result is fusion of the foot processes. The podocytes spread out over very large distances in the hypertrophied glomerular tuft and finally cause detachments from the GBM, which results in denuded areas of the GBM leading to tuft adhesion and the development of FSGS (Figs. 6 and 7).

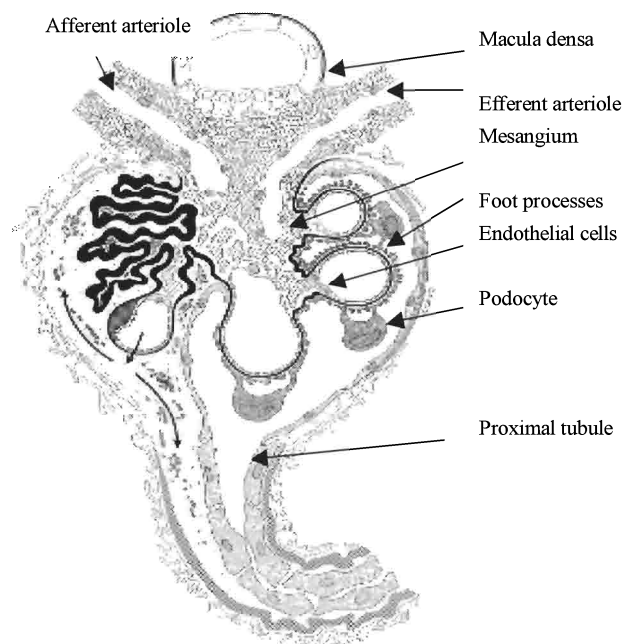


Fig.6 shows a schematic of the misdirected filtration and filtrate spreading in nephron degeneration. The GBM is shown in black. The tuft adhesion contains several collapsed capillary loops and one is partially hyalinized. The filtrate of this loop is delivered into a paraglomerular space (yellow). Arrows shows podocytes, foot processes, mesangium, endothelial cells, afferent and efferent arterioles, macula densa and proximal tubule. From Microscopy research and technique, Kriz, W; 57:189-195,2002. ©Wiley-Liss,Inc 2002

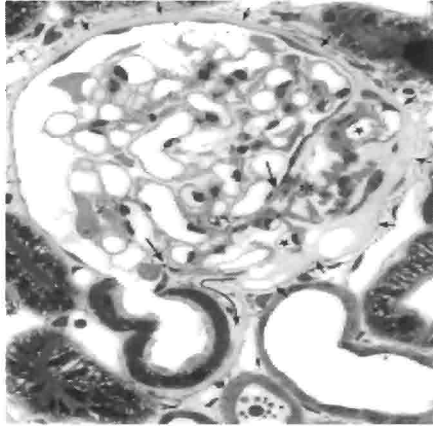


Figure. 7 shows an early stage of FSGS in a glomerular tuft (LM x 480). One part of the adherent tuft (asterisks) protrudes through a gap in Bowman's capsule (marked by two arrows) in a large paraglomerular space, which is delimited from the interstitium (small arrows). The long arrow indicates the outer aspect of the proximal tubule filled with proteinaceous fluid. From Microscopy research and technique, Kriz, W; 57:189-195,2002. ©Wiley-Liss, Inc 2002

Pathogenesis

Almost 30 years ago Shalhoub et al. proposed that MCNS was a systemic disorder of T-cell function and cell-mediated immunity (Shalhoub, 1974). An abnormal expansion of a clone of T-cells may cause production of a lymphokine toxic to the GBM, and change glomerular permeability to protein. This view is supported by the observation that MCNS patients often remits with measles (viral-associated immunosuppression) and particularly the susceptibility to pneumococcal infections.

In various studies during relapses, $CD4^{+}$ and $CD8^{+}$ T-cell subsets are expanded and the levels of cytokines rise, such as tumor necrosis factor- α (TNF- α), interleukin-8 (IL-8), and IL-13 (Garin et al., 1997; Yap et al., 1999) . Earlier studies supported enhanced expression of the IL-2R on unstimulated T cells from patients with active MCNS (Topaloglu et al., 1994). In recent studies during a more complicated course of NS and renal lesions in kidney biopsies, direct involvement of CD8 T-cells occurred (Frank et al., 2000; Lama et al., 2002) , while CD 4 T-cells decreased in relapse.

A relation with certain human leukocyte antigens (HLA) has also been reported-e.g., HLA-B8-DR3 haplotype,-DR7 and -DQ2 have been associated with NS (Ruder H et al., 1990).

Proteinuria

Two main mechanisms are responsible for the abnormal urinary excretion of proteins in glomerular diseases. The first is an increase in charge- and size-permeability of the glomerular capillary wall, which leads to the transglomerular passage of albumin and of high molecular weight protein, like albumin and IgG, that usually do not cross the glomerular barrier (Rennke et al., 1981; Farquhar, 1995). Some evidence suggests that the ultimate, more selective barrier than the GBM is the slit diaphragm (Mundel and Kriz, 1995; Tryggvason, 1999).

Glomerular hemodynamic changes also affect the permeability to macromolecules. The glomerular capillary flow rate as well as the difference in transcapillary hydraulic pressure and the afferent arteriolar concentration of plasma protein contribute to the excretion of large proteins (Brenner et al., 1977).

The second is the subsequent impairment of reabsorption in the proximal tubule of all proteins, due to an increase in work and/or toxic injury because of an increase in the load of the abnormally-filtered proteins in the tubular lumen (D'Amico and Bazzi, 2003).

TREATMENT

Guidelines for the treatment of NS are given by the ISKDC 1978. Of the various types of the NS in children, MCNS responds best to therapy (1981a). The response to steroids is of greater prognostic value than the histological features on biopsy.

Corticosteroids

Corticosteroids, mostly Prednisolone is given in initial doses of 60 mg / m² daily during the first 6 weeks and then every other day 40 mg / m² for 6 weeks (Brodehl, 1991). This treatment suppresses the immune system by regulating the expression of

several genes, through inhibition of transcription factors in the cells (coding for a number of cytokines that have an anti-inflammatory effect) and inhibit the synthesis of almost all known cytokines. Corticosteroids also act on T-lymphocytes, who go to lysis or apoptosis (i.e., programmed cell death) on high doses of corticosteroids.

Alkylating agents

Cyclophosphamide and chlorambucil have been used especially in children with frequent relapses to induce longer lasting remissions and minimize serious steroid side-effects after prolonged and repeated steroid treatment. They react with the bases in DNA and prevent cell division. The therapeutic effect is related to the duration of treatment, but severe side-effects can occur during treatment. Bone marrow toxicity requires regular controls during treatment. Long-term toxicity includes gonadal toxicity, especially in boys, and is dose-dependent.

Cyclosporine

Cyclosporine is of fungal origin and was introduced in the 1980s (Borel et al., 1976). It is a lipophilic cyclic peptide antibiotic. The main action is decreased clonal proliferation of T-cells, primarily by inhibiting IL-2 release. CsA binds to cyclophilins, which binds to and inhibits calcineurin, a calcium and calmodulin-dependent phosphatase (an enzyme that transmits signals from the T-cell receptor to the nucleus), which in turn, through NF-AT (transcription factors), leads to reduced transcriptional activation of early cytokine genes for IL-2, IL-3, IL-4, CD40L, granulocyte-macrophage colony-stimulating factor, tumor necrosis factor- α (TNF- α), and interferon- γ (Kahan, 1989). CsA also reduces induction of and clonal proliferation of cytotoxic T-cells from CD8+ precursor T-cells.

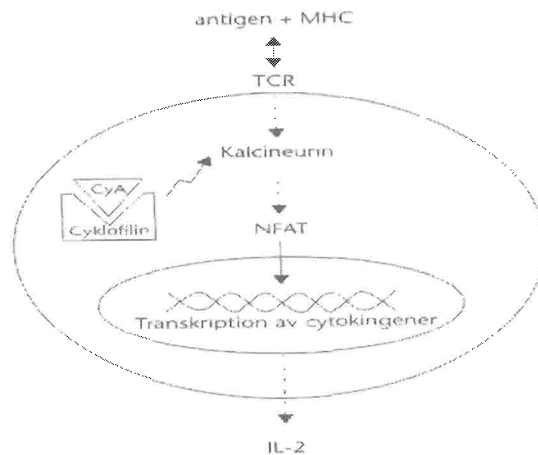


Figure 8 illustrates CsA inhibition of interleukin-2 (IL-2) activation of resting T-lymphocytes. When T-cells are activated a signal transduction occurs from TCR to the nucleus. Through calcineurin, NF-AT becomes dephosphorylated and induces transcription of cytokines. Calcineurin is inhibited by CsA. MHC (major histocompatibility-complex); TCR (T-cell receptor); NF-AT (nuclear factor of activated T-cells) (Tufvesson, 2002©Studentlitteratur).

Nephrotoxicity

Nephrotoxicity is a major concern during CsA-treatment. Acute nephrotoxicity is due to severe vasoconstriction of the efferent and afferent arterioles, reductions in renal blood flow and the glomerular filtration rate, which cause ischemia, acute tubular necrosis and hypertension. Subsequent studies indicate that these problems are dose-related, but an intrinsic renal susceptibility to CsA-induced renal vasoconstriction have also been shown (Wissmann et al., 1996). The acute nephrotoxicity is usually reversible with cessation of therapy. The only definitive diagnostic test is kidney biopsy, but no pathologic changes are specific except for

isometric vacuolization of tubular epithelium (Mihatsch et al., 1988; Kopp and Klotman, 1990; Mihatsch et al., 1994; D'Agati, 1995).

Chronic nephrotoxicity is manifested by a decline in renal function due to glomerular and vascular disease, abnormalities in tubular function, and an increase in blood pressure (Kahan, 1989). Renal biopsy reveals an hyalinosis of afferent arterioles, ischemic collapse or scarring of the glomeruli, vacuolization of the tubules, and focal areas of tubular atrophy and interstitial fibrosis ('striped fibrosis') (Mihatsch et al., 1994).

The factors responsible for chronic nephrotoxicity have not been fully elucidated. The development of interstitial fibrosis is associated with increased expression of osteopontin, (a potent macrophage chemoattractant secreted by tubular epithelial cells) (Pichler et al., 1995), chemokines (a class of cytokines which are strong chemoattractants for a variety of hematopoietic cells (Benigni et al., 1999), transforming growth factor- β (TGF- β), a stimulator of extracellular matrix production (Shihab et al., 1997; Islam et al., 2001) and an increase in the local concentrations of angiotensin II, which may explain the beneficial effects of ACE inhibitors and angiotensin II receptor antagonists (Shihab et al., 1997; Pichler et al., 1995).

Increased apoptosis (i.e., programmed cell death) occurs in kidneys exposed to CsA (Thomas et al., 1998). The decline in GFR found in CsA-treated patients seems to be reversible with cessation of CsA therapy (Hulton et al., 1994).

AIMS OF THE STUDY

1. To study the significance of glomerular volume on various types of the nephrotic syndrome in children and in experimental nephrosis (Paper I and III).
2. To investigate the significance of effacement of foot processes in renal haemodynamics in experimental nephrosis (Paper III).
3. To study the effect of oncotic pressure (serum albumin concentration) on renal haemodynamics in various types of the nephrotic syndrome in children (Paper II).
4. To investigate prospectively the effects of long-term cyclosporine treatment on renal haemodynamics and morphology in various types of the nephrotic syndrome in children (Paper IV).

PATIENTS AND CONTROLS

Paper I

To evaluate the hypothesis that increases in glomerular area and glomerular volume are an early sign of impending sclerosis, we examined retrospectively renal biopsies from 58 children (31 boys) with various types of the nephrotic syndrome. Forty patients had MCNS, 4 patients DMP and 14 patients had FSGS from start. They had attended our Centre for Paediatric Nephrology, where the renal biopsies had been taken. It has a catchment area including Northern, and Central Sweden.

Six patients were subjected to repeat biopsy because of steroid resistance. Seventeen age-matched healthy children served as the controls for the renal haemodynamic data.

To compare the glomerular volumes, the patients were matched to BSA, when comparing MCNS with FSGS patients and to age when comparing MCNS to DMP patients.

Paper II

We studied renal function in relation to the histological diagnosis and the stage of the nephrotic syndrome in 98 patients (55 boys) using clearance of inulin-and PAH under water diuresis. The stages of the disease comprised the nephrotic stage, the recovery and remission. Ninety patients were biopsied and light microscopy showed MCNS in 55, DMP in 7, FSGS in 15, mesangiocapillary glomerulonephritis (MCGN) type I or II in 10, and membranous nephropathy (MN) in 3 children. Although no biopsies were taken in 8 children, they had a clinical history of steroid-responsive nephrotic syndrome, probably MCNS and were included in the MCNS group.

The renal function tests were performed at the time of the biopsy and at additional time points as indicated by the clinical course. Thirty-six healthy children, aged 3.5-20.5 years, who had performed renal haemodynamics because of suspicion of renal disease and then were decided to be healthy, served as controls for the renal haemodynamic data.

Paper III

Animals

Puromycin aminonucleoside (PAN) is used in experimental models for minimal change nephropathy. We studied 16 male Munich-Wistar-Frömter rats, about 8 weeks of age, weighing a median of 247 (171-286) g were studied. In this strain, the glomeruli are located superficially and therefore were easily accessible for further examination of the glomerular structure (Hackbarth H et al., 1983).

The rats were housed with a 12-h light-dark cycle in which the humidity and temperature were controlled. They were fed pellets containing 20.5 % protein and 0.3% sodium chloride. They were given tap water ad libitum. Four of the rats served as controls. The other 12 rats were divided into three groups receiving daily subcutaneous injections of 1, 1.67, and 2.5 mg puromycin-aminonucleoside/100 g body weight, respectively, for 6 days.

This rat strain develops albuminuria at 10 weeks of age and foot process fusion and glomerulosclerosis at 20 weeks during normal conditions (Alt et al., 1985; Rovira-Halbach G et al., 1986; Remuzzi A et al., 1988; Remuzzi et al., 1992; Fassi et al., 1998).

Paper IV

Twenty-two therapy resistant children, 11 patients with biopsy proven MCNS and 11 with FSGS, were long-term treated with cyclosporine A (CsA). They were followed clinically and evaluated for CsA-nephrotoxicity by repeated renal biopsies and renal function with clearances of inulin- and para-aminohippuric acid (PAH) that estimate the GFR and ERPF to determine the safety and adverse effects of CsA.

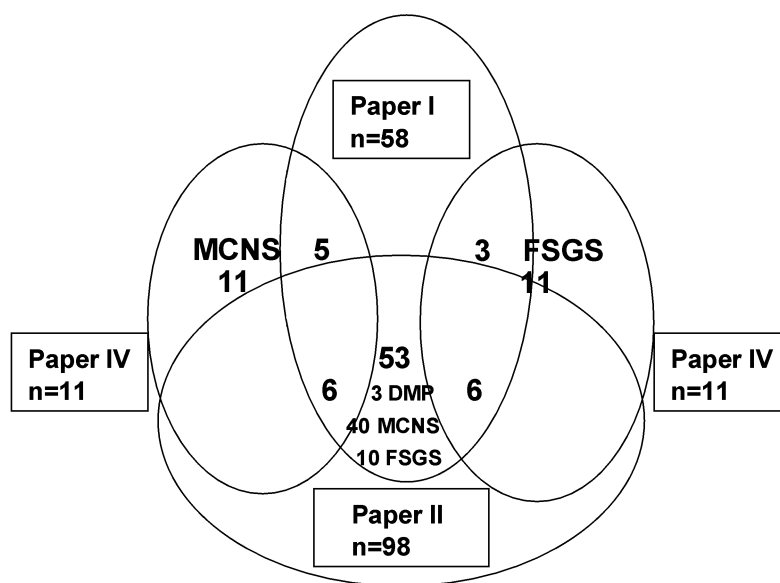


Figure 9. The number of patients with various types of the nephrotic syndrome described in Papers I, II, and IV and the amount of overlapping among the patients in each study. In Paper II, 53 patients were included from the study in Paper I. In Paper IV 6 patients each with MCNS and FSGS, from the study in Paper II were included and from Paper I 5 MCNS and 3 FSGS patients each were included.

ETHICAL ASPECTS

Approval by the local Ethics Committee at Huddinge University Hospital for Papers I, II and IV was obtained. . Patient and parental consent were obtained in the study in Paper IV. As regards Paper III, the animal care and the protocols for experiments were approved by the Swedish National Ethic's Board for Animal Research.

METHODS

RENAL FUNCTION

GFR and ERPF determined by clearances of inulin and PAH (Paper I-II and IV)

Method for measuring renal clearance was introduced by Rehberg in 1926 using exogenous creatinine as a marker (Rehberg PB, 1926).

He applied the classic formula:

$$\text{GFR} = \frac{U \cdot V}{P}$$

where U is the urine concentration of the substance (mg/mL), V is the urine flow rate (mL/min) and P is the plasma concentration of the substance (mg/mL).

Inulin, a 5200 dalton uncharged polymer of fructose, was introduced in the 1930s and is still regarded as a major reference substance for the glomerular filtration rate against which other markers are compared (Smith HW et al., 1945).

Clearance of inulin may be insensitive for the detection of early renal disease, since some nephrons initially damaged or destroyed, cause that the remaining ones compensate by increasing their filtration rate (Hostetter TH et al., 1981). Of crucial importance for the exact determination of the urinary inulin clearance is completeness of voiding, which can be controlled by ultrasonography.

In all studies renal haemodynamics was evaluated with the GFR and ERPF and determined by clearances of inulin- and PAH during water diuresis using standard clearance technique (Berg U and Bohlin A-B, 1982).

The inulin and PAH were mixed as follows: 17 ml of Inutest (Laevosan Gesellschaft, Vienna, Austria) and 3 ml of 20 % PAH, (MSD, West Point, USA). After a prime dose of inulin and PAH (0.3mL/kg), a continuous i.v. infusion (0.1mL/min for 10-20 kg body weight (BW), 0.2mL/min for 21-37 kg BW and 0.375 mL/min for > 37 kg BW) was given. Two intravenous (iv) lines were used, one in each arm. After an equilibrium

period of 1 hour, four 30-min urine collections were done and a blood sample was taken midway through each collection period. Water diuresis was induced by the oral ingestion of water 20 ml/kg BW during the first hour, followed by 5 ml/kg BW every 30 minutes up to a maximum of 1200 mL and 300 mL, respectively, regardless of the BW. This enabled the patients to empty their bladder spontaneously every 30 minutes. The completeness of bladder emptying could be checked with ultrasonography. The clearance was calculated as the mean of the four urine-collection periods.

The coefficient of variation was about 10% among clearance periods in a single test during baseline conditions. CV is defined as the mean divided by the standard deviation of the test results.

The clearance measured (mL/min) was corrected for a standard body surface area (BSA) of 1.73 m². BSA was calculated according to Haycock et.al (Haycock et al., 1978).

The levels of inulin in blood and urine were determined by the anthrone method until 1994 (Hilger HH et al., 1958) and then by an enzymatic method (Kuehnle HF et al., 1992). PAH was assessed by photometric analysis with a modified Smith technique (Bruhn C, 1951).

GFR and ERPF determined by single injection technique (Paper IV)

In children who were too young to control emptying of the bladder, clearances of inulin and PAH were determined with a single-injection technique. A bolus injection with inulin and PAH was given, after which repeated blood sampling was taken during the next 180 minutes. From the plasma disappearance rate of the substances, clearances were calculated by the two-compartment method (Jereb B et al., 1973).

Inulin has a high molecular weight and it may take more than 12 h to obtain complete equilibration of inulin in its volume of distribution- i.e. the total extracellular space. An incomplete distribution causes concentration levels lower than expected at steady state and, as a consequence, a constant overestimation of the GFR when the bolus infusion method is used (Florijn KW et al., 1994). This method is not recommended for use in nephrotic children in the oedema phase (Chantler et al., 1969; Hellerstein et al., 1993).

GFR determined by Iohexol (Paper IV)

In a few patients, the slope-clearance of iohexol was used to evaluate GFR in Paper IV (Frennby B and Sterner G, 2002). Iohexol is a non-ionic monomeric contrast medium used for uro-and angiography. Comparisons of iohexol and inulin with standard clearance technique show good agreement between the two methods (Lindblad and Berg, 1994). Iohexol is determined by high performance liquid chromatography (HPLC) or x-ray fluorescence.

For a mathematical description of changes in the plasma concentration of a substance, the body has been divided into compartments and the plasma clearance of a substance is calculated by dividing the injected dose with the area under curve (AUC). The optimal time for blood sampling depends on the assumed GFR. If the GFR is assumed to be normal the last blood sample can be drawn after 4 h. If the GFR is assumed to be below 20 ml/min, the last sample should be drawn after 24 and 36 h. (Stake et al., 1991).

GFR determined by the formula clearance (Paper IV)

The formula used is that by Schwartz modified by Counahan (Counahan R, 1976 ; Schwartz GJ et al., 1976) on the basis of serum creatinine and height as follows:
$$\text{GFR (ml/min per } 1.73\text{m}^2) = \text{Height (cm)} \times K (38) / \text{serum creatinine } (\mu\text{mol/L})$$

In Paper IV, we compared this formula with that of inulin in cyclosporine-treated children.

The level of serum creatinine was determined with a method based on a modified Jaffé reaction, a colorimetric assay based on the formation of a complex between creatinine and alkaline picrate (Masson P et al., 1981).

Effective renal plasma flow (ERPF)

ERPF is determined by the clearance of para-aminohippuric acid (PAH). PAH is filtered by the glomerulus and secreted by the tubular cells resulting in a high extraction ratio. When the concentration in plasma is low, virtually all the PAH that escapes filtration is secreted. PAH is not reabsorbed. Eight to ten percent of the total renal plasma flow (RPF) supplies the non-secreting portions of the kidneys. This is why the extraction is not complete and the clearance of PAH is therefore called ERPF instead of RPF. Thus true RPF exceeds the PAH clearance by about 10%. (Smith et al., 1945; Bruhn C, 1951).

Filtration fraction (FF)

The filtration fraction is the proportion of plasma entering the renal circulation and removed from the circulation by glomerular filtration- i.e.,

$FF \% = GFR \times 100 / ERPF$.

CONTROLS

In Papers I, II, and IV healthy children were used as controls for renal haemodynamic data. These were studied because of nocturnal enuresis, psychogenic polydipsia, suspected hypertension, which could not be verified, Münchhausen by proxy and/or an increase in serum creatinine levels, which was found to be a technical error.

In Paper I, we used 17 age-matched controls 7.9 ± 0.5 years of age (mean \pm SD). Their GFR was 119 ± 2 (mean \pm SE) and ERPF 615 ± 14 ml/min per 1.73 m^2 . FF was $19.5 \pm 0.6\%$.

In Paper II we used 36 children, aged 3.5-20.5 years. Their GFR and ERPF was 119 ± 2 (mean \pm SE) and 627 ± 14 ml/min per 1.73 m^2 , respectively. FF was $19.4\% \pm 0.5\%$.

In paper IV served 47 children, 3-19 (median 11) years of age, as controls. Their mean GFR was 116 ± 10 (mean \pm SD) and ERPF 615 ± 76 ml/min per 1.73 m^2 .

RENAL MORPHOLOGY

Histological evaluation of glomerular volume

(Paper I)

In this paper, we estimated the glomerular area and the glomerular volume.

Renal biopsies were performed percutaneously, using a Tru-Cut biopsy needle with an outer diameter of 2.0 mm (Travenol). Tissue for light microscopy (LM) was fixed in 3 % buffered formaldehyde and embedded in paraffin. Sections 2-3 μm thick were stained with Ladewig's trichrome. All biopsies were examined blindly.

The glomeruli were traced through serial LM sections at a magnification of x400 and the area calculated from the largest single tuft area in each glomerulus, using a point-counting technique with an ocular grid having a point-density square area of $423 \mu\text{m}^2$ (Weibel ER, 1979). The area was measured and the volume then calculated using the formula of a sphere ($4\pi r^3/3$). In each patient we studied a median number of 10 (4-12) glomeruli and calculated the area and volume of the single largest glomerulus in the serial LM sections. Glomeruli with detectable sclerotic changes were excluded.

Histological evaluation of cyclosporine nephrotoxicity

(Paper IV)

.

All biopsies were performed under local or general anaesthesia with ultrasound guidance, using a fixed adapter (3,5 MHz, Acuson, Mountain View, CA, USA) with an automatic biopsy device (Biopsy Bard Urological, Covington, GA, USA) and a 16-gauge needle (Manan Medical Products, Northbrook, IL, USA). Samples for light and electron microscopy and immunofluorescence were taken. All biopsy specimens were examined by one of the authors (MS) blinded to the case histories.

Biopsy samples were evaluated by light microscopy, using haematoxylin and eosin, periodic acid-Schiff, trichrome and periodic acid-silver methenamine stains, immunofluorescence (IFL) and electron microscopy. The diagnoses were made using standard histological classifications (Olson and Schwartz, 1998).

Evaluation of the morphological changes mainly followed standardised guidelines (Mihatsch et al., 1994). The total number of glomeruli was counted and the percentages of glomeruli with ischaemic changes and global or segmental sclerosis were calculated. Glomeruli were defined as ischaemic when we found wrinkling of the glomerular basement membranes and thickening of Bowman's capsules (Hughson, 1998). We graded tubular atrophy, interstitial fibrosis and hyaline arteriolopathy from none (0) to severe (3). Hyaline arteriolopathy was defined as the presence of subendothelial hyaline material- i. e., non-specific arteriolar hyalinosis. The criterion for acute CsA toxicity was the presence (+) or absence (-) of isometric vacuolisation of the tubular epithelium (D'Agati, 1995).

ANIMAL STUDY

Induction of puromycin aminonucleoside nephropathy

Puromycin aminonucleoside (PAN) nephropathy was induced by subcutaneous injection of the drug. The animals were divided into groups of 4 rats and received a daily subcutaneous injection of 1 mg (group I), 1.67 mg (group II) and 2.5 mg /100 g body weight (group III) of PAN for 6 consecutive days (Bohrer MP et al., 1977). A control group of 4 animals of the same age and weight were followed, but not treated.

Urinary albumin excretion

The rats were weighed and placed in individual metabolic cages to determine their albumin excretion in 24-h urine on the day before the first PAN injection. Another 24-h urine collection for assessing albumin excretion was done on the day after the last PAN injection. Urine albumin was determined with a nephelometric method. The 24-h urine volumes collected before starting the injections ranged between 9 and 16 (median 12) ml/24 h. Urine collections were incomplete in 2 control rats, in 2 rats before PAN administration and in 2 rats in the control group, 2 rats in group I and 1 rat in group II after the 6 PAN injections, depending on difficulties in using the metabolic cages. The 24-h urine volumes in the

control group and groups I and II ranged between 8 and 14 (median 10) ml/24h. In group III, the urine volumes ranged between 18 and 40 (median 27) ml/24 h. In the 24-h urine volumes of the rats with incomplete collections we assumed that the urine volumes were the same as the mean urine volume of each group.

Renal function

On the 7th day, the rats were anaesthetised with Inactin®. Tracheostomy was performed and indwelling polyethylene catheters were inserted into the right femoral artery for monitoring of blood pressure and blood sampling and into the right femoral vein for infusion of radioactive inulin (^3H inulin dissolved in 0.9% NaCl 50 $\mu\text{Ci/ml}$). GFR was determined by the clearance of inulin. Inulin was given as a priming dose of 0.4 ml, followed by a continuous infusion of 1.2 ml/h. The urinary bladder was catheterised. After an equilibration period of 1 hour, three 20-min urine samples were collected and blood samples were drawn in the middle of each urine sampling period. GFR was calculated as the mean of the three urine collection periods and expressed in absolute (ml/min), and in relative values (ml/min/100g).

The blood pressure was continuously recorded. We excluded rats, which developed a decrease in blood pressure during the renal function test that did not return to normal within 15 minutes. Blood and urine samples were analysed for ^3H activities with the liquid scintillation technique.

Tissue fixation

The left kidney was exposed and immobilised in a plastic micropuncture cup. After sealing the cup with agar, the kidney, while being perfused, was continuously bathed in fixative (2 % glutaraldehyde and 4% paraformaldehyde in a 0.05 M sodium phosphate buffer, pH 7.4, temperature 37° C, osmolality 675 mOsmol/kg H_2O) (Karnovsky MJ, 1965). The fixation procedure was continued for 15 minutes, during which the fixative was renewed every 30 sec. The kidney was then removed for morphometric studies and placed in a vial with the same fixative as above.

Light microscopy evaluation

The kidneys were cut into 1.5-mm thick coronal slices from which a set of two systematic random slices were collected and embedded in glycol methacrylate. The mean glomerular volume (GV) was estimated by Cavalieri's principle (Gundersen and Jensen, 1987). The tissue blocks were serially sectioned in 2.5- μm slices were stained with hematoxylin and eosin. The glomeruli were sampled in their order of appearance in the sections. The first profile of a glomerulus was sampled when its capillary loops became visible, then came the successive sections in which the following profile areas were sampled at 7.5- μm intervals, i.e., in every third section. The number of levels sampled per glomerulus ranged between 10 and 15. The area of the profiles was estimated by point-counting using an ocular grid with 19.9 μm between each point (d) at tissue level (410x). The sum of profile areas (A SUM) was estimated using the formula $A \text{ SUM} = P_{\text{tot}} \times d^2$. For the final calculation of the glomerular volume, we estimated the mean of the actual section thickness (t) by a method based on confocal microscopy (CSLM) (Brismar et al., 1996). In brief, the x-z profile of a section is scanned by CSLM, and the full width half-maximum of the intensity profile obtained in the axial direction is used to estimate the actual section thickness. The precision of the method has been shown to be $\pm 100 \text{ nm}$ (Brismar et al., 1996). The glomerular volume was then calculated as: $A \text{ SUM} \times 3t$. In each rat, at least 8 glomeruli were measured and the mean GV was calculated.

Electron microscopic quantification

For electron microscopy, systematic and random sampled tissue blocks of superficial cortex were postfixed in 1 % osmium tetroxide and embedded in Epon with standard procedures. Ultra-thin sections were stained with uranylacetate and lead citrate and studied with a Philips 420 electron microscope. From each rat, 3 or 4 superficial glomeruli were analysed. The reference space of the glomerular tuft and the delineation of the mesangium to the peripheral capillary wall were as previously reported (Gundersen HJG et al., 1980; Hirose K et al., 1980; Østerby and Gundersen, 1980). The average width of foot process was estimated as the ratio of the surface density of the glomerular basement membrane, S_v (GBM) to the length density of the filtration

slits, L_v (slits) along the peripheral capillary walls. At about 4000x, sets of 4 to 8 micrographs per glomerulus were taken in a systematic random manner by moving the specimen stage between predetermined points of which the first was chosen at random. The final magnification was corrected with a grating grid having 2160 lines per mm. A superimposed square test grid was used which had points defined by the intersections between the grid lines. The distance (d) between each point in the test grid was about 12 μ m at tissue level.

The intersections between the grid lines and the peripheral glomerular basement membrane (I), the points hitting the reference space (P) and the number of transected filtration slits along the peripheral basement membrane (Q) within the reference space were counted.

In the control group the average number of filtration slits counted in each animal was 1926 (1316 - 3219).

S_v (GBM), was calculated by the following formula:

$$S_v \text{ (GBM)} = 2 * I / (P * (2d / \text{mag})) (\mu\text{m}^{-1})$$

L_v (slits) was estimated as:

$$L_v \text{ (slits)} = 2 * Q / (P * (d^2 / \text{mag}^2)) (\mu\text{m}^{-2}),$$

where mag is the corrected final magnification. The total slit pore length was calculated as the product of glomerular volume and length density of the filtration slits.

STATISTICAL METHODS

Descriptive statistics

The distribution of variables is given as median and range (minimum-maximum) or mean and standard deviation throughout the study.

Statistical analyses

In comparisons of mean values of continuous data, considered to be normally distributed we used the unpaired *t*-test to compare two groups (Papers I, II, III, and IV). Treatment groups were compared by one-way analysis of variance (ANOVA), followed by the post-hoc tests Tukey-Kramer for all pairs and Dunnet for comparisons with a control group (Papers II, III, and IV).

ANOVA repeated measurements were used when the same children were studied at different times (Papers II and IV) when data were normally distributed. One-sample *t*-test was used to compare the slope mean to a null hypothesis (Paper IV).

The Mann-Whitney U- test (Papers I and IV) and Friedman's test (Paper IV) were used to compare continuous skewed data in two or more independent groups.

In all papers we used least squares regression to describe the relationship between continuous data (Papers I, II, III and IV).

The correlation between the semiquantitative morphological data and various continuous data was analysed with Spearman's correlation coefficient (Paper IV). We analysed the differences between formula clearance and the clearance of inulin by regression and scatter plot and the differences between these two methods were plotted against the average of the two methods (Paper IV) (Bland and Altman, 1986).

All significance tests were two-tailed and done at the 5% significance level.

The statistical analyses in these studies are summarized in Table 1.

Table 1. Statistical methods.

Statistical analyses	Paper			
	I	II	III	IV
Comparison between groups				
ANOVA one-way	x	x	x	x
ANOVA repeated measurements		x		x
Paired <i>t</i> -test	x	x	x	x
Unpaired <i>t</i> -test	x	x		
One-sample <i>t</i> -test				x
Mann-Whitney U-test	x	x		x
Friedman's two way analysis of variance				x
Correlation analysis				
Spearman's rank correlation coefficient				x
Least squares regression	x	x	x	x

RESULTS AND DISCUSSION

Glomerular volume, foot process effacement and renal hemodynamics

(Papers I and III)

Glomerular hypertrophy is thought to be an important factor in the pathogenesis of focal glomerulosclerosis (Fogo A et al., 1990).

We therefore retrospectively evaluated the glomerular surface area in kidney biopsies from 58 children with various types of the nephrotic syndrome-i.e., MCNS, DMP and FSGS (Paper I). Six patients were subjected to repeat biopsy because of steroid-resistance. The morphology was unchanged in 2, 2 showed a different immunofluorescence pattern, 1 changed from MCNS to DMP and 1 from MCNS to FSGS.

The prognosis of these morphological groups differs. Moreover, patients with MCNS and DMP may develop FSGS and those with MCNS may develop DMP (Waldherr R et al., 1978; Habib R and Churg J, 1984; Tejani A, 1985).

We measured the glomerular surface area and calculated the GV. Since GV correlated with the body surface area (BSA) and age ($P<0.001$, $r=0.45$ and $P<0.01$, $r=0.42$, respectively) patients with FSGS and DMP were matched according to their BSA and age, respectively, with corresponding MCNS patients. The difference in methods depends on one DMP patient, who was a dwarf.

Our findings showed that the glomerular volumes of FSGS and DMP patients were significantly larger than those of MCNS patients.

In all patients the median absolute GV was 1.42 and it ranged between 0.444 and $3.654 \times 10^6 \mu\text{m}^3$ in the first biopsy.

Diagn.	No.	GV	GV / BSA	Rebiopsy GV/BSA
MCNS	40	1.36 ± 0.10	2.55 ± 0.19	-
FSGS	14	$1.93 \pm 0.26^*$	$4.26 \pm 1.0^*$	-
DMP	4	1.26 ± 0.30	2.97 ± 0.84	5.13; 6.02

Table 2 shows the mean glomerular volume in absolute values ($10^6 \mu\text{m}^3$) and related to 1.73 m^2 in the first biopsy MCNS, FSGS and DMP patients. $*P<0.05$. FSGS compared to MCNS.

When we analysed the relation between glomerular volumes on the one hand, and GFR and ERPF on the other hand, in all patients, we found higher correlations with ERPF ($P<0.001$, $r=0.518$), than with GFR ($P<0.001$, $r=0.418$).

When the clinical outcomes were retrospectively related to glomerular volumes, we found, that the 5 children receiving a kidney transplant later, had a mean GV/BSA of $5.55 \times 10^6 \mu\text{m}^3$ and 2 girls who died of their nephrotic syndrome had a GV/BSA of 5.6 and $6.02 \times 10^6 \mu\text{m}^3$, respectively. Five of these 7 children had FSGS on their first examination.

Eight children examined in this study were later treated with CsA and 5 of them were placed in the MCNS group and 3 in the FSGS group (Paper IV). The mean GV/BSA of the MCNS patients treated with CsA was $2.26 \times 10^6 \mu\text{m}^3$ and of the 3 FSGS patients $3.25 \times 10^6 \mu\text{m}^3$, one of them had a GV/BSA of $6.58 \times 10^6 \mu\text{m}^3$ at the last biopsy. These findings show that glomerular hypertrophy may predict the development of a more severe disease and a transition to overt FSGS.

The positive significant correlations between GV, on the one hand, and absolute GFR and ERPF (ml/min), on the other, in all patients suggest that hyperfiltration and hyperperfusion contribute to the development of glomerular sclerosis also seen by others (Yoshida et al., 1989; Fogo and Ichikawa, 1991).

In the study by Fogo et al., patients with MCNS had a normal glomerular size, but initial biopsies in those who developed FSGS showed significant glomerular enlargement. In patients who had the nephrotic syndrome without evident segmental glomerulosclerosis, those with normal glomerular size seemed to have a good prognosis. However, those with markedly enlarged glomerular size run a high risk of developing overt FSGS (Fogo et al., 1990). None of the MCNS patients had glomerular hypertrophy. Moreover, they usually responded to corticosteroid treatment and the foot process effacement reversed, unlike in the patients with incipient FSGS (Kriz et al., 1996).

These findings reflect the actions of several factors on glomerular cells, which responded by an increasing matrix, hypertrophy, and proliferation often occurring in concert (Fogo, 2000). Thus, an increasing glomerular size is a sign of a growth response in many cases that often also induces an increase in the accumulation of matrix, the criterion of sclerosis (Fogo A et al., 1999). More recent studies have shown that ACEI inhibits not only sclerosis, but also abnormal glomerular growth, indicating that angiotensin II may act as a growth factor and induce vasoconstriction via the angiotensin II type I (AT 1) receptor (Wolf G et al., 1992; Matsusaka T et al., 1996; Sharma et al., 1998; Wolf, 1998; Ardaillou, 1999; Fogo A et al., 1999).

Numerous growth factors have been described in terms of their ability to increase matrix synthesis, including TGF- β , platelet-derived growth factor (PDGF), and others (Sharma and Ziyadeh, 1994), and vasoactive substances such as endothelin and already mentioned, angiotensin II.

In Paper III we studied rats with PAN-induced nephropathy. The mechanisms by which PAN induces nephrosis in animals have been described earlier (Fiegelson et al., 1957; Ryan and Karnovsky, 1975; Caulfield et al., 1976; Mahan et al., 1986; Coers et al., 1994). Recently, the effect of PAN was studied in a podocyte cell line. The podocyte damage found in this study involves nephrin, either directly, or indirectly via actin (Saleem et al., 2002).

The aim was to determine if the width of the foot processes, and the glomerular volume were related to GFR and fractional urine albumin excretion. In a previous study of

nephrotic children, an inverse correlation was found between foot process width and serum albumin and GFR (Bohman et al., 1984). In this study we gave rats various doses - i.e., 1mg, 1.67 mg and 2.5 mg/100 g rat weight of PAN, to induce different stages of the nephrotic syndrome (Bohrer MP et al., 1977).

We found increased glomerular volumes in the first treatment group than in the controls Moreover, the glomerular volumes decreased in groups 2 and 3, indicating more severe damage and shrinking of the glomeruli (Fig.10).

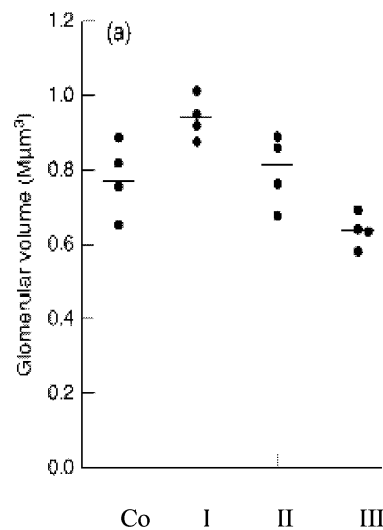


Figure 10 shows the glomerular volumes in the different groups. (Controls, grp I to III).

The glomerular volumes, as estimated by the Cavalieri principle, were more accurate than point counting the largest glomerular surface area and calculating the GV, as in Paper I. This is because the sampling is not biased by glomerular size and shape.

The PAN-treated rats showed significantly broader foot processes than the controls. The total slit pore length of the glomerulus was significantly greater in the controls than in the PAN-treated rats.

On relating the absolute GFR to GV, we found a strong positive correlation and an inverse correlation to foot process width. These findings have been reported by others (Trachtman H et al., 1993; Inokuchi et al., 1996). We also found a strong inverse correlation between fractional log urine albumin excretion and GV and a positive correlation to foot process width. This accords with the findings in children having the nephrotic syndrome (Bohman et al., 1984) (Fig. 11)

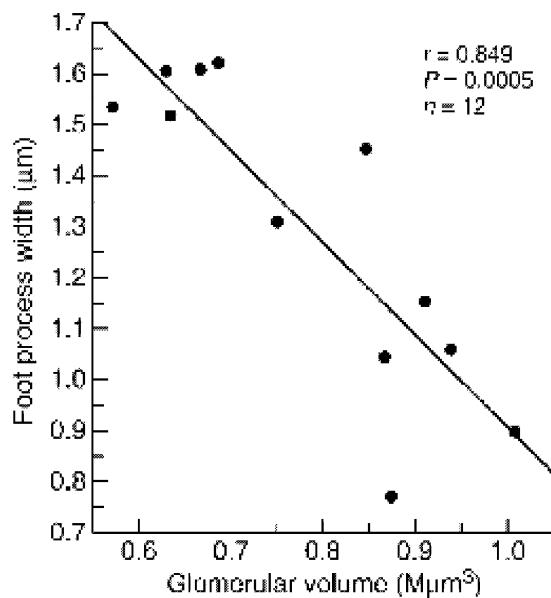


Figure 11 shows the relationship between GV and foot process width in the 12 PAN-treated rats.

When we compared the findings in Paper I, we also found a positive correlation between GFR, and even stronger to ERPF, and GV in children with the nephrotic syndrome.

It was evident in the rat study that GFR decreased with the degree of nephrosis and the degree of foot process fusion due to the reduction in slit pore length. It has been thought that this reduces glomerular capillary permeability to water and small solutes as reported by others (Drumond et al., 1994; Guasch and Myers, 1994; Ting et al., 1994). It may also explain the decrease in hydraulic permeability of the filtration barrier and the decreased GFR (Drumond et al., 1994).

Renal haemodynamics and oncotic pressure (serum albumin concentration)

(Paper II)

In this study, we showed that GFR in the nephrotic stage (s-albumin <25 mg/L) was significantly lower than in remission and in the controls, especially at the onset of the disease (Fig.12). Furthermore, ERPF was higher in the nephrotic stage than in recovery, mostly in children with histological lesions in the renal biopsy. We evaluated 98 children with various types of the nephrotic syndrome-i.e., MCNS, DMP, MCGN, MN and FSGS. The last 4 diagnoses show histological changes with LM.

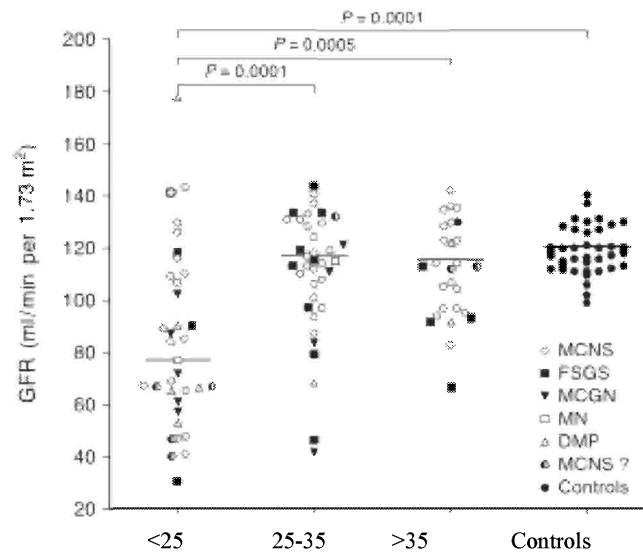
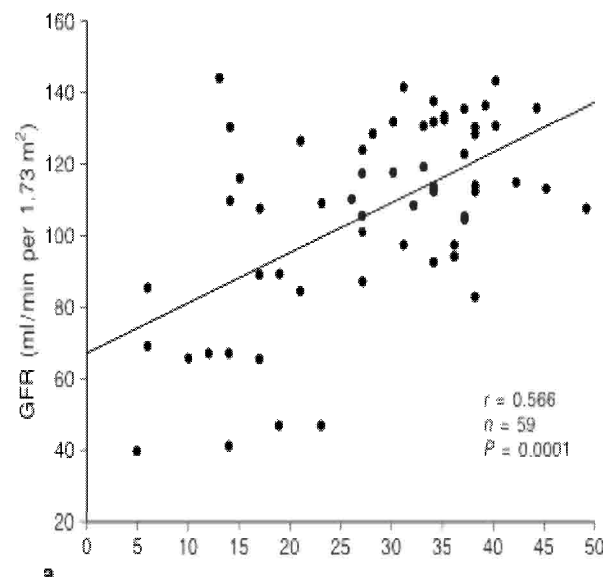


Figure 12 illustrates the GFR in different diagnostic groups and different stages of the nephrotic syndrome in relation to s-albumin (g/L).

In a comparison of GFR and serum albumin concentration, we found a direct correlation in MCNS patients ($P < 0.0001$, $r = 0.57$) (Fig.12), but ERPF in the patients with histological lesions showed an inverse correlation to the s-albumin concentration ($P = 0.002$, $r = 0.58$), which has been reported by others (Geers et al., 1984).

Figure 13 shows the correlation between s-albumin concentration (mg/L) and GFR in the MCNS patients.



We evaluated 98 children with various types of the nephrotic syndrome-i.e., MCNS, DMP, MCGN, MN and FSGS. The last 4 diagnoses show histological changes with LM.

The low GFR might have several explanations. A possible explanation for reduced GFR could be a decrease in ultrafiltration pressure. In opposite, there were factors counteracting a low GFR, such as a normal or increased ERPF reported earlier in children and adults (Berg U and Bohlin A-B, 1982; Guasch and Myers, 1994; Nyberg et al., 1994; Ting et al., 1994; Vande Walle JG et al., 1995), treatment with glucocorticoids (Levitt and Bader, 1951; Baylis and Brenner, 1978), increased ultrafiltration pressure through low plasma oncotic pressure and hypertension.

The increase in ultrafiltration pressure could instead be seen as a compensatory mechanism to counteract a greatly decreased ultrafiltration coefficient. A direct

relationship between the plasma oncotic pressure and the ultrafiltration coefficient are shown in animals and humans (Baylis et al., 1977; Guasch and Myers, 1994).

Renal function and morphology during long-term treatment with cyclosporine (CsA)

(Paper IV)

Between 1987 and 2002, 22 children, 11 with MCNS and 11 with FSGS, were prospectively followed during CsA treatment with repeated renal biopsies and renal hemodynamic studies. The indications for CsA treatment were severe steroid side-effects or steroid-resistance (Capodicasa et al., 1986; Tejani, 1987; Niaudet et al., 1988).

The CsA nephrotoxicity is of great concern and also the decline in renal function with this treatment (Habib R and P, 1994; Mihatsch et al., 1994; Inoue et al., 1999).

The clinical follow-up shows that all proteinuric MCNS patients and 7 proteinuric FSGS children went into remission in a median of 2 months. Four MCNS and 4 FSGS patients had no relapse during CsA treatment, two FSGS children remained proteinuric, and one of them progressed to end-stage renal failure (ESRF). Another patient was found to have ESRF after 118 months. CsA was stopped in 4. Two of them went into long-term remission. Six FSGS patients are still on continuous CsA treatment. When CsA was discontinued in the MCNS patients, they all relapsed.

The fact that CsA dependence develops in MCNS patients instead of corticosteroid dependence alleviates the corticosteroids side-effects, but with time, the patient runs the risk of developing CsA nephrotoxicity. The fact that most of our patients relapsed after the withdrawal of CsA agrees with the findings of many authors (Melocoton et al., 1991; Niaudet P and Habib R, 1994; Gregory MJ et al., 1996; Singh et al., 1999). Many patients who have relapses will respond when CsA is reinstituted but not in all patients (Sairam et al., 2002).

Secondly, on repeated evaluations of GFR and ERPF in MCNS patients, GFR declined from the first to the last investigation from a median of 110 to 93 ml/min per 1.73m². GFR on the first and last investigations were also significantly lower than those of the controls. ERPF declined significantly from 521 to 468 ml/min per 1.73m² and differed significantly from the values in controls. The annual change was – 3.8 and – 24 ml/min per 1.73m².

Most investigations in the literature were done with creatinine-based renal function tests in MCNS children and no significant changes were found (Kitano Y et al., 1990; Tanaka et al., 1993; Inoue Y et al., 1999; Seikaly et al., 2000). In a recent study in adults with uveitis, a significant decline in GFR occurred, but not in ERPF (Isnard Bagnis et al., 2002). In the evaluation of renal function after CsA treatment was discontinued, both GFR and ERPF became normal in 4 of 5 children. In one study of children during 27 months Hulton et al. also found an increase in GFR after treatment was stopped (Hulton et al., 1994). However, hardly any studies have assessed hemodynamics adequately (Neuhaus et al., 1992; Hulton et al., 1994; Isnard Bagnis et al., 2002).

Thirdly, the mild tubulo-interstitial nephrotoxic changes found in half of our MCNS patients developed after a median of 34 months. This accords with the findings of Iijima et al., who stated that treatment with CsA for more than 24 months entails a significant risk for CsA toxicity (Iijima K et al., 2002), as well as with the data of Habib and Niaudet, who reported that such changes became worse with the duration of treatment (Habib R and P, 1994).

In 11 FSGS patients a significant reduction in GFR from a median of 96 to 77 ml/min per 1.73m² occurred from the first to the last investigation and ERPF declined from 581 to 398 ml/min per 1.73m². The annual change was - 4.5 and - 36 ml/min per 1.73m². A similar reduction in renal function in such patients has also been reported by others (Zietse R et al., 1988; Ingulli and Tejani, 1995; Lieberman KV and Tejani A, 1996; Cattran et al., 1999). In two studies the incidence of ESRF was 24 %

(Ingulli et al., 1995; Chishti et al., 2001). Of our 11 patients, 2 received a kidney replacement therapy.

Reductions in GFR and ERPF and morphological changes in the group of FSGS patients were difficult to distinguish from increasing severity of the disease, but CsA toxicity probably contributed. In these patients, the risk of renal toxicity also increases with the duration of CsA and the total dose of CsA.

Renal hemodynamics did not change during the first 2 years of treatment, but declined slightly thereafter in both groups. In most patients, we found no correlation between renal hemodynamics and the morphological changes. In children with MCNS, the changes in renal function seemed to be reversible and the morphological changes were mild. In the FSGS patients, the declines in GFR and ERPF were more marked and may have been due to increasing severity of the disease as well as to CsA toxicity. Many of the FSGS patients became free of protein in the urine, which have been shown to indicate a more favorable prognosis (Rydel et al., 1995; Korbett, 1999; Martinelli et al., 2001).

CONCLUDING REMARKS

Glomerular volumes of DMP and FSGS patients were significantly larger than those of MCNS patients. Steroid-resistant patients also showed larger glomeruli than steroid-sensitive patients. Thus increased GV at onset seems to predict a more severe disease.

The decline in GFR seen in the nephrotic patients could not be attributed to a decline in renal plasma flow, since ERPF was normal or even increased in some patients.

An increase in ultrafiltration pressure owing to a reduction in oncotic pressure in nephrotic patients, would have counteracted a decrease in GFR.

In the animal study, the decline in GFR was due to a decrease infiltration slit length caused by severe foot process fusion, thereby decreasing the ultrafiltration coefficient and is in agreement with a previous study in children with MCNS.

An increase in ultrafiltration pressure owing to a reduction in oncotic pressure and to some extent increased systemic blood pressure in nephrotic patients, seems to be a compensatory factor counteracting the decreased ultrafiltration coefficient.

In CsA treated MCNS patients the nephrotoxic changes were very mild, but seemed to progress with time. GFR and ERPF was unchanged during the first two years, but declined slightly thereafter and seemed to be reversible after cessation of CsA. Therefore, CsA can also be used for longer periods in selected MCNS patients, if renal function and morphology are adequately monitored.

In children with CsA treated FSGS, reductions in GFR and ERPF and more marked morphological changes were difficult to distinguish from increasing severity of the disease, but CsA toxicity probably contributed.

The high incidence of reduction in proteinuria in CsA treated FSGS children indicates that early initiation of this treatment may retard progression of the disease.

FUTURE PERSPECTIVES

Certain areas are of special interest and importance.

Although cure rates of the idiopathic nephrotic syndrome has improved because of advances in immunosuppressive therapy, the pathogenesis and course of the disease remain controverse.

Cell-mediated immunity and T-cell activation, cytokine response and their effect on the podocytes have become an intense research area.

The molecular mechanisms underlying NS have yet to be clarified. The identification of the genes that play a central role in the immunopathogenesis is of importance.

The immunosuppressive therapy has improved through calcineurin inhibitors, but drug-related side-effects are matters of concern.

Therapy resistant nephrotic syndrome, with persistent disordered lipoprotein metabolism, has a high risk in developing premature cardiovascular disease. Treatment of lipid abnormalities is not readily accomplished because of concerns about side-effects.

ACKNOWLEDGEMENTS

Jag vill tacka alla er som på olika sätt hjälpt mig slutföra detta arbete:

Ulla Berg, min handledare i forskning och klinik, som med otrolig entusiasm och vilja, har fått mig att fullfölja detta arbete. Du är också ett föredöme i det kliniska arbetet. Ingenting är för svårt för Dig!

Sven-Olof Bohman, min förste handledare, avliden sedan flera år, som introducerade mig i experimentell forskning och morfologi på njurpatologen. Din generositet och omsorg in i det sista glömmar jag inte.

Agne Larsson, professor på enheten för pediatrik, för att skapa en kreativ akademisk miljö på Barnens sjukhus och för att jag beretts möjlighet att forska inom Enheten för Pediatrik.

Ann-Britt Bohlin, klinikchef, för kreativt samarbete och stöd genom åren på njursektionen.

Georg Jaremko, numera patolog på KS, som med noggrannhet och tålamod hjälpte mig att fullfölja mitt experimentella arbete.

Magnus Söderberg, njurpatolog på Huddinge, för morfologisamarbete och trevliga diskussioner.

Gun Forsander, diabetesbarnläkare, numera på Drottning Silvias Barn- och Ungdomssjukhus för samarbete om nefrosbarn.

Birgir Jakobson, för stöd genom åren på nefrosektionen.

Birgitta Sundelin, njurpatolog på KS, som det är roligt att diskutera klinik och patologi med.

Svante, för kloka synpunkter på mitt arbete.

Kajsa, Märta, Stella, Torun, Mia och Gianni för att ni är goda vänner och arbetskamrater på njursektionen och för att ni haft tålamod under den här tiden.

Birgitta, Helena, Anette, Annki, Elisabeth, Kicki, Carina, Monica och Annika, vårdpersonal och sekreterare på njursektionen. Ni är otroligt kompetenta och trevliga.

Annelie Hansson, Beatrice Sandvik, Ann-Marie Moutakefi, för hjälp på patologlab. och med EM-bilder.

Alla trevliga kollegor på Barnens sjukhus som uppmuntrat mig under den här tiden.

Elisabeth och Ulla, institutionens sekreterare, som hjälpt med alla dessa papper.
Dr. Zoe Walsh and editor Francis Walsh, for fast and professional language revision .

Karin, min syster, som bor långt bort, men ändå engagerat sig i min forskning.

Johanna och Andreas, mina bästa supportere och härliga barn. Ni är det bästa i mitt liv.

Kalle, min blivande svärson, som uppmuntrat mig och hjälpt mig med synpunkter på forskningen.

Maria, Ann-Marie, Meg för er entusiasm och uppmuntran.

Göran för Din akvarell till omslaget på boken.

Anders, för det stöd jag fått när jag behövt det.

Mina tidigare kollegor på Barnkliniken, Danderyds sjukhus, särskilt Anders, Bosse och Lars.

Alla nefrosbarn med föräldrar, som jag lärt känna. Jag har lärt mig mycket genom er.

This work was supported from grants from Karolinska Institutet, The Samariten Foundation, the Sven Jerring Foundation, The Swedish Association of Kidney Diseased (Rnj), Frimurareorden, Barnhuset, Tornspiran Foundation and The Swedish Medical Research Foundation (Nos.5937 and 6864)

REFERENCES

- ISKDC, (1981a) The primary nephrotic syndrome in children. Identification of patients with minimal change nephrotic syndrome from initial response to prednisone. International Study of Kidney Disease in Children. *J Pediatr* 98, 561-564.
- ISKDC, (1981b) Primary nephrotic syndrome in children: Clinical significance of histopathological variants of minimal change and of diffuse mesangial hypercellularity. A Report of the International Study of Kidney Disease in Children. *Kidney Int* 20, 765-771.
- Alt, J., Maess, B. and Hackbarth, H. (1985) Species and strain differences in urinary protein excretion. *Renal Physiol* 8, 301-309.
- Ardailou, R. (1999) Angiotensin II receptors. *J Am Soc Nephrol* 10 (suppl 11), S30-S39.
- Barratt, T., Mc Laine, P. and Soothill, J. (1970) Albumin excretion as a measure of glomerular dysfunction in children. *Arch Dis Child* 45, 496-501.
- Baylis, C. and Brenner, B. (1978) Mechanism of the glucocorticoid-induced increase in glomerular filtration rate. *Am J Physiol* 234(2), F166-F170.
- Baylis, C., Ichikawa, I., Willis, W., Wilson, C. and Brenner, B. (1977) Dynamics of glomerular ultrafiltration. IX. Effects of plasma protein concentration. *Am J Physiol* 232(1), F58-F71.
- Bell, E. (1929) Lipoid nephrosis. *Am J Pathol* 5, 587-622.
- Benigni, A., Bruzzi, I. and Mister, M. (1999) Nature and mediators of renal lesions in kidney transplant patients given cyclosporine treatment for more than one year. *Kidney Int* 55, 674-685.
- Berg U and Bohlin A-B. (1982) Renal hemodynamics in minimal change nephrotic syndrome in childhood. *Int J Ped Nephrol* 3, 187-192.
- Bland, J. and Altman, D. (1986) Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* Feb 8, 307-310.
- Bohman, S.-O., Jaremkö, G., Bohlin, A.-B. and Berg, U. (1984) Foot process fusion and glomerular filtration rate in minimal change nephrotic syndrome. *Kidney Int* 25, 696-700.
- Bohrer MP, Baylis C, Robertson CR and Brenner BM. (1977) Mechanisms of the puromycin-induced defects in the transglomerular passage of water and macromolecules. *J Clin Invest* 60, 152-161.

- Bonilla-Felix, M., Parra, C., Dajani, T., Ferris, M., Swinford, R., Portman, R. and Verani, R.(1999) Changing patterns in the histopathology of idiopathic nephrotic syndrome in children. *Kidney Int* 55, 1885-1890.
- Borel, J., Feurer, C., Gubler, H. and Stahelin, H.(1976) Biological effect of cyclosporin A: a new anti-lymphocytic agent. *Agents Actions* 6, 468-475.
- Boute N, Gribouval O, Roselli S, Benessy F, Lee H, Fuchshuber A, Dahan K, GublerM-C, Niaudet P and Antignac C.(2000) NPHS2, encoding the glomerular protein podocin, is mutated in autosomal recessive steroid-resistant nephrotic syndrome. *Nature Genet* 24, 349-354.
- Bradley SE and Tyson CJ.(1948) The 'nephrotic syndrome'. *N Engl J Med* 238, 223-227, 260-266.
- Brenner, B., Bohrer, M. and Baylis, C.(1977) Determinants of glomerular permselectivity: Insights derived from observations in vivo. *Kidney Int* 12, 229-237.
- Brenner , B., Hostetter, T. and Humes, H.(1978) Molecular basis of proteinuria of glomerular origin. *N Engl J Med* 298, 826-.
- Brismar, H., Patwardhan, A., Jaremko, G. and Nyengaard, J.(1996) Thickness estimation of fluorescent sections using CSLM. *J Microsc* 184, 106-116.
- Brodehl, J.(1991) The treatment of minimal change nephrotic syndrome: lessons learned from multicentre co-operative studies. *Eur J Pediatr* 150, 380-387.
- Bruhn C.(1951) A rapid method for the determination of para-aminohippuric acid in kidney function tests. *J Lab Clin Med* 37, 955-958.
- Burgess E, Curtis M and Benediktsson H.(1990) Membranoproliferative glomerulonephritis presenting as malignant hypertension. *Nephron* 54, 369-370.
- Cameron, J.(1985) Five hundred years of the nephrotic syndrome: 1484-1984. *The Ulster Medical Journal* 54 (Suppl.), S5-S19.
- Cameron, J., Turner, D., Heaton, J. and al., e.(1983) Idiopathic mesangiocapillary glomerulonephritis. Comparison of types I and II in children and adults and long-term prognosis. *Am J Med* 74, 175-192.
- Cameron JS and Hicks J.(2002) The origins and development of the concept of a 'nephrotic syndrome'. *Am J Nephrol* 22, 240-247.
- Capodicasa, G., De Santo, N., Nuzzi, F. and Giordano, C.(1986) Cyclosporine A in nephrotic syndrome of childhood - A 14 month experience. *Int J Pediatr Nephrol* 7, 69-72.

- Cattran, D., Appel, G., Hebert, L., Hunsicker, L., Pohl, M., Hoy, W., Maxwell, D. and Kunis, C.(1999) A randomized trial of cyclosporine in patients with steroid-resistant focal segmental glomerulosclerosis. North America Nephrotic Syndrome Study Group. *Kidney Int* 56, 2220-2226.
- Caulfield, J.P., Reid, J.J. and Farquhar, M.G.(1976) Alterations of the glomerular epithelium in acute aminonucleoside nephrosis: Evidence for formation of occluding junctions and epithelial cell detachment. *Lab Invest* 34, 43-59.
- Chantler, C., Garnett, E.S., Parsons, V. and Veall, N.(1969) Glomerular filtration rate measurement in man by the single injection method using ⁵¹Cr-EDTA. *Clin Sci* 37, 169-180.
- Chishti, A., Sorof, J., Brewer, E. and Kale, A.(2001) Long-term treatment of focal segmental glomerulosclerosis in children with cyclosporine given as a single daily dose. *Am J Kidney Dis* 38, 754-60.
- Churg J, Habib R and White RHR.(1970) Pathology of the nephrotic syndrome in children. *Lancet* 1, 1299-1302.
- Coers, W., Huitema, S., van der Horst, M.L.C. and Weening, J.J.(1994) Puromycin aminonucleoside and adriamycin disturb cytoskeletal and extracellular matrix protein organization, but not protein synthesis of cultured glomerular epithelial cells. *Exp Nephrol* 2, 40-50.
- D'Agati, V. (1995) Morphologic Features of Cyclosporin Nephrotoxicity. In: A, T (Ed) *Contrib Nephrol*, Vol. 114. Karger, Basel, p. 84-110.
- D'Amico, G. and Bazzi, C.(2003) Pathophysiology of proteinuria. *Kidney Int* 63, 809-825.
- Detwiler, R., Falk, R., Hogan, S. and Jennette, J.(1994) Collapsing glomerulopathy: a clinically and pathologically distinct variant of focal segmental glomerulosclerosis. *Kidney Int* 45, 1416-1424.
- Drumond, M. and Deen, W.(1994) Structural determinants of glomerular capillary hydraulic permeability. *Am J Physiol* 266, F1-F12.
- Drumond, M.C., Kristal, B., Myers, B.D. and Deen, W.M.(1994) Structural basis for reduced glomerular filtration capacity in nephrotic humans. *J Clin Invest* 94, 1187-1195.
- Epstein, A.(1927) Thyroid therapy and thyroid tolerance in nephrosis. *JAMA* 40, 73-79.
- Farnsworth, J.(1950) Metabolic changes associated with the administration of adrenocorticotropin in the nephrotic syndrome. *Proc Soc Exp Biol Med* 74, 60-62.

- Farquhar, M.(1995) The primary glomerular filtration barrier: Basement membrane or epithelial slits? *Kidney Int* 8, 197-211.
- Fassi, A., Sangalli, F., Maffi, R., Colombi, F., Mohamed, E.I., Brenner, B.M., Remuzzi, G. and Remuzzi, A.(1998) Progressive glomerular injury in the MWF rat is predicted by inborn nephron deficit. *J Am Soc Nephrol* 9, 1399-1406.
- Fiegelson, E., Drake, J. and Recant, L.(1957) Experimental aminonucleoside nephrosis in rats. *J Lab & Clin Med* 50, 437-446.
- Fogo, A.(2000) Glomerular hypertension, abnormal glomerular growth, and pregression of renal disease. *Kidney Int* 57 (Suppl 75), S15-S21.
- Fogo, A.(2001) Minimal change disease and focal segmental glomerulosclerosis. *Nephrol Dial Transplant* 16 (suppl 6), 74-76.
- Fogo A, Hawkins EP, Berry PL, Glick AD, Chiang ML, MacDonell RC Jr and Ichikawa I.(1999) Glomerular hypertrophy in minimal change disease predicts subsequent progression to focal segmental glomerulosclerosis. *Kidney Int* 38, 115-123.
- Fogo, A. and Ichikawa, I.(1991) Evidence for a pathogenic linkage between glomerular hypertrophy and sclerosis. *Am J Kid Dis* 17, 666-669.
- Fogo, A., Yoshida, Y., Ichikawa, I. and Glick, A.(1988) Hypertrophic glomeruli and possible relationship to glomerular sclerosis. *Lab Invest* 58, 31 A.
- Frank, C., Herrmann, M., Fernandez, S., Dirnecker, D., Böswald, M., Kolowos, W., Ruder, H. and Haas, J.-P.(2000) Dominant T cells in idiopathic nephrotic syndrome of childhood. *Kidney Int* 57, 510-517.
- Garin, E., West, L. and Zheng, W.(1997) Effect of interleukin-8 on glomerular sulfated compounds and albuminuria. *Pediatr Nephrol* 11, 274-279.
- Garin EH, Donnelly WH, Geary D and Richard GA.(1983) Nephrotic syndrome and diffuse mesangial proliferative glomerulonephritis in children. *Am J Dis Child* 137, 109-113.
- Geers, A., Koomans, H., Boer, P. and Dorhout Mees, E.(1984) Plasma and blood volumes in patients with the nephrotic syndrome. *Nephron* 38, 170-173.
- Gregory, MJ, Smoyer WE, Sedman A, Kershaw DB, Valentini RP, Johnson K and Bunchman TE.(1996) Long-term cyclosporine therapy for pediatric nephrotic syndrome: a clinical and histologic analysis. *J Am Soc Nephrol* 7, 543-549.
- Groffen, A., Veerkamp, J., Monnens, L. and van den Huevel, L.(1999) Recent insights into the structure and functions of heparan sulfate proteoglycans in the human glomerular basement membrane. *Nephrol Dial Transplant* 14, 2119-2129.

- Guasch, A., Deen, W. and Myers, B.(1993) Charge selectivity of the glomerular filtration barrier in healthy and nephrotic humans. *J Clin Invest* 92, 2274-2282.
- Guasch, A. and Myers, B.D.(1994) Determinants of glomerular hypofiltration in nephrotic patients with minimal change nephropathy. *J Am Soc Nephrol* 4, 1571-1581.
- Gundersen HJG, Seefeldt T and Østerby R.(1980) Glomerular epithelial foot processes in normal man and rats. *Cell Tissue Res* 205, 147-155.
- Gundersen, H.J.G. and Jensen, E.B.(1987) The efficiency of systematic sampling in stereology and its prediction. *J Microsc* 147, 229-263.
- Habib, R (1973) Editorial: Focal glomerular sclerosis. *Kidney Int* 4, 355-361.
- Habib R and Churg J. (1984) Minimal change disease, mesangial proliferative glomerulonephritis and focal sclerosis: Individual entities or a spectrum of disease? In: Robinson., RR (Ed) *Nephrology 1*. Springer Verlag., New York Berlin Heidelberg Tokyo., p. 634-644.
- Habib R and P, N.(1994) Comparison between pre-and posttreatment renal biopsies in children receiving ciclosporine for idiopathic nephrosis. *Clin Nephrol* 42, 141-146.
- Habib, R. and Kleinknecht, C.(1971) The primary nephrotic syndrome of childhood: Classification and clinicopathologic study of 406 cases. *Pathol Annu* 6, 417-474.
- Haycock, G., Schwartz, G. and al., e.(1978) Geometric method for measuring body surface area: a height-weight formula validated in infants, children, and adults. *J Pediatr* 93, 62-66.
- Hellerstein, S., Berenbom, M., Alon, U. and Warady, B.(1993) The renal clearance and infusion clearance of inulin are similar, but not identical. *Kidney Int* 44, 1058-1061.
- Hirose K, Tsuchida H, Østerby R and Gundersen HJG.(1980) A strong correlation between glomerular filtration rate and filtration surface in diabetic kidney function. *Lab Invest* 43, 434-437.
- Howie AJ.(1986) Changes at the glomerular tip: a feature of membranous nephropathy and other disorders associated with proteinuria. *J Pathol* 150, 13-20.
- Hughson, M. (1998) End-Stage Renal Disease. In: Jenette JC, Olson JL, Schwartz MM and Silva FG (Eds), In: *Heptinstall's Pathology of the Kidney*, 5th ed. Lippincott-Raven, Philadelphia, p. 1388.
- Hulton, S.-A., Jadresic, L., Shah, V., Trompeter, R., Dillon, M. and Barratt, T.(1994) Effect of cyclosporin A on glomerular filtration rate in children with minimal change nephrotic syndrome. *Pediatr Nephrol* 8, 404-407.

- Ichikawa, I., Rennke, H., Hoyer, J., Badr, K., Schor, N., Troy, J., Lechenne, C. and Brenner, B.(1983) Role for intrarenal mechanisms in the impaired salt excretion of experimental nephrotic syndrome. *J Clin Invest* 71, 91-103.
- Iijima K, Hamahira K, Tanaka R, Kobayashi A, Nozu K, Nakamura H and Yoshikawa N.(2002) Risk factors for cyclosporine-induced tubulointerstitial lesions in children with minimal change nephrotic syndrome. *Kidney Int* 61, 1801-1805.
- Ingulli, E., Singh, A., Baqi, N., Ahmad, H., Moazami, S. and Tejani, A.(1995) Aggressive, long-term cyclosporine therapy for steroid-resistant focal segmental glomerulosclerosis. *J Am Soc Nephrol* 5, 1820-1825.
- Ingulli, E. and Tejani, A.(1995) Focal segmental glomerulosclerosis in children. *Curr Opin Pediatr* 7, 176-181.
- Inokuchi, S., Shirato, I., Kobayashi, N., Koide, H., Tomino, Y. and Sakai, T.(1996) Re-evaluation of foot process effacement in acute puromycin aminonucleoside nephrosis. *Kidney Int* 50, 1278-1287.
- Inoue, Y., Iijima, K., Nakamura, H. and Yoshikawa, N.(1999) Two-year cyclosporin treatment in children with steroid-dependent nephrotic syndrome. *Pediatr Nephrol* 13, 33-38.
- ISKDC.(1978) Nephrotic syndrome in children: Prediction of histopathology from clinical and laboratory characteristics at time of diagnosis. A Report of the International Study of Kidney Disease in Children. *Kidney Int* 13, 159-165.
- ISKDC.(1979) International trials and pediatric nephrology {editorial}.International Study of Kidney disease in children. *N Engl J Med* 300, 254-255.
- ISKDC.(1981) Primary nephrotic syndrome in children:Clinical significance of histopathological variants of minimal change and of diffuse mesangial hypercellularity. A Report of the International Study of Kidney Disease in Children. *Kidney Int* 20, 765-771.
- Islam, M., Burke, J.J., McGowan, T., Zhu, Y., Dunn, S., McCue, P., Kanalas, J. and Sharma, K.(2001) Effect of anti-transforming growth factor- β antibodies in cyclosporine-induces renal dysfunction. *Kidney Int* 59, 498-506.
- Isnard Bagnis, C., Tezenas Du Montcel, S., Beaufils, H., Jouanneau, C., Chantal Jaudon, M., Maksun, P., Mallet, A., Lehoang, P. and Deray, G.(2002) Long-term renal effects of low-dose cyclosporine in uveitis-treated patients:Follow-up study. *J Am Soc Nephrol* 13, 2962-2968.
- Iversen, P. and Brun, C.(1951) Aspiration biopsy of the kidney. *Am J Med* 11, 323-330.
- Kahan, B.(1989) Drug therapy: cyclosporine. *N Engl J Med* 321, 1725-1938.

- Kanwar, Y., Liu, Z., Kashihara, N. and Wallner, E.(1991) Current status of the structural and functional basis of glomerular filtration and proteinuria. *Semin Nephrol* 11, 390-413.
- Kark, R., Pirani, C., Pollak, V., Muehrcke, R. and Blainey, J.(1958) The nephrotic syndrome: a common disorder with many causes. *Ann Intern Med* 49, 751-774.
- Karnovsky MJ.(1965) A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. *J Cell Biol* 27, 137 A.
- Kestilä, M., Lenkkeri, U., Männikkö, M., Lamerdin, J., McCready, P., Putaala, H., Ruotsalainen, V., Morita, T., Nissinen, M., Herva, R., Kashtan, C.E., Peltonen, L., Holmberg, C., Olsen, A. and Tryggvason, K.(1998) Positionally cloned gene for a novel glomerular protein - nephrin - is mutated in congenital nephrotic syndrome. *Molecular Cell* 1, 575-582.
- Kitano Y, Yoshikawa N, Tanaka R, Nakamura H, Ninomiya M and Ito H.(1990) Cyclosporin treatment in children with steroid-dependent nephrotic syndrome. *Pediatr Nephrol* 4, 474-477.
- Kopp, J. and Klotman, P.(1990) Cellular and molecular mechanisms of cyclosporin nephrotoxicity. *J Am Soc Nephrol* 1, 162-179.
- Korbet, S.(1999) Clinical picture and outcome of primary focal segmental glomerulosclerosis. *Nephrol Dial Transplant* 14 [Suppl 3], 68-73.
- Kriz, W., Hackenthal, E., Nobiling, R., Sakai, T. and Elger, M.(1994a) A role for podocytes to counteract capillary distension. *Kidney Int* 45, 369-376.
- Kriz, W., Kretzler, M., Nagata, M., Provoost, A.P., Shirato, I., Uiker, S., Sakai, T. and Lemley, K.V.(1996) A frequent pathway to glomerulosclerosis: Deterioration of tuft architecture-podocyte damage-segmental sclerosis. *Kidney Blood Pressure Res* 19, 245-253.
- Kriz, W., Mundel, P. and Elger, M. (1994b) The contractile apparatus of podocytes is arranged to counteract GBM expansion. In: Koide, H and Hayashi, T (Eds), *Contrib Nephrol*, Vol. 107, Extracellular matrix in the kidney. Karger, Basel, p. 1-9.
- Lama, G., Luongo, I., Tirino, G., Borriello, A., Carangio, C. and Salsano, M.(2002) T-lymphocyte populations and cytokines in childhood nephrotic syndrome. *Am J Kidney Dis* 39, 958-965.
- Letter, L.(1931) Nephrosis. *Medicine* 10, 135-242.
- Levitt, M. and Bader, M.(1951) Effect of cortisone and ACTH on fluid and electrolyte distribution in man. *Am J Med* 11, 715-723.

- Lieberman KV and Tejani A.(1996) A randomized double-blind placebo-controlled trial of cyclosporine in steroid-resistant idiopathic focal segmental glomerulosclerosis in children. *J Am Soc Nephrol* 7, 56-63.
- Lindblad, H. and Berg, U.(1994) Comparative evaluation of iohexol and inulin clearance for glomerular filtration rate determinations. *Acta Paediatr* 4, 418-422.
- Löwenborg, E., Forsander, G. and Berg, U.(2001) A national audit of nephrotic syndrome in Sweden: Initial treatment and 1-4 years follow up (Abstract). *Ped Nephrol* 16, C111.
- Mahan, J.D., Sisson-Ross, S. and Vernier, R.L.(1986) Glomerular basement membrane anionic charge site changes early in aminonucleoside nephrosis. *Am J Pathol* 125, 393-401.
- Martinelli, R., Okumura, A., Pereira, L. and Rocha, H.(2001) Primary focal segmental glomerulosclerosis in children: prognostic factors. *Pediatr Nephrol* 16, 658-661.
- Matsusaka T, Hymes J and Ichikawa I.(1996) Angiotensin in progressive renal diseases: Theory and practice. *J Am Soc Nephrol* 7, 2025-2043.
- Mc Enery, P., Alexander, S., Sullivan, K. and Tejani, A.(1993) Renal transplantation in children and adolescents: the 1992 Annual Report of the North American Pediatric Renal Transplant Cooperative Study. *Ped Nephrol* 7, 711-720.
- Melocoton, T., Kamil, E., Cohen, A. and Fine, R.(1991) Long-term cyclosporine A treatment of steroid-resistant and steroid-dependent nephrotic syndrome. *Am J Kidney Dis* 18, 583-588.
- Mihatsch, M., Antonovych, T., Bohman, S.-O., Habib, R., Helmchen, U., Noel, L., Olsen, S., Sibley, R., Kemper, E. and Feutren, G.(1994) Cyclosporin A nephropathy: standardization of the evaluation of kidney biopsies. *Clin Nephrol* 41, 23-32.
- Mihatsch, M., Thiel, G. and Ryffel, B.(1988) Histopathology of cyclosporine nephrotoxicity. *Transplant Proc* 20, 759-771.
- Muehrcke, R., Kark, R. and Pirani, C.(1955) Technique of percutaneous kidney biopsy in the prone position. *J Urol* 74, 267-277.
- Mundel, P. and Kriz, W.(1995) Structure and function of podocytes: an update. *Anat Embryol* 192, 385-397.
- Neuhaus, T., Burger, H., Klingler, M., Fanconi, A. and Leumann, E.(1992) Long-term low-dose cyclosporin A in steroid dependent nephrotic syndrome of childhood. *Eur J Pediatr* 151, 775-778.

- Niaudet P and Habib R.(1994) Cyclosporine in the treatment of idiopathic nephrosis. *J Am Soc Nephrol* 5, 1049-1056.
- Niaudet, P., Tete, M., Broyer, M. and Habib, R.(1988) Cyclosporin and childhood idiopathic nephrosis. *Transplant Proc* 20, 265-268.
- Nyberg, E., Bohman, S.-O. and Berg, U.(1994) Glomerular volume and renal function in children with different types of the nephrotic syndrome. *Pediatr Nephrol* 8, 285-289.
- Olson, J. and Schwartz, M. (1998) The nephrotic syndrome: minimal change disease, focal segmental glomerulosclerosis, and miscellaneous causes. In: Jenette JC, Olson JL, Schwartz MM and Silva FG (Eds), In: *Heptinstall's Pathology of the Kidney*, 5th ed. Lipincott-Raven, Philadelphia, p. 187-257.
- Pichler, R., Franceschini, N., Young, B. and al., e.(1995) Pathogenesis of cyclosporine nephropathy: Roles of angiotensin II and osteopontin. *J Am Soc Nephrol* 6, 1186-1196.
- Remuzzi A, Puntorieri S, Mazzoleni A and Remuzzi G.(1988) Sex related differences in glomerular ultrafiltration and proteinuria in Munich-Wistar rats. *Kidney Int* 34, 481-486.
- Remuzzi, A., Puntorieri, S., Alfano, M., Macconi, D., Abbate, M., Bertani, T. and Remuzzi, G.(1992) Pathophysiologic implications of proteinuria in a rat model of progressive glomerular injury. *Lab Invest* 67(5), 572-579.
- Rennke, H.(1995) Secondary membranoproliferative glomerulonephritis. *Kidney Int* 47, 643-656.
- Rennke, H., OLsson, J. and Venkatachalam, M.(1981) Glomerular filtration of macromolecules: Normal mechanisms and the pathogenesis of proteinuria. *Contr Nephrol* 24, 30-41.
- Rennke HG and KLein PS.(1989) Pathogenesis and significance of nonprimary focal and segmental glomerulosclerosis. *Am J Kidney Dis* 13, 443-456.
- Routsalainen, V., Ljungberg, P., Wartiovaara, J., Lenkkeri, U., Kestila, M., Jalanko, H., Holmberg, C. and Tryggvason, K.(1999) Nephrin is specifically located at the slit diaphragm of glomerular podocytes. *Proc Natl Acad Sci USA* 96, 7962-7967.
- Rovira-Halbach G, Alt JM, Brunkhorst R, Frei U, Kühn K and Stolte H.(1986) Single nephron hyperfiltration and proteinuria in a newly selected rat strain with superficial glomeruli. *Renal Physiol* 9, 317-325.
- Ruder H, Schärer K, Opelz G, Lenhard V, Waldherr R, Müller-Wiefel DE, Wingen AM and Dippel J.(1990) Human leukocyte antigens in idiopathic nephrotic syndrome in children. *Pediatr Nephrol* 4, 478-481.

- Ryan, G. and Karnovsky, M.(1975) An ultrastructural study of the mechanisms of proteinuria in aminonucleoside nephrosis. *Kidney Int* 8, 219-232.
- Rydel, J., Korbet, S., Borok, R. and Schwartz, M.(1995) Focal segmental glomerular sclerosis in adults: presentation, course and response to treatment. *Am J Kidney Dis* 25, 534-542.
- Sairam, V., Kalia, A., Rajaraman, S. and Travis, L.(2002) Secondary resistance to cyclosporin A in children with nephrotic syndrome. *Pediatr Nephrol* 17, 842-846.
- Sakai, T. and Kriz, W.(1987) The structural relationship between mesangial cells and basement membrane of the renal glomerulus 176, 373-386.
- Saleem, M., Ni, L., Witherden, I., Tryggvason, K., Ruotsalainen, V., Mundel, P. and Mathieson, P.(2002) Co-localization of nephrin, podocin, and the actin cytoskeleton. *Am J Pathol* 61, 1459-1466.
- Savage, C.(1994) The biology of the glomerulus: Endothelial cells. *Kidney Int* 45, 314-319.
- Seikaly, M., Prashner, H., Nolde-Hurlbert, B. and Browne, R.(2000) Long-term clinical and pathological effects of cyclosporin in children with nephrosis. *Pediatr Nephrol* 14, 214-217.
- Shalhoub, R.(1974) Pathogenesis of lipoid nephrosis: A disorder of T-cell function. *Lancet* 2(7880), 556-560.
- Sharma, K. and Ziyadeh, F.(1994) the emerging role of transforming growth factor-beta in kidney diseases. *Am J Physiol* 266, F829-F842.
- Sharma, M., Sharma, R., Greene, A., McCarthy, E. and Savin, V.(1998) Documentation of angiotensin II receptors in glomerular epithelial cells. *Am J Physiol* 274, F623-F627.
- Shihab, F., Bennet, W., Tanner, A. and Andoh, T.(1997) Angiotensin II blockade decreases TGF-beta1 and matrix proteins in cyclosporine therapy. *Kidney Int* 52, 660-673.
- Singh, A., Tejani, C. and Tejani, A.(1999) One-center experience with cyclosporine in refractory nephrotic syndrome in children. *Pediatr Nephrol* 13, 26-32.
- Smith, H., Finkelstein, N., Aliminos, L., Crawford, B. and Graber, M.(1945) The renal clearances of substituted hippuric acid derivatives and other aromatic acids in dog and man. *J Clin Invest* 24, 388-404.
- Smoyer WE and Mundel P.(1998) Regulation of podocyte structure during development of nephrotic syndrome. *J Mol Med* 76, 172-183.

- Srivastava, T., Simon, S. and Alon, U.(1999) High incidence of focal segmental glomerulosclerosis in nephrotic syndrome of childhood. *Pediatr Nephrol* 13, 13-18.
- Stake, G., Monn, E., Rootwelt, K. and Monclair, T.(1991) The clearance of iohexol as a measure of the glomerular filtration rate in children with chronic renal failure. *Scand J Clin Lab Invest* 51, 729-734.
- Tanaka, R., Yoshikawa, N., Kitano, Y., Ito, H. and Nakamura, H.(1993) Long-term ciclosporin treatment in children with steroid-dependent nephrotic syndrome. *Pediatr Nephrol* 7, 249-252.
- Tarshish P, Tobin JN, Bernstein J and Edelmann CM Jr.(1997) Prognostic significance of the early course of minimal change nephrotic syndrome: Report of the International Study of Kidney Disease in Children. *J Am Soc Nephrol* 8(5), 769-776.
- Tejani, A.(1987) Cyclosporine treatment in patients with primary glomerular diseases. *Int J Pediatr Nephrol* 8, 1-3.
- Tejani A.(1985) Morphological transition in minimal change nephrotic syndrome. *Nephron* 39, 157-159.
- Thomas, S., Andoh, T., Pichler, R. and al., e.(1998) Accelerated apoptosis characterizes cyclosporine-associated interstitial fibrosis. *Kidney Int* 53, 897-908.
- Ting, R., Kristal, B. and Myers, B.(1994) The biophysical basis of hypofiltration in nephrotic humans with membranous nephropathy. *Kidney Int* 45, 390-397.
- Topaloglu, R., Saatci, U., Arikan, M., Canpinar, H., Bakkaloglu, A. and Kansu, E.(1994) T-cell subsets, interleukin-2 receptor expression and production of interleukin-2 in minimal change nephrotic syndrome. *Ped Nephrol* 8, 649-652.
- Trachtman H, Futterweit S, Schwob N, Maesaka J and Valderrama E.(1993) Recombinant human growth hormone exacerbates chronic puromycin aminonucleoside nephropathy in rats. *Kidney Int* 44, 1281-1288.
- Tryggvason, K.(1999) Unraveling the mechanisms of glomerular ultrafiltration:Nephrin, a key component of the slit diaphragm. *J Am Soc Nephrol* 10, 2440-2445.
- Tufvesson, G. (2002) Immunosuppressiv behandling:. In: G.Tufvesson, CJa (Ed) Transplantation. Studentlitteratur, Lund.
- Vande Walle JG, Donckerwolcke RAMG, van Isselt JW, Derkx FHM, Joles JA and Koomans HA.(1995) Volume regulation in children with early relapse of minimal change nephrosis with or without hypovolaemic symptoms. *Lancet* 346, 148-152.

- Waldherr R, Gubler MC, Levy M, Broyer M and Habib R.(1978) The significance of pure diffuse mesangial proliferation in idiopathic nephrotic syndrome. Clin Nephrol 10, 171-179.
- Wissmann, C., Frey, F., Ferrari, P. and Uehlinger, D.(1996) Acute cyclosporine-induced nephrotoxicity in renal transplants:The role of the transplanted kidney. J Am Soc Nephrol 7, 2677-2681.
- Wolf, G.(1998) Angiotensin II is involved in the progression of renal disease: Importance of non-hemodynamic mechanisms. Nephrologie 19, 451-456.
- Wolf G, Haberstroh U and Neilson EG.(1992) Angiotensin II stimulates the proliferation and biosynthesis of type I collagen in cultured murine mesangial cells. Am J Pathol 140, 95-107.
- Yap, H., Cheung, W., Murugasu, B., Sim, S., Seah, C. and Jordan, S.(1999) Th1 and Th 2 cytokine mRNA profiles in childhood nephrotic syndrome. Evidence for increased IL-13 mRNA expression in relapse. J Am Soc Nephrol 10, 529-537.
- Yoshida, Y., Fogo, A. and Ichikawa, I.(1989) Glomerular hemodynamic changes vs. hypertrophy in experimental glomerular sclerosis. Kidney Int 35, 654-660.
- Yoshikawa N, Ito H, Akamatsu R, Matsuyama S, Hasagawa O, Nakahara C and Matsuo T.(1986) Focal segmental glomerulosclerosis with and without nephrotic syndrome in children. J Pediatr 109, 65-70.
- Zietse R, Wenting GJ, Kramer P, Schalekamp MA and Weimar W.(1988) Fractional excretion of protein: a marker of the efficacy of cyclosporine A treatment in nephrotic syndrome. Transplant Proc 20, 280-284.
- Østerby, R. and Gundersen, H.J.G.(1980) Fast accumulation of basement membrane material and the rate of morphological changes in acute experimental diabetic glomerular hypertrophy. Diabetologia 18, 493-500.