



LUND UNIVERSITY

Diagnostic and prognostic value of proteinuria in chronic renal diseases

Bakoush, Omran

2004

[Link to publication](#)

Citation for published version (APA):

Bakoush, O. (2004). *Diagnostic and prognostic value of proteinuria in chronic renal diseases*. [Doctoral Thesis (compilation), Nephrology]. Omran Bakoush, Griffelvägen 46, 245 64, Hjärup,.

Total number of authors:

1

General rights

Unless other specific re-use rights are stated the following general rights apply:

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

Read more about Creative commons licenses: <https://creativecommons.org/licenses/>

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

LUND UNIVERSITY

PO Box 117
221 00 Lund
+46 46-222 00 00

Diagnostic and prognostic value of proteinuria in chronic renal diseases

Clinical Studies

Omran Bakoush

Department of Nephrology, Faculty of Medicine

University Hospital in Lund, Sweden



LUND
UNIVERSITY

Lund 2004

Cover figure. Scanning electron micrograph demonstrating the endothelial surface of a glomerular capillary from the kidney of a normal rat. Numerous endothelial pores, or fenestrae, are evident (Magnification $\times 21,400$).

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

In the name of the God, Most Gracious, Most Merciful

وَمَا أُوتِيْتُمْ مِّنَ الْعِلْمِ إِلَّا قَلِيْلًا

"Of Knowledge it is only A little that is communicated To you"

(From Holly Quran- Surat Al Israa, Ayat 85)

To all of you, who have made my life worth living

My parents

My sister and brothers

My teachers

My close friends

CONTENTS

LIST OF PUBLICATIONS	6
ABBREVIATIONS	7
ABSTRACT	8
INTRODUCTION	10
The kidneys	10
Glomerular filtration	11
The permeability characteristic of the glomerular capillary wall	12
The glomerular filter	14
Proteinuria pathogenesis and sequelae	16
Assessment of proteinuria	18
Albumin	19
Immunoglobulin G	19
Immunoglobulin M	20
Protein HC	20
Glomerular diseases	21
IgA nephropathy	22
Mesangial proliferative glomerulonephritis	22
Membranous glomerulopathy	22
Minimal change nephropathy	23
Focal segmental glomerulosclerosis	23
Diabetic nephropathy	23
Hypertensive nephrosclerosis	24
AIMS OF THE PRESENT STUDIES	26
MATERIALS AND METHODS	27
Calculations	29
Theoretical analysis	30
Statistical Methods	31
RESULTS AND DISCUSSION	32
CONCLUSIONS	39

FUTURE PERSPECTIVES	40
ACKNOWLEDGEMENTS	41
REFERENCES	43
PAPERS I-V	56

LIST OF PUBLICATIONS

- I. **Bakoush O, Torffvit O, Rippe B, Tencer J:** High proteinuria selectivity index based upon IgM is a strong predictor of poor renal survival in glomerular diseases. *Nephrol Dial Transpl.* 16 (7):1357-1363, 2001.
- II. **Bakoush O, Torffvit O, Rippe B, Tencer J:** Renal function in proteinuric glomerular diseases correlates to changes in urine IgM excretion but not to the degree of albuminuria. *Clin Nephrol.* (59): 345-352.2003.
- III. **Bakoush O, Grubb A, Rippe B, Tencer J:** Urine excretion of protein HC in proteinuric glomerular diseases correlates to urine IgG but not to albuminuria. *Kidney Int.* (60): 1904-1909, 2001.
- IV. **Bakoush O, Tencer J, Tapia J, Rippe B, Torffvit O:** Higher urinary IgM excretion in type II diabetic nephropathy compared to type I diabetic nephropathy. *Kidney Int* (61): 203-208, 2002.
- V. **Bakoush O, Tencer J, Torffvit O, Tenstad O, Skogvall I, Rippe B:** Characterization of increased glomerular albumin permeability in old spontaneously hypertensive rats (SHR), submitted.

ABBREVIATIONS

ACI	Albumin creatinine index
α_1 -M	alpha1-microglobulin
Cr-EDTA	chromium ethylene diamine tetra-acetic acid
DN	Diabetic Nephropathy
ESRD	End Stage Renal Disease
GBM	Glomerular Basement membrane
GCW	Glomerular Capillary Wall
GFR	Glomerular Filtration Rate
GN	Glomerulonephritis
θ	“Theta”. Glomerular sieving coefficient or fractional clearance
θ_{alb}	Theta of native albumin
$\theta_{\text{n-alb}}$	Theta of neutral albumin
HMW	High Molecular Weight
HRP	Horseradish peroxidase
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgG ₂	Immunoglobulin G2
IgG ₄	Immunoglobulin G4
IgM	Immunoglobulin M
kDa	Kilo Dalton
LMW	Low Molecular Weight
MW	Molecular Weight
PCI	Protein Creatinine Index
Å	Ångström

ABSTRACT

To the extent that increased urinary protein excretion is an indicator of alterations of the glomerular capillary wall (GCW) and appearance of tubulointerstitial damage, proteinuria can be a good marker of the overall severity of the glomerular and tubulointerstitial damage, and therefore, the prognosis of glomerular diseases. Studies I, II, and III show that it is the type of proteinuria, rather than the degree of albuminuria, that predicts the progression in renal, proteinuric diseases. For instance, we found that the quantity of urinary IgM correlated to the decrease of glomerular filtration rate (GFR) in primary glomerular diseases, irrespective of the degree of albuminuria. 21% of patients with initial proteinuria with high IgM content developed end-stage renal failure compared to none of the patients with proteinuria with low IgM content. Patients who maintained high urinary IgM excretion during the course of glomerular disease showed a more rapid GFR decline over time compared to patients with maintained low IgM excretion despite persistent high degree of albuminuria (study II). Changes in urinary IgG, but not in albumin excretion, during the course of the glomerular disease, correlated to changes in urinary protein HC excretion (study III). Protein HC is a marker of impairment of the proximal tubular function.

In study IV, we observed that patients with type 2 DN had a higher urinary excretion of high molecular weight proteins (IgG and IgM) than patients with type 1 DN, despite similar degree of albuminuria. This suggests partly different patho-physiological mechanisms in diabetic nephropathy (DN) in type 1 and type 2 diabetes mellitus. Patients with type 2 DN have a better preserved ratio of urinary excretion of IgG₂/IgG₄ than type 1 DN patients, indicating that the charge selectivity is less impaired in type 2 DN.

Finally, old but not young hypertensive rats (study V) develop proteinuria as a result of a dysfunction of the glomerular capillary filter, affecting primarily its size-selectivity. The

changes are functionally compatible with the appearance in the glomerular barrier of an increased number of unselective pores.

Conclusions: During the course of glomerular diseases a maintained low urinary excretion of IgG or IgM indicates a salutary prognosis. Different patho-physiological mechanisms of albuminuria in type 1 and type 2 diabetes have been found, and hypertension induced proteinuria is primarily a size-selective disorder.

INTRODUCTION

The process of urine formation begins with the filtration of nearly protein-free fluid across the glomerular capillary wall (GCW), as first experimentally demonstrated by Wearn and Richards in 1924 (1). In 1842, William Bowman described the structure of the glomerulus, and later on, Ludwig argued that the initial event in the process of urine formation involves separation from the plasma of a protein-free ultrafiltrate by the walls of the glomerular capillaries (2, 3). In 1896, Starling described the mechanisms responsible for the ultrafiltrate formation, namely the magnitude and direction of the hydraulic and colloid osmotic pressures across capillary walls (4). Since 1970's, tracer macromolecules of well defined size and shape such as dextrans, Ficoll, and proteins have been extensively used to characterize the permeability of the GCW. The fractional clearances of such test probes have proven to be determined by the size- and charge-selectivity of the capillary wall, and by the charge, shape, deformability and size of the transported macromolecules (5, 6). Glomerular diseases are characterized by defects in both size- and charge selectivity of the GCW and result in so-called "glomerular proteinuria" (6). The main interest of this thesis, and the studies it is based on, is the diagnostic and prognostic value of the urinary excretion of endogenous proteins in glomerular diseases.

The kidneys

The kidneys are situated on both sides of the posterior part of the abdomen, behind the peritoneum. Each kidney is about 11-12 cm long and weighs about 150 gram. The kidneys contains of a total of two millions glomerulae (7, 8). The glomerulus is a lobulated network of convoluted capillary blood vessels surrounded by the Bowman's capsule, Fig.1 (7, 8). The total length of the, 9-12 μm in diameter capillaries, in a single glomerulus is 9.5 mm, giving

an overall capillary length of 19 km, and a glomerular surface area of $\approx 1 \text{ m}^2$ in the kidneys (7-10).

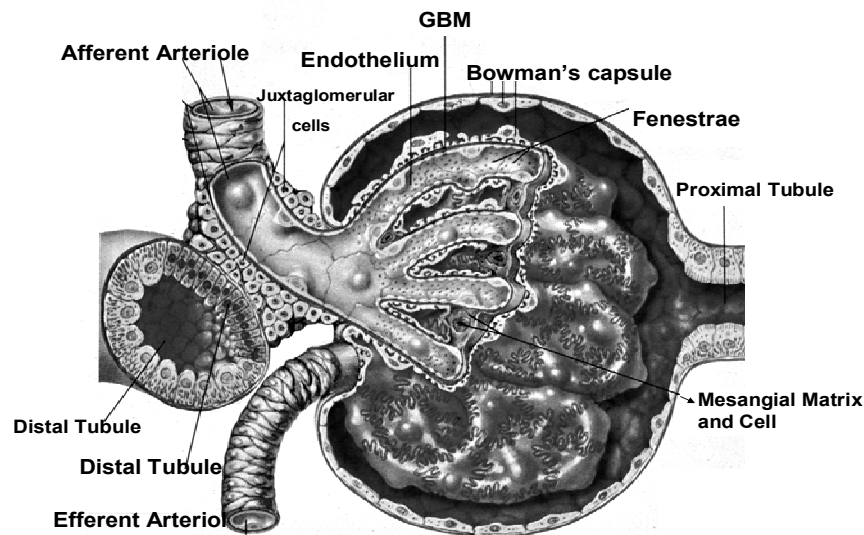


Figure 1. Schematic diagram of the glomerulus.

Glomerular filtration

The major function of the glomerulus is to produce an ultrafiltrate from the blood using the GCW as a filter. The glomerular filtration process differs from the transcapillary exchange process as in other organs in two ways. First, the GCW almost completely excludes plasma proteins of the size of albumin (radius 36\AA) or larger from the filtrate. Second, the glomeruli exhibit an extraordinary high permeability-surface area product (PS) to water and small solutes and also a very high capillary filtration capacity (6). Fluid movement across the glomerulus is, similar to the conditions in other capillaries, governed by the Starling forces, i.e. the effective hydrostatic pressure gradient minus the effective oncotic pressure gradient (4). The glomerular filtration rate (GFR) can thus be described by:

$$GFR = L_p S \times (\Delta P - \Delta \Pi)$$

Where, L_p represent the hydraulic conductivity of the GCW, and S is the surface area available for filtration. ΔP denotes the hydrostatic pressure in the glomerular capillaries

minus the hydrostatic pressure in the Bowman's space, and $\Delta\Pi$ the effective oncotic pressure in the glomerular capillaries minus that in the Bowman's space. If L_pS is 4 ml/min/mmHg/100g of kidney weight in humans, and $\Delta P \approx (52-15)$ mmHg, while $\Delta\Pi \approx (28-0)$ mmHg, then, the GFR in man equals $4 \times 3 \times [(52-15) - (28-0)] \approx 120$ ml/min. GFR can be measured clinically using molecules that are freely filtered across the glomerulus and that are not bound to plasma proteins nor are absorbed or secreted by the tubules, e.g. inulin or Cr EDTA. Normal GFR in females is 95 ± 20 ml/min and in males 120 ± 25 ml/min (11).

The size selective function of the GCW has been extensively investigated by measuring transglomerular filtration of tracer macromolecules (6). The filtrate-to-plasma concentration ratio of a test macromolecule (e.g. albumin) towards a reference solute such as Cr EDTA, which appears in Bowman's space in the same concentration as in plasma water, is denoted "fractional clearance" or "sieving coefficient" (θ) of the transported macromolecule through the GCW. It is a convenient way to measure permselectivity, varying from 0, when the test molecule is impermeable, to 1, when the molecule is not measurably restricted at normal GFR (6). Note that θ is not a constant, but varies with the GFR (12).

The permeability characteristics of the glomerular capillary wall: Despite the extremely low resistance to the flux of water, the human glomerular filter very efficiently restricts the passage of macromolecules from blood into Bowman's space, see table 1 (12-19). The passage of low molecular weight (LMW) proteins, e.g. proteins smaller than 30 kDa MW, and with a radius smaller than 25 Å, is almost completely unrestricted in normal individuals (6, 20, 21). The estimated albumin concentration in normal glomerular ultrafiltrate is only about 20 mg/L compared to approximately 40000 mg/L concentration of the protein in human plasma (19, 22). Thus, the glomerular sieving coefficient of albumin is $5-6 \times 10^{-4}$ (12, 15-19).

In normal individuals the transport of negatively charged albumin is restricted by a factor of 7-10 compared to equally sized, uncharged macromolecules, table 1 (12, 14).

Table 1. Glomerular sieving coefficient (θ) of macromolecules of different size, charge and configuration in normal rats.

Protein	Radius	θ	References
Anionic HRP	32 Å	0.007	13
Native HRP	30 Å	0.06	13
Dextran	30 Å	0.37	13
Native albumin	36 Å	0.0006	12,15-19
Neutral albumin	36 Å	0.006	12,17
Ficoll	35 Å	0.008	14
IgG	55 Å	0.005	15,18
α_2 macroglobulin	90 Å	0.000029	18

This view of the glomerular filter as a highly size- and charge-selective barrier has been challenged recently by Comper and his associates (23). The authors found that θ for albumin was nearly a 100-fold higher than previously reported, and they found little evidence for charge-selectivity of the glomerular filter (23, 24). If this were correct, then no less than 600 g of albumin would pass the human glomerular filter every day! Therefore they had to postulate a non-degradative ‘retrieval pathway’ to account for the reabsorption to plasma of almost the entire filtered load of albumin. Furthermore, a substantial fraction of urinary proteins was reported to be degraded and excreted in the final urine as protein fragments. It was proposed that a reduced protein ‘retrieval’ to plasma, or reduced protein degradation, would be mainly responsible for the increased urinary protein excretion occurring in a number of proteinuric disorders. This concept was specifically tested by Ohlson et al in the isolated perfused kidney model. However, they found considerable evidence that the classical view is still the most acceptable (25).

The most widely used description of macromolecular transport across the GCW indicates that the glomerular filter is perforated by pores having either a continuous log-normal distribution of radii, or by of two discrete populations of pores (12, 18, 26, 27). This hypothetical description of GCW is not based on ultra-structural analyses but on a hydrodynamic theory of hindered solute transport through water filled pores, as first modelled by Pappenheimer et al (28). The two-pore theory of capillary permeability adequately describes the fractional clearance data obtained in experimental and in clinical studies (12, 18, 29-32). In the “two-pore with a shunt model” the vast majority of pores are "small pores". The small pores exhibit a radius of $\approx 29\text{\AA}$ vis-à-vis negatively charged, rigid, spherical proteins, and a radius of 37-38 \AA vis-à-vis uncharged macromolecules (12, 18). The second pore population consists of a very small number of “large pores” of radius 90-115 \AA (18). The small pores are essentially impermeable to macromolecules the size of albumin or larger. Such molecules are normally transported by convection across the large pores (27). In addition to the two pores, the GCW may display “shunts”, which are very sporadic physiological “membrane defects”, large enough to allow the transport of very large proteins and even red blood cells (29, 33). Proteins such as IgM (radius 120 \AA) are able to pass the GCW only through these shunts (18). Conceivably, a repairing apparatus normally seals these shunts, and an increased transport of IgM indicates unsealing of the shunts and/or increased density of these defects in the GCW (29, 33).

The glomerular filter

The GCW consists of the glomerular basement membrane (GBM), an endothelial cell layer, and an epithelial cell layer (Fig.2). Both the cell layers are coated with a negatively charged surface coat (10-60 nm thick), called the glycocalyx (9). Furthermore, a much larger exclusion area extending from the endothelial surface for anionic macromolecules, possibly

composed of glycosylated macromolecules and adsorbed plasma proteins, has been described, denoted the “endothelial surface layer” (ESL) (34). The fenestrae between the endothelium cells are 50-60 nm in diameter, and also appear to be filled with plugs of glycocalyx or ESL up to 90 nm in height. They are thought to provide the GCW with size- and, most importantly, with charge-selectivity (9, 34, 35).

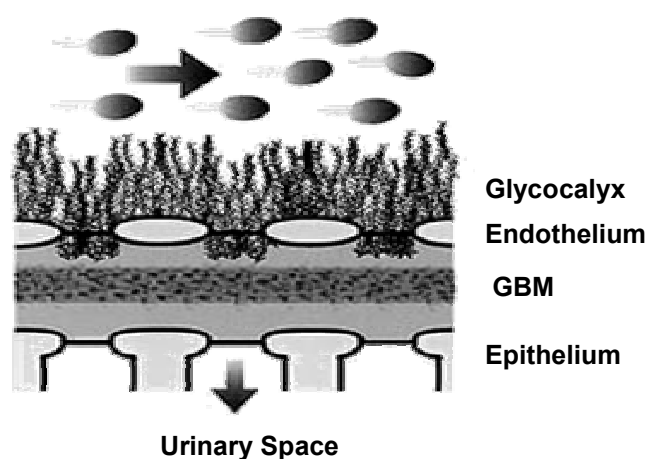


Figure 2. A schematic diagram of the glomerular capillary wall showing the luminal surface coat lining the endothelium and filling out the fenestrae. The ultrafiltrate passes through the downstream layers into the urinary space (Bowman’s capsule).

The GBM is a gel like material, 200 nm in width in rats, and 300-400 nm in humans, and is composed of tightly cross-linked extracellular matrix proteins, such as type IV collagen, laminin, nidogen and proteoglycans (36). Type IV collagen and laminin provide strength and flexibility to the GBM and also an adhesion surface for endothelial cells and podocytes. The heparan sulfate proteoglycans, perlecan and agrin, may contribute to the charge-selective permeability of the GBM (37), although this was recently questioned (38). The epithelial cells, the podocytes, cover the external surface of the GBM. They are highly specialized cells forming multiple interdigitating foot processes leaving in between them filtration slits, spanned by a “slit diaphragm”, 30 to 40 nm in width (39). The foot processes stabilize the glomerular architecture by counteracting the distension of the GBM (40). Nephrin is a major

structural component of the slit diaphragm, and the absence of nephrin, or any other slit diaphragm associated proteins, e.g. podocin, CD2-associated protein, Neph1, or alpha-actinin-4 leads to proteinuria and the nephrotic syndrome (41-45).

It is still not recognized which of the substructures of the GCW represents the ultimate permeability barrier, serving to retain plasma proteins in the circulation (46, 47). The blood flow is of great importance to maintain the GFR, and hence, the glomerular barrier function and, under normal hemodynamic conditions, albumin may be restricted already at the endothelial level, Fig.2 (33, 34, 47-49). In addition, the absence of concentration polarization within the GBM raises the possibility that the glycocalyx-filled fenestrae play a greater role in the size-selectivity than the GBM or the slit-diaphragm (9, 12, 50). The charge selectivity of the GCW is attributed mainly to the glycocalyx and/or ESL and to the heparan sulphate proteoglycans of the GBM (35, 37). Orosomuroid and albumin are among serum proteins that are thought to have a role in determining capillary permeability by maintaining and reinforcing the charge barrier (51-53). In all proteinuric diseases, whether immune complex mediated or not, the podocytes show structural changes with effaced foot processes, and occasionally separation from the GBM (40). Because of limited capacity of podocytes to proliferate, even after injury, podocyte loss or low podocyte number per glomerulus, may contribute to the development and progression of glomerulosclerosis and proteinuria (40, 54-57).

Proteinuria, pathogenesis and sequelae

An abnormal excretion of proteins in the urine is the hallmark of experimental and clinical glomerular diseases. Proteinuria is an indicator of alterations in the GCW and in tubular

protein uptake. It can be a good marker of the overall severity of the glomerular and tubulointerstitial damage, and therefore, of the prognosis of glomerular diseases.

The proteins filtered into the primary urine are normally reabsorbed via receptor-mediated endocytosis in the proximal convoluted tubules (19, 58). Megalin and cubilin are the two receptors known to be important for normal tubular reabsorption of filtered proteins (58, 59). The absorbed proteins are completely hydrolysed within the lysosomes and their resulting amino-acids cross the basolateral membrane to be returned to the circulation (58). Normally, the proximal tubules reabsorb approximately 90–95% of the filtered albumin while LMW proteins, such as protein HC, or light chains, are reabsorbed almost completely (19).

Tubulointerstitial injury causes an impairment of proximal tubular uptake of filtered proteins which leads to increased urinary excretion of LMW proteins, the characteristic feature of tubular proteinuria (20, 21). The normal upper limit of the total daily urine protein excretion is less than 150 mg, and normal urine consists of 20-30 mg albumin, 10-20 mg of LMW proteins, and 40-60 mg of secretory proteins, such as Tamm-Horsfall protein and IgA (60-62). In glomerular injuries, altered size- and charge-selective properties of the GCW result in increased filtered load of albumin and HMW proteins (63). When the reabsorptive capacity of the proximal tubules is exceeded, these proteins appear in the final urine, a phenomenon called “glomerular proteinuria”, although it also includes a component of “tubular” proteinuria.

Proteinuria may in itself contribute to ongoing renal injury by causing mesangial and tubulointerstitial damage (64, 65). In fact, proteinuria is the major determinant of progressive renal failure (66-68). The most widely proposed cause of tubular injury in proteinuric glomerular disease is the extensive tubular uptake of filtered plasma proteins, including

growth factors and complement factors, cytokines and protein bound substances, such as fatty acids, carried by the filtered albumin. These factors may induce tubular production of vasoactive and inflammatory cytokines, causing an invasion of the interstitium by inflammatory cells (69-75). In highly selective proteinuria, i.e. an almost pure urinary loss of albumin, the tubulointerstitial damage is infrequent, and almost all patients retain normal kidney function (76-78). With non-selective proteinuria, an increasing number of patients develop tubulointerstitial damage and progress to renal failure (79-81). It is generally believed that impairment of the charge-selectivity of the glomerular filter is the predominant lesion in selective proteinuria (82). The predominant lesion in non-selective proteinuria is a size-selectivity dysfunction, with the functional appearance of unselective “large pores” and or “shunts” in the glomerular filter (63).

Assessment of proteinuria

The clinical indication for proteinuria assessment is the screening for, and follow-up of renal diseases. During the screening, albuminuria is usually detected by dipstick methods, e.g. Albustix[®], in which the albumin concentration may be underestimated in diluted urine. The amount of protein in urine collected over a 24-hour period is used as the “golden standard” for measurement of proteinuria. However, because of the high risk of collection errors in a 24-hour sampling, and for practical and economic purposes, many investigators prefer to use urinary protein to creatinine index (PCI) instead (83-93). In pathological conditions, the high correlation to the 24 hour urine protein excretion, makes PCI in a single voided urine specimens accepted in clinical routine practice (68, 85, 86, 89-94). Reference values for PCI are: < 3.8 mg/mmol for albumin, < 0.8 mg/mmol for IgG, and <0.7 mg/mmol for protein HC in random urine specimen (61, 62, 91, 94).

The selectivity of proteinuria, assessed by the selectivity index (SI), is measured by the comparison of the clearance of high molecular weight proteins, e.g. IgG or IgM, with the clearance of transferrin or albumin as markers of intermediate size proteins (29, 95). SI based on α_2 -macroglobulin or IgM is of better predictive value than SI based on IgG (29).

Albumin is the most abundant circulating plasma protein, (69 kDa MW, serum concentration 38-40g/L) and has a variety of functions (96). These include maintenance of the oncotic pressure, buffering of acid-base changes, and transport of multiple bioactive substances such as fatty acids, steroid hormones and vitamins. Liver is the major site of synthesis of albumin, and its breakdown mainly occurs in the endothelial cells (96). The absolute level of serum albumin reflects not only the level of its synthesis and breakdown, but also its volume of distribution, the availability of amino acid precursors, and the albumin loss into urine, intestinal lumen and from the skin (97).

Immunoglobulins, or antibodies, are a complex group of functionally and structurally related proteins that protect the organism from invasion by pathogenic microorganisms and their toxic products. The basic structure of the immunoglobulin molecule consists of a monomer that contains four polypeptide chains, two heavy chains (each of 50 kDa MW) and two light chains (kappa or lambda, each of 20 kDa MW) linked by disulfide bonds (98). There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG and IgM antibodies.

Immunoglobulin G (IgG) is a monomeric molecule of 150 kDa, which predominates in secondary or memory immune response against infectious organisms. It is found on surface of memory B cells and predominantly in the blood. It constitutes 75% of serum immunoglobulins. Normal serum concentration of IgG is 5-15g/l. There are four subclasses of IgG, differing in the number of disulfide bonds and in the length of the hinge region. IgG₁ has

the highest concentration in human serum (3-10g/l), followed in order by IgG₂ (1-3.5 g/l), IgG₃ (0.3-1g/l), and IgG₄ (0.2-0.5g/l) (99).

Since IgG₂ is neutral and IgG₄ is negatively charged, a low value of urine IgG₂/IgG₄ ratio reflects loss of charge selectivity of the glomerular capillary wall.

Immunoglobulin M (IgM) is the third most common serum Ig with serum concentration of 0.5-4g/l. IgM is composed of five complete Ig units linked by disulfide bonds to form a pentamer with a molecular weight of 950 kDa (98, 99). IgM is the first immunoglobulin to be made by the foetus and the first antibody made by a virgin B cells when it is stimulated by antigen. As a consequence of its pentameric structure, IgM is a good agglutinating and complement fixing immunoglobulin, very efficient in clumping and lysis of microorganisms.

Protein HC (alias α_1 -microglobulin) is a human complex forming glycoprotein, heterogeneous in charge, and was first described by Tejler and Grubb (100). It is composed of a single polypeptide chain and its physiological function is unknown (101). Complexes between protein HC and IgA and between protein HC and albumin are present in most human biological fluids but they are not present in normal urine and rarely in pathological urine (102). Only the free form of protein HC is filtered through the GCW, and thus, found in normal urine. Protein HC is relatively stable in urine and is also stable at low urinary pH values. The high sensitivity of increased urinary excretion of protein HC makes the determination of the urinary excretion of protein HC an ideal instrument for demonstration of proximal tubular disorders (101-106).

Glomerular diseases

Disorders of glomerular structure and function are the leading cause of end stage renal disease (ESRD) (107, 108). Glomerular diseases may be primary or secondary to systemic disease such as diabetes or SLE (109, 110).

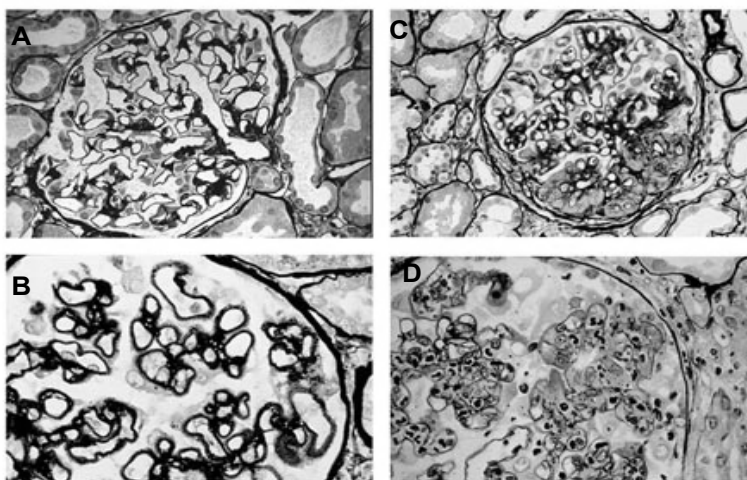


Figure 3. Four patterns of glomerulus histology. (A) minimal change, (B) membranous, (C) focal glomerulosclerosis and (D) Crescentic GN.

See table 2.

Table 2. Histological classification of glomerular diseases.

Non proliferative changes	Proliferative changes
<ul style="list-style-type: none"> •Primary renal disorder Minimal change Nephropathy Focal Segmental glomerulosclerosis Membranous glomerulopathy 	<ul style="list-style-type: none"> IgA nephropathy Mesangioproliferative glomerulonephritis Mesangiocapillary glomerulonephritis Focal segmental glomerulonephritis Crescentic glomerulonephritis
<ul style="list-style-type: none"> •Secondary renal disorder Diabetic nephropathy Nephrosclerosis 	<ul style="list-style-type: none"> Lupus nephritis Hepatitis induced GN

The term glomerulonephritis (GN) is used to denote diseases characterized by intraglomerular inflammation and cellular proliferation. Both humoral and cell-mediated immune mechanisms play a part in the pathogenesis of the glomerular inflammation (109-112). Non-proliferative forms of nephropathies, characterized by chronic non-inflammatory mechanisms of renal

injury, may also induce an increase in glomerular permeability and proteinuria without significant histological alterations in the glomeruli, Fig.3 (113, 114).

IgA nephropathy (IgA N) is a mesangioproliferative GN characterised by diffuse mesangial deposition of Immunoglobulin A. It is the most common form of GN worldwide (110). The disease occurs in all ages with a peak incidence in the second and third decades of life. Males are affected up to six times more frequently than females. IgA N presents in 50-60% of cases with episodes of gross hematuria that is frequently associated with respiratory- or gastrointestinal tract infection. In 30% of cases it presents with persistent microscopic hematuria and proteinuria, and occasionally, in 10% of cases, with nephrotic syndrome or rapidly progressive nephritis (113). The disease progresses to ESRD in 20-40% of cases (110, 115, 116).

Mesangial proliferative glomerulonephritis is found in 5 to 10% of renal biopsies (117). The patho-histological findings are characterised by mesangial cell proliferation and increase in mesangial matrix. Circulating immune complexes are present in 50 to 70% of cases. The disease usually presents with proteinuria, often in the nephrotic range. It accounts for 3-5% of patients with idiopathic nephrotic syndrome (117). Corticosteroid treatment leads to remission in 20 to 60% of the cases. Frequent relapses, partial remissions and glucocorticoid dependence are not uncommon (113).

Membranous glomerulopathy is the most common cause of idiopathic nephrotic syndrome in adult Caucasians (118). It is characterised by immune deposits of IgG and complement components predominantly on the subepithelial surface of the GCW. The disease is usually idiopathic but may be secondary to drugs (gold, penicillamine), infections (hepatitis B virus),

autoimmune diseases (SLE), or malignancy (118). Patients present typically with the nephrotic syndrome, and in 20% of cases, with non-nephrotic range proteinuria. Spontaneous remission occurs in 40% of patients while ~30% develop progressive renal failure (118-120). Treatment with cytotoxic agents and prednisolone is indicated in progressive cases (118, 119).

Minimal change nephropathy is responsible for 90% of nephrotic syndromes in children and 20% of nephrotic syndromes in adults (121). Morphologic changes are apparent only on electron microscopy that shows diffuse effacement of foot processes of the podocytes. The pathogenesis is unknown, but probably linked to T-cell mediated immunity (122). Clinically, the disease is characterised by the abrupt onset of nephrotic syndrome, normal renal function, and normotension. Spontaneous remissions occur in 40% of cases, and progression to renal failure is very rare (121, 123).

Focal segmental glomerulosclerosis (FSGS) has characteristic pathological features of focal and segmental glomerular scars found on light microscopy of renal biopsy (121). The disease is a common cause of nephrotic syndrome in adults (20 to 30%), especially in black males (124). Renal failure occurs in more than 50% of patients within 10 years (121, 125). FSGS is idiopathic, but may associate with intravenous drug use and is found in 20% of heterosexual HIV-positive persons (126). A non-immunoglobulin circulating factor seems to account to cases that recur after kidney transplantation (127).

Diabetic nephropathy (DN) is a (microvascular) complication of both type 1 and type 2 diabetes, that is associated with ESRD and premature death from cardiovascular disease (128, 129). Histologically, DN is characterised by diffuse or nodular mesangial expansion with thickening of the GBM (130). In patients with type 2 DN, particularly those with

hypertension, renal biopsy shows significant nephrosclerosis as well. Hyperglycaemia and increased intraglomerular pressure cause an increased synthesis of several cytokines and growth factors, in particular transforming growth factor- β (TGF- β). The cytokines have been identified to stimulate matrix production or inhibit matrix degradation and thus, lead to glomerulosclerosis and proteinuria (131, 132). The earliest clinical evidence of DN is appearance of low degree albuminuria, referred to as microalbuminuria ($>30\text{mg/day}$) (133, 134). Untreated, 20-40% of type 1 diabetes patients with microalbuminuria will progress to overt nephropathy over a period of 10 years (135-138). Once overt nephropathy occurs the GFR starts to fall at an average rate of 4-6 ml/min/year (135, 139). DN has become the most common cause of ESRD in Europe and US (108). As the incidence of type 2 diabetes increases, and the age of onset declines, the burden of DN will further increase in the future. Improved glycemic control has proven to dramatically decrease the incidence of DN (135, 140-142). Treatment with renin angiotensin system blockers and certain calcium channel blockers can reduce the progression to ESRD (143-146).

Hypertensive nephrosclerosis is the characteristic renal lesion associated with essential hypertension. Hypertension is considered present when the systolic blood pressure (SBP) is 140 mm Hg or higher, the diastolic blood pressure (DBP) is 90 mm Hg or higher, or the patient is on antihypertensive medication (147). It is estimated that 24% of the adult population is hypertensive, and 6% of hypertensive patients are at risk for progression to ESRD (147, 148). Systolic hypertension is a powerful predictor of development of renal injury, and the uncontrolled hypertension accounts for 27% of all new cases in ESRD (147-151). Histologically, hypertensive nephrosclerosis is characterized by myointimal hyperplasia of interlobular and afferent arteriolar vessels, hyaline arteriosclerosis especially of the latter, wrinkling collapse of the glomerular tuft and, commonly, global glomerulosclerosis (147).

Hypertension may cause renal damage as result of glomerular ischemia and hypoperfusion induced by progressive narrowing of the lumina of preglomerular arteries and arterioles. By contrast, afferent vasodilatation in remnant nephrons of individuals with hypertension, especially those with a low number of nephrons, transmit the systemic hypertension to glomerular capillaries, which may lead to progressive renal failure (152-154). Renal susceptibility genes play a role in the development of nephrosclerosis (153). Increased awareness of high blood pressure and treatment of hypertension has contributed to a dramatic reduction in morbidity and mortality attributable to hypertension (155).

AIMS OF THE PRESENT STUDIES

1. To study the urinary excretion of IgM and albumin as prognostic markers in a number of proteinuric glomerular diseases in order to examine whether pathophysiological and patho-morphological differences may influence the renal outcome (Study I).
2. To re-examine correlations between the renal function and alterations (regression or progression) of urinary excretion of IgM and albumin in the course of proteinuric glomerular diseases (Study II)
3. To study the effect on renal tubular function of urine excretion of large proteins by examining the correlation between the degree of tubular damage, assessed by the level of urine α_1 -microglobulin (protein HC), on the one hand, and urinary levels of IgG and albumin on the other hand, in chronic glomerular diseases (Study III).
4. To investigate the potential difference in the patho-physiology between patients with diabetic nephropathy in type 1 diabetes mellitus (type 1 DN) and type 2 diabetes mellitus (type 2 DN) by comparing the patterns of urinary excretion of proteins of different size and charge (study IV).
5. To examine the mechanisms of albuminuria resulting from severe, longstanding hypertension by measuring the transglomerular transport of native and neutral albumin in spontaneously hypertensive rats (SHR) of various age.

MATERIALS AND METHODS

The characteristic of the patients in the studies I to IV are shown in tables 3 and 4.

Table 3. Number of patients (male/female) in studies I-IV

Diagnosis	I	II	III	IV
	Male/Female	Male/Female	Male/Female	Male/Female
Mesangioproliferative GN	17/15	4/6	12/12	-
IgA nephropathy	14/2	3/2	4/3	-
Membranous GN	12/7	10/4	8/4	-
Minimal change nephropathy	5/3	3/3	3/3	-
Nephrosclerosis	8/1	1/1	6/0	12/1
Diabetes type 1	-	-	-	15/7
Diabetes type 2	-	-	-	18/2
Healthy subjects	-	-	-	14/2
Total	84	37	56	71

Table 4. Inclusion and Exclusion criteria in studies I-IV

Criteria	Studies
Inclusion criteria	
•Albumin creatinine index >20 mg/mmol	I, III, IV
>100 mg/mmol	II
•Serum creatinine < 250 µmol/L	II, IV
< 400 µmol/L	I, III
Exclusion criteria	
•Diabetic Nephropathy	I, II, III
•Systemic diseases	I-IV
•Rapidly progressive glomerulonephritis	I-IV

The patients in the first three studies were all participants in a large investigation program of glomerular diseases being conducted at the Nephrology Department, University Hospital of Lund, Sweden.

In study I we followed renal function in 84 patients with biopsy verified glomerular disease over a median of 41 (± 3) months. The patients were subdivided into groups with low (≤ 0.002) and high (> 0.002) proteinuria selectivity index based upon the IgM / albumin clearance ratio (IgM-SI), and into groups with low (≤ 200 mg/mmol) and high (> 200 mg/mmol) albumin creatinine index (ACI).

In study II we followed 37 proteinuric patients (21 males and 16 females) with glomerular disease and significant initial albuminuria for a mean of 44 (± 3.6) months. The comparisons between the patients were made according to the findings at the end of the study, by dividing them into three groups. One group had decreasing albuminuria (by more than 50%), one group had persisting albuminuria and low (< 0.04 mg/mmol creatinine), urinary IgM excretion and the last group had persisting albuminuria and high (≥ 0.04 mg/mmol) urinary IgM excretion.

In study III, we studied the relationship between urinary excretion of IgG, albumin, and protein HC in 56 patients (33 males and 23 females) with glomerular disease at the time of the diagnostic renal biopsy and after a mean of 49 follow-up months.

In study IV urinary albumin, IgG2, IgG4 and IgM were assessed in 20 patients (18 males and 2 females) with albuminuria and biopsy verified diabetic nephropathy due to type 2 diabetes, along with 22 (15 males and 7 females) patients with type 1 diabetes and macroalbuminuria (Tables 3 and 4). The measurements were compared with those in a control group consisting

of 13 (12 males and one female) patients with nephrosclerosis due to systemic hypertension, and with a second control group consisting of 16 (14 males and 2 females) healthy controls.

In study V, we assessed the glomerular sieving coefficients (θ) for neutral albumin ($\theta_{n\text{-alb}}$) and for native (negatively charged) albumin (θ_{alb}) in spontaneously hypertensive rats (SHR) of age 3, 9, and 14 months in comparison with age matched normal control Wistar rats (NCR). The hypothesis was that increases in the glomerular permeability of both negatively charged and neutral albumin would indicate a preferential size-selective dysfunction of the glomerular capillary wall (GCW), while an increased permeability to negatively charged albumin, as compared to neutral albumin, predominantly would indicate a charge selectivity dysfunction of the GCW. The glomerular sieving coefficients (θ) was assessed using a tissue (renal) uptake technique together with urinary sampling, described at some length elsewhere. Renal tracer protein clearance was calculated from the amount of tracer radioactivity accumulated in the two kidney cortices + the TCA precipitable urine tracer activity (collected during the tracer infusion period) divided by the plasma tracer “area under curve” (AUC). Protein sieving coefficients θ were calculated by dividing the measured protein clearance by the GFR. The GFR was assessed using the plasma to urine clearance of $^{51}\text{Cr-EDTA}$.

Calculations

The “gold standard” techniques for measurement of GFR using renal inulin or iothalamate clearances are time consuming and difficult to perform. However, given its simplicity, low cost, and widespread use, serum creatinine and creatinine clearance have been relied upon as the principal indicator of renal function in clinical and epidemiological studies (156-158). Various formulas for conversion of serum creatinine into creatinine clearance have been developed. The Cockcroft-Gault formula is probably the most used (159), where:

$$\text{Creatinine clearance (Ccr)} = \frac{(140 - \text{age}) \times \text{weight}}{\text{Serum creatinine } (\mu\text{mmol/L})} (\times 1.23 \text{ for men})$$

A more recently developed formula, based on data derived from the Modification of Diet in Renal Disease (MDRD) study, correlates well with measured GFR, and is of use in patients with mild to moderate renal insufficiency but is inaccurate in patients with normal or above normal GFR (160, 161), namely:

$$\text{GFR} = \{186 \times (\text{serum creatinine mg/dl})^{-1.154} \times (\text{age})^{-0.203} (\times 0.742 \text{ if female}).$$

IgG2/IgG4 ratio was calculated as:

$$\frac{\text{Urine IgG2 concentration (mg/l)}}{\text{Urine IgG4 concentration (mg/l)}}$$

IgM-selectivity index (IgM-SI) was calculated according to the formula (29, 162):

$$\frac{\text{Urine IgM} \times \text{Serum Albumin}}{\text{Serum IgM} \times \text{Urine Albumin}}$$

Theoretical analysis

According to the two-pore model, the glomerular clearance of any protein larger than the assumed small-pore radius is determined by its convective transport across large pores. Thus, its large-pore clearance (Cl_L) is just the product of the large pore volume flow (Jv_L) and the (large pore) reflection coefficient (σ_L), or actually $(1 - \sigma_L)$, of the solute:

$$Cl_L = Jv_L(1 - \sigma_L) \quad (1)$$

Since native albumin is negatively charged, it would be completely excluded from the small pore pathway in the glomerular filter, and thus be confined to convective transport through large pores (12). Based on that consideration, the large pore volume flow Jv_L could be

determined from the sieving coefficient of native albumin (θ_{alb}) and from GFR assuming a fixed value (100Å) for the large pore radius (18).

$$\theta_{alb} = \frac{Jv_L}{GFR} (1 - \sigma_L) \quad (2)$$

Charge selectivity defect: If charge selectivity is lost, then native albumin, which is normally excluded from the small pore pathway, will be able to penetrate the small pores, leading to a selective increment in θ_{alb} , so that it will eventually approach the sieving coefficient of neutral albumin (θ_{n-alb}).

Size selectivity defect: If enlarged, less selective pores are formed when permeability is increased, then charge and size selectivity will be changed in parallel, leading to increases in both θ_{alb} and θ_{n-alb} . If new large pores are formed, then, because of the presence of negative charges in the large pores, the changes in θ_{alb} would be less pronounced than those in θ_{n-alb} , according to the following equation:

$$\theta_{alb} \propto \frac{(1 - \sigma_L)_{alb}}{(1 - \sigma_L)_{n-alb}} \theta_{n-alb} \quad (3)$$

For 100 Å large pores $(1 - \sigma_L)_{alb}$ will be 0.398 (accounting for negative charges on albumin and on the pore walls according to the Debye Hückel theory of ion-ion interaction) and $(1 - \sigma_L)_{n-alb}$ will be 0.593.

Statistical methods

The data in the tables are expressed as medians and ranges or means \pm SE (study II and V). Statistical comparison between the patient groups was performed with non-parametric Mann-Whitney test, or Kruskal Wallis test when applicable. Correlation was tested using Spearman's correlation coefficient. $P < 0.05$ was selected as the level of significance. Urine

concentrations of IgM below the detection limit were set at 0.01 mg/mmol and urinary HC-CI and IgG-CI below the detection limit were set at 0.1 mg/mmol. The statistical package for social science (SPSS, version 10) was used. $P \leq 0.05$ (or when appreciate $P \leq 0.01$) was selected as the level of significance.

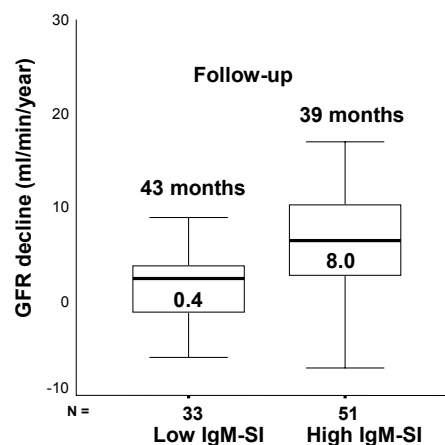
RESULTS AND DISCUSSION

Studies (I - III)

Clinical and experimental data indicate that glomerular proteinuria affects the progression of renal impairment in glomerular diseases by enhancing the formation of tubulointerstitial fibrosis (163). However, several recent reports suggest that it may not be albumin per se, but rather other factors associated with the enhanced urinary leakage of plasma proteins, such as complement factors or protein-bound inflammatory cytokines (e.g. TGF- β), that might cause these sequelae (70, 164).

Studies I-II show that an increased urine IgM excretion predicts an unfavourable outcome, while a decreased urine excretion of this protein correlates to a more salutary prognosis in patients with primary

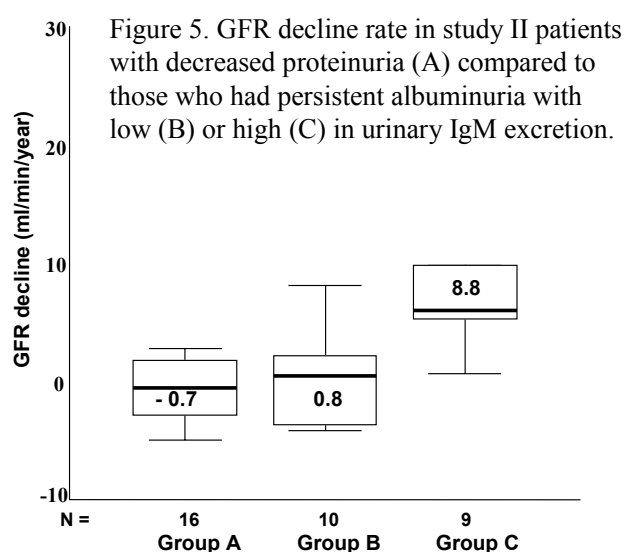
Figure 4. GFR decline rate in study I patients with low respectively high values of IgM-selectivity index.



glomerular diseases. This is true also when the total proteinuria, often presented as albuminuria, is persisting. In study I, patients with a high IgM based selectivity index (IgM-

SI) significantly decreased their renal function, by an average of 8 ml/min/year (Fig.4). Furthermore, 21% of the patients in this group developed end stage renal failure (ESRD) during the study time. In comparison, patients with low IgM-SI on average maintained their kidney function unaltered during observation time, although they had a higher degree of albuminuria (411 mg/mmol) than the patients in the high IgM-SI group (151 mg/mmol) ($p<0.001$). None of the patients in low IgM-SI group developed ESRD.

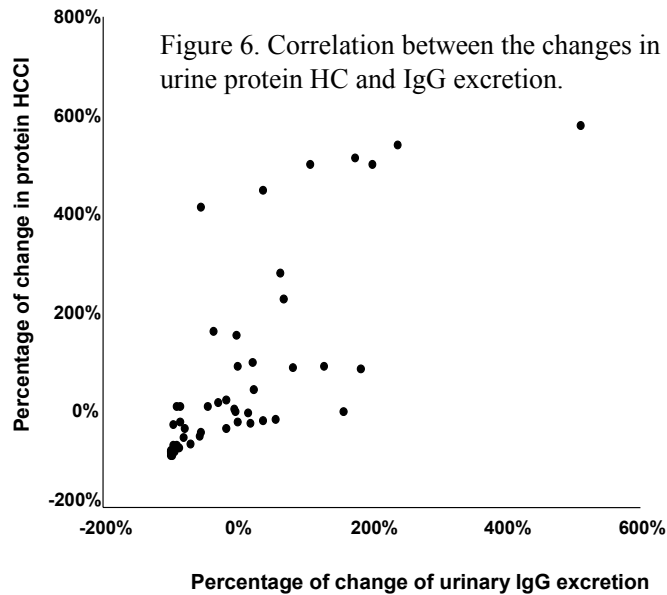
Observations of patients with glomerular diseases with long-time persistent albuminuria in study II showed that only patients with persistent high urinary IgM excretion decreased significantly in renal function, while those with reduced urine IgM excretion preserved their renal function



(Fig.5). In both studies, an increased urine IgM excretion was the strongest single predictor of the GFR decline ($r=0.73$, $p<0.001$).

The pivotal role of albuminuria in the progression of renal tubular function impairment in glomerular diseases was further questioned in study III, where its relation to the urinary excretion of protein HC (reflecting the impairment of tubular protein uptake) was examined. We found that changes in urinary excretion of protein HC in a single patient during the follow-up time were much more strongly ($r^2=0.7$) associated with changes in the urine IgG excretion than with changes in the degree of albuminuria ($r^2=0.29$) (Fig.6). In consistency

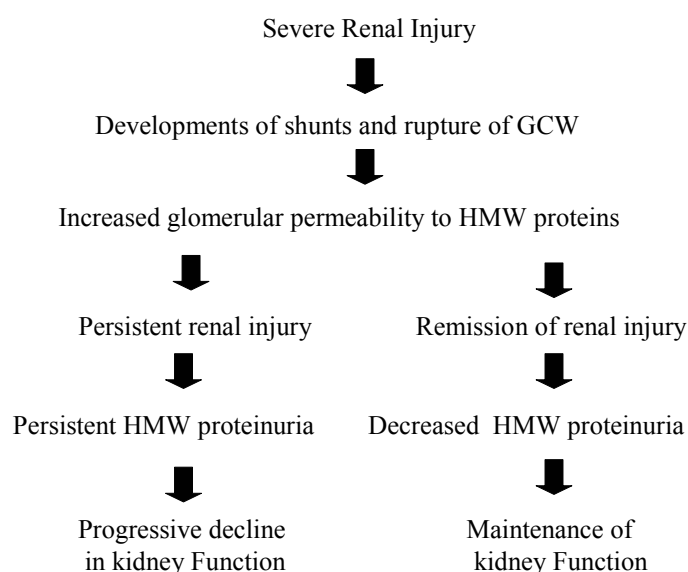
with other recent investigations, these studies (I-III) shows a significant correlation between non-selective proteinuria and the degree of tubulointerstitial damage (77).



According to the “two-pore with a shunt” theory, macromolecules of the size of albumin or larger are normally transported through large pores of the GCW (26, 27). Since the population of these pores is, under normal conditions, relatively small, the transport of albumin across the GCW is usually low. Very large proteins, such as IgM, are able to pass the GCW only through the extremely rare shunts (18, 33). In case of loss of charge selectivity of the glomerular filter the “effective” small pore radius increases (by 8 Å) from ~29 to ~37 Å, thus enabling albumin to escape in large quantities through the small pores. Proteins larger than albumin are, however, still unable to pass through the small pore pathway. Thus, selective proteinuria is usually a consequence of a charge-selective defect rather than a size-selective disorder. This situation is conceivably the case in minimal charge nephropathy, but also in some other glomerular diseases (165). Once the glomerular disease produces alterations in the size-selective properties of the GCW, the urine contains an increased amount of large proteins

such as IgG and IgM. However, whereas the increased urinary excretion of IgG reflects increased density of “large pores” in the GCW, the occurrence of IgM in the final urine conceivably reflects a markedly increased population of highly unselective pathways, i.e. shunts. Recently, varying degrees of ultrastructural defects in the glomeruli, measuring 15-200 nm in diameter, were revealed in nephrotic patients by transmission electron microscopy using a tissue negative staining method. These ultrastructural defects were not seen in normal renal tissue (166). Thus, proteins of size of IgM could make a sensitive marker of such defects, and an increased urinary IgM excretion apparently would predict a more severe glomerular injury and poorer renal outcome in glomerular diseases (Fig. 7).

Figure 7. Pathophysiology of progressive nephropathy



Isolated albuminuria may represent alterations in either the charge- or size-selective properties of the GCW, or both. These alterations do not necessarily correlate to a gross damage of the GCW, which in turn, could explain the lack of correlation between albuminuria and the progress of the renal function impairment or development of interstitial fibrosis.

Study IV

Proteinuria is widely regarded as a hallmark of nephropathy in both type 1 and type 2 diabetes (128, 129). Until now, few studies have compared type 1 and type 2 DN (167).

Indeed, urinary IgM excretion as marker of the size-selective injury in type 2 DN has, to our knowledge, not been studied before at all. In study IV, the patients with type 1 DN and type 2 DN did not differ with regard to

Figure 8. Urinary IgM excretion in healthy individuals and in patients with diabetic nephropathy due to type 1 and type 2 diabetes.

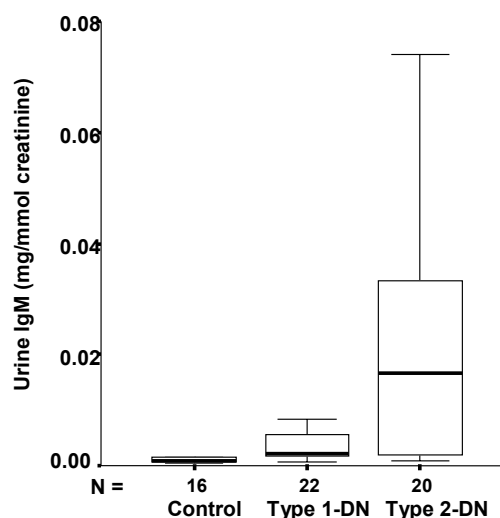
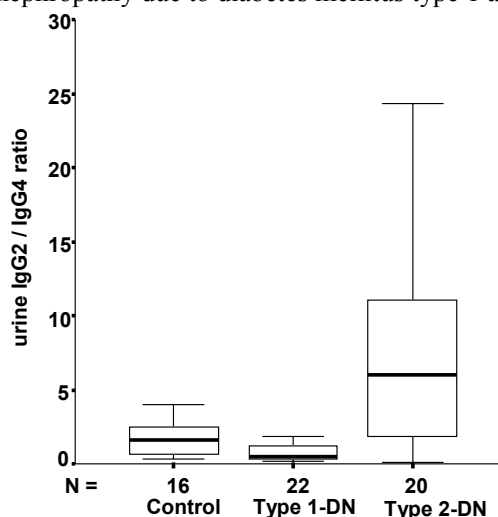


Figure 9. urine IgG2/IgG4 ratio in healthy individuals and patients with diabetic nephropathy due to diabetes mellitus type 1 and type 2.



the degree of albuminuria, serum creatinine or urine protein HC concentration. However, compared to patients with type 1 DN, the type 2 DN patients showed an increased urine excretion of IgG, suggesting a more severe size-selective dysfunction in type 2 DN. The size-selective properties of the glomerular capillary wall were relatively intact in type 1 DN. The appearance of IgM in the urine in type 2 DN patients can be interpreted to reflect a markedly increased population of highly non-selective “shunt”-pathways (Fig.8). The urine IgG2/IgG4 ratio was high in type 2 DN and low in type 1 DN patients ($p < 0.01$) (Fig.9). This suggests that the charge selectivity of the glomerular barrier in type 2 DN patients is preserved. Thus, while

an impairment of the charge selectivity of the GCW is probably the major cause of proteinuria in early type 1 diabetes, the proteinuria in type 2 DN is primarily due to a size-selective dysfunction. It is likely that the presence of hypertension and an increased vascular resistance in patients with type 2 DN may induce ischemia and structural changes in the glomeruli resulting in proteinuria mainly reflecting a size-selective dysfunction in type 2 DN (168, 169).

Study V

The major result from study V is that the glomerular sieving coefficient of native albumin (θ_{alb}) in SHR, was normal during the first 9 months of hypertension, but significantly increased in old animals, as compared to that in age matched NCR (Fig.10). The glomerular sieving coefficient of native albumin (θ_{alb}) in SHR increased from $5.0 (\pm 0.5) \times 10^{-4}$ at 3 months, to $7.6 (\pm 0.8) \times 10^{-4}$ at 9 months, and to $12.9 (\pm 0.9) \times 10^{-4}$ at 14 months of age ($p < 0.001$), while θ_{alb} did not change significantly with age in NCR, remaining at $7.0 (\pm 0.5) \times 10^{-4}$ at 3 months and 9 months and at $7.2 (\pm 0.9) \times 10^{-4}$ at 14 months of age, respectively. Thus, in SHR, it takes more than half a lifetime of hypertension to develop proteinuria and kidney damage.

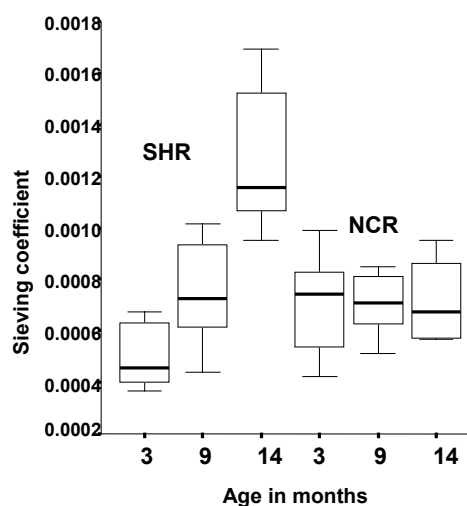


Figure 10. Sieving coefficient (θ_{alb}) of native albumin in animals studied (SHR and NCR) at 3, 9 and 14 months of age.

Furthermore, the glomerular disturbance developed during long-standing hypertension is of size-selective nature, and not represented by a primary charge-selective defect of the GCW. Thus, the ratio of neutral to negative albumin clearance was maintained high throughout the

life span of the hypertensive rats, and the increases θ_{alb} in old SHR was significantly correlated with increases in θ_{n-alb} ($r=0.86$, $p<0.001$). This may, according to pore theory, be explained by the creation of an increased number of rather unselective pores of intermediate radius (64.2 Å; *cf.* small and large pores). All the animals studied (NCR and SHR) thus showed a generally higher clearance of neutral albumin (7 fold) than of native (negatively charged) albumin, indicating a normally marked influence of charge on transglomerular protein transport.

The albumin sieving coefficient (0.0007) obtained by the present tissue renal uptake technique or earlier micropuncture studies (0.00062) (19) along with a recent report by Norden et al, (170) of the absence of a significant amount of albumin degradation products in normal urine, contradicts the recent reports that the glomerulus is normally highly leaky to albumin (23). Our data reconfirm the old concept of the GCW as a highly charge- and size-selective barrier to the passage of macromolecules of the size of albumin or larger.

CONCLUSIONS

- The findings of the present studies indicate that it is possible to predict the rate of progression in renal diseases in patients with non-diabetic glomerulopathies by the type of proteinuria rather than by the degree of albuminuria.
- High urinary IgM excretion correlates to a decreased GFR in primary glomerular diseases regardless of the degree of albuminuria. In parallel, low urinary IgM excretion indicates beneficial prognosis in these diseases.
- Since IgM can be predicted to pass the glomerular barrier entirely through large shunts or defects in the glomerular capillary wall, a decreasing urine content of IgM might be considered as a sign of recovery of the glomerular damage
- The difference in the proteinuria patterns in type 1 and type 2 diabetic nephropathy, in addition to the clinical and functional differences, suggests mutually different pathophysiological mechanisms of nephropathy in the two entities of diabetic renal disease.
- In old age, hypertensive rats develop proteinuria as a result of a dysfunction of the glomerular capillary wall, affecting primarily its size selectivity. This conceivably occurs by the appearance of an increased number of rather unselective pores in the glomerular filtration barrier in untreated, long-standing hypertension.

FUTURE PERSPECTIVES

An increased use of non-invasive diagnostic and prognostic approaches in glomerular diseases would be beneficial to the patients and also be cost-effective. The studies presented in this thesis illustrate the use of urinary proteins as markers of glomerular and tubular dysfunction and the prognosis of proteinuric renal diseases. The studies have raised several questions requiring further investigation. Identification, at early stages of glomerular disorders, of patients with poor renal prognosis suggests more intensive clinical follow-up of such patients and provides insights for the design of future therapeutic studies on e.g. urinary IgM excretion. An interesting issue would be to evaluate the effects of ACE-inhibitor treatment and/or immunosuppressive drug treatment of this group of patients. Further studies are needed to elucidate the association between urine IgM excretion and the urinary excretion of other biologically active proteins, such as TGF- β or complement factors, to further evaluate the mechanisms of glomerular disease and the progression to renal failure.

Finally, the permeability properties of the glomerular filter in healthy and diseased conditions might be further studied using proteins of different size and charge, e.g. albumin, IgG and IgM, in order to add further understanding to the function of the glomerular sieving process and the mechanisms of glomerular injury. Although, at present, this can only be made in animal models of glomerular diseases, this may help in improving the medical care of individuals with glomerular diseases.

ACKNOWLEDGEMENTS

These studies were carried out in a close connection to my clinical work at Department of Nephrology, Lund University Hospital. I am deeply thankful to Prof. *Bengt Rippe* for introducing me to the clinical work in nephrology and for his guidance and encouragement during years of nephrology specialist training, and for his continuous generous support during research period, for providing me a pleasant atmosphere and for his scientific spirit, constructive criticism and revisions of manuscripts.

I wish also to express my sincere gratitude to all who helped and supported me during this work and in particular to:

Associate Professor *Jan Tencer*, my principal tutor, who have done a tremendous work in improving and organising the fantastic unique glomerulonephritis investigating program (Örestad register). I am grateful that I have shared your vast knowledge in this research field, for enthusiasm and excellent guidance, rapid reply to all my wondering even during holidays and evening times. Your help was irreplaceable in the preparation of the original manuscripts.

Associate professor *Ole Torffvit*, Associate professor *Olav Tenstad* and Professor *Anders Grubb*, my co-authors, for stimulatory discussions, shearing idea, and fantastic laboratory co-operations. Putting your laboratory facilities under my demand were of a great help for production of this work.

Juan Tapia, *Ingela Skogvall*, co-authors, for help with taking care of kidney biopsies.

Åsa Pettersson, *Nermina Jagansac*, *Veronica Lindström*, for great help with analysis of urinary proteins.

Anna Rippe for providing her great professional experience in animal experiment and specially tissue renal uptake technique.

Kerstin Wihlborg for being an excellent efficient secretary and always willing to help.

My colleagues, *nephrologists* at nephrology clinics in south Sweden for the great help provided with the clinical studies and data collection. Without your excellent cooperation never will be such a fantastic glomerulonephritis register.

Last and not least, you people working hardly behind lights, making everything arranged and fixed at exact time, the *secretaries* and *nurses* at nephrology department especially at out-patient clinic and medical ward 9, for helping with patients' medical records, arrangements of patients' appointments, and collection of urine and blood samples. Without your fantastic help this work could not be done.

The *general peoples committee of Libya* and *Libyan embassy* at Stockholm for financial support of my clinical training at Sweden.

My *parents, brothers and sister*, for their never ending love, encouragement, continuous support, care and concern, and always being there for me.

Finally my profound thanks go to *Manal, Nagimaldin, Mohamed and Sana* (my wife and children) for loving support, patience, understanding, and making everything worthwhile.

This work was supported by grants from Swedish Medical Research Council, Skåne Medical Research Council, Medical Faculty of Lund University, and grants from Riksförbundet för Njursjuka and Swedish Society for Medical Research.

REFERENCES

1. Weam JT, Richards AN. Observations on the composition of glomerular urine, with particular reference to the problem of reabsorption in the renal tubule. *Am J Physiol* 1924;171:209-227.
2. Bowman W. On the structure and use of the Malpighian bodies of the kidney, with observations on the circulation through that gland. *Philos trans Soc* 1842;132:57-80.
3. Ludwig C. *Beitrage zur Lehre vom Mechanismus der Harnsecretion*. Marburg: Elwert 1843.
4. Starling E. On the absorption of fluids from the connective tissue spaces. *J Physiol (London)* 1896;19:312-316.
5. Deen WM, Lazzara MJ, Myers BD. Structural determinants of glomerular permeability. *Am J Physiol Renal Physiol* 2001;281(4):F579-96.
6. Anderson S, Tank JE, Brenner BM. Renal and Systemic Manifestations of Glomerular Disease. In: Brenner BM, Levine SA, editors. *Brenner & Rector's The Kidney*. Sixth ed. Philadelphia: W. B. Saunders Company; 2000. p. 1871-1900.
7. Kriz W, Elgar M. Renal Anatomy. In: Johnson RJ, Feehally J, editors. *Comprehensive Clinical Nephrology*. First ed. London: Harcourt Publisher Limited; 2000. p. 1.1-1.10.
8. Hoy WE, Douglas-Denton RN, Hughson MD, Cass A, Johnson K, Bertram JF. A stereological study of glomerular number and volume: Preliminary findings in a multiracial study of kidneys at autopsy. *Kidney Int* 2003;63(s83):s31-s37.
9. Rostgaard J, Qvortrup K. Sieve plugs in fenestrae of glomerular capillaries - site of the filtration barrier? *Cells Tissues Organs* 2002;170(2-3):132-8.
10. Bohle A, Aeikens B, Eenboom A, Fronholt L, Plate WR, Xiao JC, et al. Human glomerular structure under normal conditions and in isolated glomerular disease. *Kidney Int Suppl* 1998;67:S186-8.
11. Toto RD. Conventional measurement of renal function utilizing serum creatinine, creatinine clearance, inulin and para-aminohippuric acid clearance. *Curr Opin Nephrol Hypertens* 1995;4(6):505-9; discussion 503-4.
12. Lund U, Rippe A, Venturoli D, Tenstad O, Grubb A, Rippe B. Glomerular filtration rate dependence of sieving of albumin and some neutral proteins in rat kidneys. *Am J Physiol Renal Physiol* 2003;284(6):F1226-34.
13. Rennke HG, Patel Y, Venkatachalam MA. Glomerular filtration of proteins: clearance of anionic, neutral, and cationic horseradish peroxidase in the rat. *Kidney Int* 1978;13(4):278-88.
14. Oliver JD, 3rd, Anderson S, Troy JL, Brenner BM, Deen WH. Determination of glomerular size-selectivity in the normal rat with Ficoll. *J Am Soc Nephrol* 1992;3(2):214-28.

15. Galaske RG, Baldamus CA, Stolte H. Plasma protein handling in the rat kidney: micropuncture experiments in the acute heterologous phase of anti-GBM-nephritis. *Pflugers Arch* 1978;375(3):269-77.
16. Oken DE, Kirschbaum BB, Landwehr DM. Micropuncture studies of the mechanisms of normal and pathologic albuminuria. *Contrib Nephrol* 1981;24:1-7.
17. Bertolatus JA, Hunsicker LG. Glomerular sieving of anionic and neutral bovine albumins in proteinuric rats. *Kidney Int* 1985;28(3):467-76.
18. Tencer J, Frick IM, Oquist BW, Alm P, Rippe B. Size-selectivity of the glomerular barrier to high molecular weight proteins: upper size limitations of shunt pathways. *Kidney Int* 1998;53(3):709-15.
19. Tojo A, Endou H. Intrarenal handling of proteins in rats using fractional micropuncture technique. *Am J Physiol* 1992;263(4 Pt 2):F601-6.
20. Maack T, Johnson V, Kau ST, Figueiredo J, Sigulem D. Renal filtration, transport, and metabolism of low-molecular-weight proteins: a review. *Kidney Int* 1979;16(3):251-70.
21. Norden AG, Scheinman SJ, Deschodt-Lanckman MM, Lapsley M, Nortier JL, Thakker RV, et al. Tubular proteinuria defined by a study of Dent's (CLCN5 mutation) and other tubular diseases. *Kidney Int* 2000;57(1):240-9.
22. Landwehr DM, Carvalho JS, Oken DE. Micropuncture studies of the filtration and absorption of albumin by nephrotic rats. *Kidney Int* 1977;11(1):9-17.
23. Russo LM, Bakris GL, Comper WD. Renal handling of albumin: a critical review of basic concepts and perspective. *Am J Kidney Dis* 2002;39(5):899-919.
24. Osicka TM, Panagiotopoulos S, Jerums G, Comper WD. Fractional clearance of albumin is influenced by its degradation during renal passage. *Clin Sci (Lond)* 1997;93(6):557-64.
25. Ohlson M, Sorensson J, Haraldsson B. Glomerular size and charge selectivity in the rat as revealed by FITC-ficoll and albumin. *Am J Physiol Renal Physiol* 2000;279(1):F84-91.
26. Deen WM, Bridges CR, Brenner BM, Myers BD. Heteroporous model of glomerular size selectivity: application to normal and nephrotic humans. *Am J Physiol* 1985;249(3 Pt 2):F374-89.
27. Rippe B, Haraldsson B. Transport of macromolecules across microvascular walls: the two-pore theory. *Physiol Rev* 1994;74(1):163-219.
28. Pappenheimer J, Renkin E, Borrero L. Filtration, diffusion and molecular sieving through peripheral capillary membranes. A contribution to the pore theory of capillary permeability. *Am J Physiol* 1951;167:13-46.

29. Tencer J, Torffvit O, Thysell H, Rippe B, Grubb A. Proteinuria selectivity index based upon alpha 2-macroglobulin or IgM is superior to the IgG based index in differentiating glomerular diseases. Technical note. *Kidney Int* 1998;54(6):2098-105.
30. Torffvit O, Rippe B. Size and charge selectivity of the glomerular filter in patients with insulin-dependent diabetes mellitus: urinary immunoglobulins and glycosaminoglycans. *Nephron* 1999;83(4):301-7.
31. Tencer J, Torffvit O, Thysell H, Rippe B, Grubb A. Urine IgG2/IgG4-ratio indicates the significance of the charge selective properties of the glomerular capillary wall for the macromolecular transport in glomerular diseases. *Nephrol Dial Transplant* 1999;14(6):1425-9.
32. Ala-Houhala I, Koskinen M, Ahola T, Harmoinen A, Kouri T, Laurila K, et al. Increased glomerular permeability in patients with nephropathia epidemica caused by Puumala hantavirus. *Nephrol Dial Transplant* 2002;17(2):246-52.
33. Schurek HJ, Neumann KH, Flohr H, Zeh M, Stolte H. The physiological and pathophysiological basis of glomerular permeability for plasma proteins and erythrocytes. *Eur J Clin Chem Clin Biochem* 1992;30(10):627-33.
34. Hjalmarsson C, Johansson BR, Haraldsson B. Electron microscopic evaluation of the endothelial surface layer of glomerular capillaries. *Microvasc Res* 2004;67(1):9-17.
35. Ciarimboli G, Hjalmarsson C, Bokenkamp A, Schurek HJ, Haraldsson B. Dynamic alterations of glomerular charge density in fixed rat kidneys suggest involvement of endothelial cell coat. *Am J Physiol Renal Physiol* 2003;285(4):F722-30.
36. Miner JH. Renal basement membrane components. *Kidney Int* 1999;56(6):2016-24.
37. Groffen AJ, Veerkamp JH, Monnens LA, van den Heuvel LP. Recent insights into the structure and functions of heparan sulfate proteoglycans in the human glomerular basement membrane. *Nephrol Dial Transplant* 1999;14(9):2119-29.
38. Rossi M, Morita H, Sormunen R, Airene S, Kreivi M, Wang L, et al. Heparan sulfate chains of perlecan are indispensable in the lens capsule but not in the kidney. *Embo J* 2003;22(2):236-45.
39. Ohno S, Hora K, Furukawa T, Oguchi H. Ultrastructural study of the glomerular slit diaphragm in fresh unfixed kidneys by a quick-freezing method. *Virchows Arch B Cell Pathol Incl Mol Pathol* 1992;61(5):351-8.
40. Pavenstadt H, Kriz W, Kretzler M. Cell biology of the glomerular podocyte. *Physiol Rev* 2003;83(1):253-307.
41. Wolf G, Stahl RA. CD2-associated protein and glomerular disease. *Lancet* 2003;362(9397):1746-8.

42. Boute N, Gribouval O, Roselli S, Benessy F, Lee H, Fuchshuber A, et al. NPHS2, encoding the glomerular protein podocin, is mutated in autosomal recessive steroid-resistant nephrotic syndrome. *Nat Genet* 2000;24(4):349-54.
43. Kaplan JM, Kim SH, North KN, Rennke H, Correia LA, Tong HQ, et al. Mutations in ACTN4, encoding alpha-actinin-4, cause familial focal segmental glomerulosclerosis. *Nat Genet* 2000;24(3):251-6.
44. Patrakka J, Kestila M, Wartiovaara J, Ruotsalainen V, Tissari P, Lenkkeri U, et al. Congenital nephrotic syndrome (NPHS1): features resulting from different mutations in Finnish patients. *Kidney Int* 2000;58(3):972-80.
45. Gubler MC. Podocyte differentiation and hereditary proteinuria/nephrotic syndromes. *J Am Soc Nephrol* 2003;14(Suppl 1):S22-6.
46. Tryggvason K, Wartiovaara J. Molecular basis of glomerular permselectivity. *Curr Opin Nephrol Hypertens* 2001;10(4):543-9.
47. Ryan GB, Karnovsky MJ. Distribution of endogenous albumin in the rat glomerulus: role of hemodynamic factors in glomerular barrier function. *Kidney Int* 1976;9(1):36-45.
48. Vink H, Duling BR. Capillary endothelial surface layer selectively reduces plasma solute distribution volume. *Am J Physiol Heart Circ Physiol* 2000;278(1):H285-9.
49. Rippe B. What is the role of albumin in proteinuric glomerulopathies? *Nephrol Dial Transplant* 2004;19(1):1-5.
50. Smithies O. Why the kidney glomerulus does not clog: a gel permeation/diffusion hypothesis of renal function. *Proc Natl Acad Sci U S A* 2003;100(7):4108-13.
51. Fujihara CK, Arcos-Fajardo M, Brandao De Almeida Prado E, Jose Brandao De Almeida Prado M, Sesso A, Zatz R. Enhanced glomerular permeability to macromolecules in the Nagase albuminemic rat. *Am J Physiol Renal Physiol* 2002;282(1):F45-50.
52. Curry FE, Rutledge JC, Lenz JF. Modulation of microvessel wall charge by plasma glycoprotein orosomucoid. *Am J Physiol* 1989;257(5 Pt 2):H1354-9.
53. Haraldsson BS, Johnsson EK, Rippe B. Glomerular permselectivity is dependent on adequate serum concentrations of orosomucoid. *Kidney Int* 1992;41(2):310-6.
54. Lemley KV, Lafayette RA, Safai M, Derby G, Blouch K, Squarer A, et al. Podocytopenia and disease severity in IgA nephropathy. *Kidney Int* 2002;61(4):1475-85.

- 55.Kriz W, Lemley KV. The role of the podocyte in glomerulosclerosis. *Curr Opin Nephrol Hypertens* 1999;8(4):489-97.
- 56.Lemley KV. A basis for accelerated progression of diabetic nephropathy in Pima Indians. *Kidney Int* 2003;63(s83):s38-s42.
- 57.Nakamura T, Ushiyama C, Suzuki S, Hara M, Shimada N, Ebihara I, et al. The urinary podocyte as a marker for the differential diagnosis of idiopathic focal glomerulosclerosis and minimal-change nephrotic syndrome. *Am J Nephrol* 2000;20(3):175-9.
- 58.Christensen EI, Birn H. Megalin and cubilin: synergistic endocytic receptors in renal proximal tubule. *Am J Physiol Renal Physiol* 2001;280(4):F562-73.
- 59.Leheste JR, Rolinski B, Vorum H, Hilpert J, Nykjaer A, Jacobsen C, et al. Megalin knockout mice as an animal model of low molecular weight proteinuria. *Am J Pathol* 1999;155(4):1361-70.
- 60.Fairley KF. Investigation of renal disease, Urinalysis. In: Johnson RJ, Feehally J, editors. *Comprehensive Clinical Nephrology*. First ed. London: Harcourt Publisher Limited; 2000. p. 4.1-4.10.
- 61.Tencer J, Thysell H, Grubb A. Analysis of proteinuria: reference limits for urine excretion of albumin, protein HC, immunoglobulin G, kappa- and lambda-immunoreactivity, orosomuroid and alpha 1-antitrypsin. *Scand J Clin Lab Invest* 1996;56(8):691-700.
- 62.Hjorth L, Helin I, Grubb A. Age-related reference limits for urine levels of albumin, orosomuroid, immunoglobulin G and protein HC in children. *Scand J Clin Lab Invest* 2000;60(1):65-73.
- 63.D'Amico G, Bazzi C. Pathophysiology of proteinuria. *Kidney Int* 2003;63(3):809-25.
- 64.Abbate M, Zoja C, Corna D, Capitanio M, Bertani T, Remuzzi G. In progressive nephropathies, overload of tubular cells with filtered proteins translates glomerular permeability dysfunction into cellular signals of interstitial inflammation. *J Am Soc Nephrol* 1998;9(7):1213-24.
- 65.Eddy AA. Proteinuria and interstitial injury. *Nephrol Dial Transplant* 2004;19(2):277-81.
- 66.Iseki K, Ikemiya Y, Iseki C, Takishita S. Proteinuria and the risk of developing end-stage renal disease. *Kidney Int* 2003;63(4):1468-74.
- 67.Keane WF, Brenner BM, De Zeeuw D, Grunfeld JP, McGill J, Mitch WE, et al. The risk of developing end-stage renal disease in patients with type 2 diabetes and nephropathy: The RENAAL Study. *Kidney Int* 2003;63(4):1499-1507.

68. Ruggenenti P, Gaspari F, Perna A, Remuzzi G. Cross sectional longitudinal study of spot morning urine protein:creatinine ratio, 24 hour urine protein excretion rate, glomerular filtration rate, and end stage renal failure in chronic renal disease in patients without diabetes. *Bmj* 1998;316(7130):504-9.
69. Hsu SI, Couser WG. Chronic progression of tubulointerstitial damage in proteinuric renal disease is mediated by complement activation: a therapeutic role for complement inhibitors? *J Am Soc Nephrol* 2003;14(7 Suppl 2):S186-91.
70. Wang SN, LaPage J, Hirschberg R. Role of glomerular ultrafiltration of growth factors in progressive interstitial fibrosis in diabetic nephropathy. *Kidney Int* 2000;57(3):1002-14.
71. Burton CJ, Combe C, Walls J, Harris KP. Secretion of chemokines and cytokines by human tubular epithelial cells in response to proteins. *Nephrol Dial Transplant* 1999;14(11):2628-33.
72. Kamijo A, Kimura K, Sugaya T, Yamanouchi M, Hase H, Kaneko T, et al. Urinary free fatty acids bound to albumin aggravate tubulointerstitial damage. *Kidney Int* 2002;62(5):1628-37.
73. Sheerin NS, Sacks SH. Leaked protein and interstitial damage in the kidney: is complement the missing link? *Clin Exp Immunol* 2002;130(1):1-3.
74. Wang SN, Lapage J, Hirschberg R. Glomerular ultrafiltration and apical tubular action of IGF-I, TGF-beta, and HGF in nephrotic syndrome. *Kidney Int* 1999;56(4):1247-51.
75. Zoja C, Donadelli R, Colleoni S, Figliuzzi M, Bonazzola S, Morigi M, et al. Protein overload stimulates RANTES production by proximal tubular cells depending on NF-kappa B activation. *Kidney Int* 1998;53(6):1608-15.
76. Bazzi C, Petrini C, Rizza V, Arrigo G, Beltrame A, Pisano L, et al. Urinary excretion of IgG and alpha(1)-microglobulin predicts clinical course better than extent of proteinuria in membranous nephropathy. *Am J Kidney Dis* 2001;38(2):240-8.
77. Bazzi C, Petrini C, Rizza V, Arrigo G, D'Amico G. A modern approach to selectivity of proteinuria and tubulointerstitial damage in nephrotic syndrome. *Kidney Int* 2000;58(4):1732-41.
78. Branten AJ, van den Born J, Jansen JL, Assmann KJ, Wetzels JF. Familial nephropathy differing from minimal change nephropathy and focal glomerulosclerosis. *Kidney Int* 2001;59(2):693-701.
79. Woo KT, Lau YK, Wong KS, Chiang GS. ACEI/ATRA therapy decreases proteinuria by improving glomerular permselectivity in IgA nephritis. *Kidney Int* 2000;58(6):2485-91.
80. Reichert LJ, Koene RA, Wetzels JF. Urinary IgG excretion as a prognostic factor in idiopathic membranous nephropathy. *Clin Nephrol* 1997;48(2):79-84.

81. Laurent J, Philippon C, Lagrue G, Laurent G, Weil B, Rostoker G. Proteinuria selectivity index--prognostic value in lipid nephrosis and related diseases. *Nephron* 1993;65(2):185-9.
82. Fox JG, Quin JD, O'Reilly DS, Boulton-Jones JM. Glomerular charge selectivity in primary glomerulopathies. *Clin Sci (Lond)* 1994;87(4):421-5.
83. Abitbol C, Zilleruelo G, Freundlich M, Strauss J. Quantitation of proteinuria with urinary protein/creatinine ratios and random testing with dipsticks in nephrotic children. *J Pediatr* 1990;116(2):243-7.
84. Steinhauslin F, Wauters JP. Quantitation of proteinuria in kidney transplant patients: accuracy of the urinary protein/creatinine ratio. *Clin Nephrol* 1995;43(2):110-5.
85. Newman DJ, Pugia MJ, Lott JA, Wallace JF, Hiar AM. Urinary protein and albumin excretion corrected by creatinine and specific gravity. *Clin Chim Acta* 2000;294(1-2):139-55.
86. Claudi T, Cooper JG. Comparison of urinary albumin excretion rate in overnight urine and albumin creatinine ratio in spot urine in diabetic patients in general practice. *Scand J Prim Health Care* 2001;19(4):247-8.
87. Assadi FK. Quantitation of microalbuminuria using random urine samples. *Pediatr Nephrol* 2002;17(2):107-10.
88. Ruggenenti P, Perna A, Mosconi L, Pisoni R, Remuzzi G. Urinary protein excretion rate is the best independent predictor of ESRF in non-diabetic proteinuric chronic nephropathies. "Gruppo Italiano di Studi Epidemiologici in Nefrologia" (GISEN). *Kidney Int* 1998;53(5):1209-16.
89. Torng S, Rigatto C, Rush DN, Nickerson P, Jeffery JR. The urine protein to creatinine ratio (P/C) as a predictor of 24-hour urine protein excretion in renal transplant patients. *Transplantation* 2001;72(8):1453-6.
90. Neithardt AB, Dooley SL, Borensztajn J. Prediction of 24-hour protein excretion in pregnancy with a single voided urine protein-to-creatinine ratio. *Am J Obstet Gynecol* 2002;186(5):883-6.
91. Hofmann W, Guder WG. A diagnostic programme for quantitative analysis of proteinuria. *J Clin Chem Clin Biochem* 1989;27(9):589-600.
92. Chitalia VC, Kothari J, Wells EJ, Livesey JH, Robson RA, Searle M, et al. Cost-benefit analysis and prediction of 24-hour proteinuria from the spot urine protein-creatinine ratio. *Clin Nephrol* 2001;55(6):436-47.
93. Quadri KH, Bernardini J, Greenberg A, Laifer S, Syed A, Holley JL. Assessment of renal function during pregnancy using a random urine protein to creatinine ratio and Cockcroft-Gault formula. *Am J Kidney Dis* 1994;24(3):416-20.
94. Kouri T, Harmoinen A, Laurila K, Ala-Houhala I, Koivula T, Pasternack A. Reference intervals for the markers of proteinuria with a standardised bed-rest collection of urine. *Clin Chem Lab Med* 2001;39(5):418-25.

95. Joachim GR, Cameron JS, Schwartz M, Becker EL. Selectivity of protein excretion in patients with the nephrotic syndrome. *J Clin Invest* 1964;43:2332-2346.
96. Stolz A. Liver Physiology and Metabolic Function. In: Feldman M, Friedman LS, Sleisenger MH, editors. *Sleisenger & Fordtran's Gastrointestinal and Liver Disease Pathophysiology/Diagnosis/Management*. 7th ed. Philadelphia: SAUNDERS An Imprint of Elsevier Science; 2002. p. 1202-1227.
97. Pratt DS, Kaplan MM. Tests of the liver's biosynthetic capacity. In. 11.3 ed: *Up To Date*; 2003.
98. Ballantyne J, Mandle R, Bing DH. Immunoglobulins: Polyclonal and Monoclonal Antibodies. In: Hoffman R, Benz EJ, Shattil SJ, Furie B, Cohen HJ, Silberstein LE, et al., editors. *HEMATOLOGY: Basic Principles and Practice*. 3rd ed. New York: Churchill Livingstone; 2000. p. 640-650.
99. Bonilla FA. Structure of immunoglobulins. In. 11.3 ed: *UpToDate*; 2003.
100. Grubb AO, Lopez C, Tejler L, Mendez E. Isolation of human complex-forming glycoprotein, heterogeneous in charge (protein HC), and its IgA complex from plasma. Physicochemical and immunochemical properties, normal plasma concentration. *J Biol Chem* 1983;258(23):14698-707.
101. Weber MH, Verwiebe R. Alpha 1-microglobulin (protein HC): features of a promising indicator of proximal tubular dysfunction. *Eur J Clin Chem Clin Biochem* 1992;30(10):683-91.
102. Grubb A. Diagnostic value of analysis of cystatin C and protein HC in biological fluids. *Clin Nephrol* 1992;38 Suppl 1:S20-7.
103. Mantur M, Kemoni H, Dabrowska M, Dabrowska J, Sobolewski S, Prokopowicz J. alpha1-microglobulin as a marker of proximal tubular damage in urinary tract infection in children. *Clin Nephrol* 2000;53(4):283-7.
104. Kido T, Honda R, Yamada Y, Tsuritani I, Ishizaki M, Nogawa K. alpha 1-Microglobulin determination in urine for the early detection of renal tubular dysfunctions caused by exposure to cadmium. *Toxicol Lett* 1985;24(2-3):195-201.
105. Teppo AM, Honkanen E, Ahonen J, Gronhagen-Riska C. Changes of urinary alpha1-microglobulin in the assessment of prognosis in renal transplant recipients. *Transplantation* 2000;70(8):1154-9.
106. Tsukahara H, Hiraoka M, Kuriyama M, Saito M, Morikawa K, Kuroda M, et al. Urinary alpha 1-microglobulin as an index of proximal tubular function in early infancy. *Pediatr Nephrol* 1993;7(2):199-201.
107. Maisonneuve P, Agodoa L, Gellert R, Stewart JH, Buccianti G, Lowenfels AB, et al. Distribution of primary renal diseases leading to end-stage renal failure in the United States, Europe, and Australia/New Zealand: results from an international comparative study. *Am J Kidney Dis* 2000;35(1):157-65.

- 108.Excerpts from United States Renal Data System 1999 Annual Data Report. *Am J Kidney Dis* 1999;34(2 Suppl 1):S1-176.
- 109.Johnson RJ, Feehally J. Introduction to Glomerular Disease: Pathogenesis and Classification. In: Johnson RJ, Feehally J, editors. *Comprehensive Clinical Nephrology*. First ed. London: Harcourt Publisher Limited; 2000. p. 20.1-20.8.
- 110.Hricik DE, Chung-Park M, Sedor JR. Glomerulonephritis. *N Engl J Med* 1998;339(13):888-99.
- 111.Schena FP, Gesualdo L, Grandaliano G, Montinaro V. Progression of renal damage in human glomerulonephritides: is there sleight of hand in winning the game? *Kidney Int* 1997;52(6):1439-57.
- 112.Salant DJ, Adler S, Darby C, Capparell NJ, Groggel GC, Feintzeig ID, et al. Influence of antigen distribution on the mediation of immunological glomerular injury. *Kidney Int* 1985;27(6):938-50.
- 113.Falk RJ, Jennette JC, Nachman PH. Primary Glomerular Disease. In: Brenner BM, Levine SA, editors. *Brenner & Rector's The Kidney*. Sixth ed. Philadelphia: W.B. SAUNDERS COMPANY; 2000. p. 1263-1349.
- 114.Ghiggeri GM, Artero M, Carraro M, Perfumo F. Permeability plasma factors in nephrotic syndrome: more than one factor, more than one inhibitor. *Nephrol Dial Transplant* 2001;16(5):882-5.
- 115.D'Amico G. Natural history of idiopathic IgA nephropathy: role of clinical and histological prognostic factors. *Am J Kidney Dis* 2000;36(2):227-37.
- 116.Chida Y, Tomura S, Takeuchi J. Renal survival rate of IgA nephropathy. *Nephron* 1985;40(2):189-94.
- 117.Haas M, Meehan SM, Karrison TG, Spargo BH. Changing etiologies of unexplained adult nephrotic syndrome: a comparison of renal biopsy findings from 1976-1979 and 1995-1997. *Am J Kidney Dis* 1997;30(5):621-31.
- 118.Couser WG. Membranous Nephropathy. In: Johnson RJ, Feehally J, editors. *Comprehensive Clinical Nephrology*. First ed. London: Harcourt Publisher Limited; 2000. p. 24.1-24.12.
- 119.Cattran DC. Idiopathic membranous glomerulonephritis. *Kidney Int* 2001;59(5):1983-94.
- 120.Schieppati A, Mosconi L, Perna A, Mecca G, Bertani T, Garattini S, et al. Prognosis of untreated patients with idiopathic membranous nephropathy. *N Engl J Med* 1993;329(2):85-9.
- 121.Mason PD. Minimal Change Disease and Primary Focal Segmental Glomerulosclerosis. In: Johnson RJ, Feehally J, editors. *Comprehensive Clinical Nephrology*. First ed. London: Harcourt Publisher Limited; 2000. p. 22.1-22.10.
- 122.Grimbert P, Audard V, Remy P, Lang P, Sahali D. Recent approaches to the pathogenesis of minimal-change nephrotic syndrome. *Nephrol Dial Transplant* 2003;18(2):245-8.

123. Zeis PM, Kavazarakis E, Nakopoulou L, Moustaki M, Messaritaki A, Zeis MP, et al. Glomerulopathy with mesangial IgM deposits: long-term follow up of 64 children. *Pediatr Int* 2001;43(3):287-92.
124. Braden GL, Mulhern JG, O'Shea MH, Nash SV, Ucci AA, Jr., Germain MJ. Changing incidence of glomerular diseases in adults. *Am J Kidney Dis* 2000;35(5):878-83.
125. Cattran DC, Rao P. Long-term outcome in children and adults with classic focal segmental glomerulosclerosis. *Am J Kidney Dis* 1998;32(1):72-9.
126. Rao TKS, Chander PN. Secondary Focal Segmental Glomerulosclerosis. In: Johnson RJ, Feehally J, editors. *Comprehensive Clinical Nephrology*. First ed. London: Harcourt Publisher Limited; 2000. p. 23.1-23.10.
127. Savin VJ, Sharma R, Sharma M, McCarthy ET, Swan SK, Ellis E, et al. Circulating factor associated with increased glomerular permeability to albumin in recurrent focal segmental glomerulosclerosis. *N Engl J Med* 1996;334(14):878-83.
128. Wolf G, Ritz E. Diabetic nephropathy in type 2 diabetes prevention and patient management. *J Am Soc Nephrol* 2003;14(5):1396-405.
129. Parving H-H, Osterby R, Ritz E. Diabetic Nephropathy. In: Brenner BM, Levine SA, editors. *Brenner & Rector's THE KIDNEY*. Sixth ed. Philadelphia: W.B. SAUNDERS COMPANY; 2000. p. 1731-1773.
130. Dalla Vestra M, Saller A, Bortoloso E, Mauer M, Fioretto P. Structural involvement in type 1 and type 2 diabetic nephropathy. *Diabetes Metab* 2000;26 Suppl 4:8-14.
131. Ziyadeh FN, Sharma K. Overview: combating diabetic nephropathy. *J Am Soc Nephrol* 2003;14(5):1355-7.
132. Ziyadeh FN, Hoffman BB, Han DC, Iglesias-De La Cruz MC, Hong SW, Isono M, et al. Long-term prevention of renal insufficiency, excess matrix gene expression, and glomerular mesangial matrix expansion by treatment with monoclonal antitransforming growth factor-beta antibody in db/db diabetic mice. *Proc Natl Acad Sci U S A* 2000;97(14):8015-20.
133. Arun CS, Stoddart J, Mackin P, MacLeod JM, New JP, Marshall SM. Significance of microalbuminuria in long-duration type 1 diabetes. *Diabetes Care* 2003;26(7):2144-9.
134. Raptis AE, Viberti G. Pathogenesis of diabetic nephropathy. *Exp Clin Endocrinol Diabetes* 2001;109 Suppl 2:S424-37.
135. Nyberg G, Blohme G, Norden G. Constant glomerular filtration rate in diabetic nephropathy. Correlation to blood pressure and blood glucose control. *Acta Med Scand* 1986;219(1):67-72.
136. Caramori ML, Fioretto P, Mauer M. The need for early predictors of diabetic nephropathy risk: is albumin excretion rate sufficient? *Diabetes* 2000;49(9):1399-408.

137. Forsblom CM, Groop PH, Ekstrand A, Groop LC. Predictive value of microalbuminuria in patients with insulin-dependent diabetes of long duration. *Bmj* 1992;305(6861):1051-3.
138. Mathiesen ER, Romm B, Storm B, Foght H, Deckert T. The natural course of microalbuminuria in insulin-dependent diabetes: a 10-year prospective study. *Diabet Med* 1995;12(6):482-7.
139. Hovind P, Rossing P, Tarnow L, Smidt UM, Parving HH. Progression of diabetic nephropathy. *Kidney Int* 2001;59(2):702-9.
140. Sustained effect of intensive treatment of type 1 diabetes mellitus on development and progression of diabetic nephropathy: the Epidemiology of Diabetes Interventions and Complications (EDIC) study. *Jama* 2003;290(16):2159-67.
141. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. *Lancet* 1998;352(9131):837-53.
142. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. The Diabetes Control and Complications Trial Research Group. *N Engl J Med* 1993;329(14):977-86.
143. Nielsen FS, Rossing P, Gall MA, Smidt UM, Chen JW, Sato A, et al. Lisinopril improves endothelial dysfunction in hypertensive NIDDM subjects with diabetic nephropathy. *Scand J Clin Lab Invest* 1997;57(5):427-34.
144. Brenner BM, Cooper ME, de Zeeuw D, Keane WF, Mitch WE, Parving HH, et al. Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. *N Engl J Med* 2001;345(12):861-9.
145. Lewis EJ, Hunsicker LG, Bain RP, Rohde RD. The effect of angiotensin-converting-enzyme inhibition on diabetic nephropathy. The Collaborative Study Group. *N Engl J Med* 1993;329(20):1456-62.
146. Lewis EJ, Hunsicker LG, Clarke WR, Berl T, Pohl MA, Lewis JB, et al. Renoprotective effect of the angiotensin-receptor antagonist irbesartan in patients with nephropathy due to type 2 diabetes. *N Engl J Med* 2001;345(12):851-60.
147. Johnson RJ, Kurokawa K, Bakris GL. Pathogenesis and Clinical Course of Essential Hypertension. In: Johnson RJ, Feehally J, editors. *Comprehensive Clinical Nephrology*. First ed. London: Harcourt Publisher Limited; 2000. p. 38.1-38.12.

148. Shulman NB, Ford CE, Hall WD, Blaufox MD, Simon D, Langford HG, et al. Prognostic value of serum creatinine and effect of treatment of hypertension on renal function. Results from the hypertension detection and follow-up program. The Hypertension Detection and Follow-up Program Cooperative Group. *Hypertension* 1989;13(5 Suppl):I80-93.
149. Young JH, Klag MJ, Muntner P, Whyte JL, Pahor M, Coresh J. Blood pressure and decline in kidney function: findings from the Systolic Hypertension in the Elderly Program (SHEP). *J Am Soc Nephrol* 2002;13(11):2776-82.
150. Perera G. Hypertensive vascular disease: description and natural history. *J Chronic Dis* 1955;1:33-42.
151. Klag MJ, Whelton PK, Randall BL, Neaton JD, Brancati FL, Ford CE, et al. Blood pressure and end-stage renal disease in men. *N Engl J Med* 1996;334(1):13-8.
152. Mountokalakis TD. The renal consequences of arterial hypertension. *Kidney Int* 1997;51(5):1639-53.
153. Luke RG. Hypertensive nephrosclerosis: pathogenesis and prevalence. Essential hypertension is an important cause of end-stage renal disease. *Nephrol Dial Transplant* 1999;14(10):2271-8.
154. Sanchez-Lozada LG, Tapia E, Johnson RJ, Rodriguez-Iturbe B, Herrera-Acosta J. Glomerular hemodynamic changes associated with arteriolar lesions and tubulointerstitial inflammation. *Kidney Int Suppl* 2003(86):S9-14.
155. Toto RD, Mitchell HC, Smith RD, Lee HC, McIntire D, Pettinger WA. "Strict" blood pressure control and progression of renal disease in hypertensive nephrosclerosis. *Kidney Int* 1995;48(3):851-9.
156. Hsu CY, Chertow GM, Curhan GC. Methodological issues in studying the epidemiology of mild to moderate chronic renal insufficiency. *Kidney Int* 2002;61(5):1567-76.
157. Coresh J, Toto RD, Kirk KA, Whelton PK, Massry S, Jones C, et al. Creatinine clearance as a measure of GFR in screenees for the African-American Study of Kidney Disease and Hypertension pilot study. *Am J Kidney Dis* 1998;32(1):32-42.
158. Lemann J, Bidani AK, Bain RP, Lewis EJ, Rohde RD. Use of the serum creatinine to estimate glomerular filtration rate in health and early diabetic nephropathy. Collaborative Study Group of Angiotensin Converting Enzyme Inhibition in Diabetic Nephropathy. *Am J Kidney Dis* 1990;16(3):236-43.
159. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron* 1976;16(1):31-41.
160. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med* 1999;130(6):461-70.

161. Vervoort G, Willems HL, Wetzels JF. Assessment of glomerular filtration rate in healthy subjects and normoalbuminuric diabetic patients: validity of a new (MDRD) prediction equation. *Nephrol Dial Transplant* 2002;17(11):1909-13.
162. Cameron J, Whiter R. Selectivity of proteinuria in children with nephrotic syndrome. *Lancet* 1965;1:463-465.
163. Remuzzi G, Bertani T. Pathophysiology of progressive nephropathies. *N Engl J Med* 1998;339(20):1448-56.
164. Nangaku M, Pippin J, Couser WG. Complement membrane attack complex (C5b-9) mediates interstitial disease in experimental nephrotic syndrome. *J Am Soc Nephrol* 1999;10(11):2323-31.
165. Guasch A, Deen WM, Myers BD. Charge selectivity of the glomerular filtration barrier in healthy and nephrotic humans. *J Clin Invest* 1993;92(5):2274-82.
166. Ota Z, Shikata K, Ota K. Nephrotic tunnels in glomerular basement membrane as revealed by a new electron microscopic method. *J Am Soc Nephrol* 1994;4(12):1965-73.
167. Jerums G, Allen TJ, Cooper ME. Triphasic changes in selectivity with increasing proteinuria in type 1 and type 2 diabetes. *Diabet Med* 1989;6(9):772-9.
168. Keller CK, Bergis KH, Fliser D, Ritz E. Renal findings in patients with short-term type 2 diabetes. *J Am Soc Nephrol* 1996;7(12):2627-35.
169. Nelson RG, Pettitt DJ, Baird HR, Charles MA, Liu QZ, Bennett PH, et al. Pre-diabetic blood pressure predicts urinary albumin excretion after the onset of type 2 (non-insulin-dependent) diabetes mellitus in Pima Indians. *Diabetologia* 1993;36(10):998-1001.
170. Norden AG, Sharratt P, Cutillas PR, O'Driscoll E, Gardner SC, Unwin RJ. Quantitative Amino Acid and Proteomic Analysis of Normal and Fanconi Urine: No Evidence for Significant Excretion of Plasma Protein Fragments in Normal Urine. *J Am Soc Nephrol* 2003;14(Abstracts issue):284A.