JUHANI MYLLYMÄKI

Clinical Features and Long Term Prognosis in Two Forms of Mesangial Glomerulonephritis, IgA Nephropathy and IgM Nephropathy

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UNIVERSITY OF TAMPERE

To my family

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ABSTRACT

The present study concerned two primary forms of glomerulonephritis, IgA nephropathy (IgAN) and IgM nephropathy (IgMN). The etiology and pathogenesis of both diseases are still unresolved. A traditional manifestation of IgAN is macroscopic hematuria associated with respiratory tract infection. However, in IgAN as also in IgMN the initial symptoms may be only microscopic hematuria or on the other hand nephrotic range proteinuria. Hypertension is particularly common at the time of diagnosis of IgAN and IgMN and becomes even more so during the course of disease. The incidence of terminal renal insufficiency in IgAN or IgMN cannot be strictly determined due to variation in patient populations in different studies. It would seem that in 10-20% of patients end-stage renal disease (ESRD) develops during 10-20 years of follow-up. There are many studies concerning risk factors for progression in IgAN. Certain clinical and histopathological parameters (e.g. high-grade proteinuria, elevated serum creatinine, hypertension, glomerulosclerosis or tubulointerstitial changes) are commonly recognized as independently inducing the development of renal insufficiency in IgAN. Recently new risk factors have been reported, for example hyperurichemia, obesity and hyperlipidemia, their role in the pathogenesis of renal damage remaining however obscure. Likewise the importance of active inflammation seen in renal biopsy remains undefined. Previous studies have shed little light on risk factors in IgMN.

Patients with marked renal insufficiency have a clearly elevated risk of vascular diseases (VDs). It appears that minor renal dysfunction also affects metabolic changes which may constitute a risk of develop VDs. However, it is not clear whether patients with some renal disease without renal insufficiency as evaluated by traditional methods carry a higher risk of VDs compared to the general population.

The present study was undertaken to further clarify the clinical picture and prognostic factors in IgAN and in IgMN. Immunological parameters were also under investigation, one focus being on role of newer parameters, especially hyperurichemia, in inducing renal histopathological changes in IgAN. The significance of intrarenal inflammatory cell infiltrations in the natural course of IgAN was established. An assessment of the prevalence and risk factors of VDs in IgAN was one of the main aims.

In the present work 223 IgAN patients and 110 IgMN patients were studied. Almost all IgAN patients were adults, whereas about one third of the original IgMN population was

children. A cohort comprising 203 persons ≥30 years of age collected from the same residential area served as a control group representing the general population in studying the prevalence of VDs in IgAN. The mean follow-up time was eight years in IgMN and ten in IgAN. Renal biopsies from were examined by light-microscopy and immunohistochemical methods. Clinical data, such as biochemical parameters, blood pressure measurements and data on VDs were collected. Data on the control group were collected from the Health 2000 Survey organized by the National Public Health Institute of Finland.

Of renal histopathological changes, serum uric acid correlated most strongly with tubulointerstitial changes and blood pressure with vascular changes in IgAN. Also a level of serum triglycerides appeared to be associated with renal morphological changes. Further studies are needed to evaluate the effect of uric acid-lowering therapy on the prognosis of IgAN. Tubulointerstitial inflammation, especially CD3+ T-lymphocyte infiltrations and IL-1β expression, constitutes a poor prognosis in IgAN. In future evaluation the level of intrarenal inflammation may direct prevailing therapy practices.

Vascular diseases are clearly more common in IgAN patients than in the general population. Male gender, hypertension, renal insufficiency, smoking and high serum triglyceride concentration were independently associated with some manifestation of VDs. Progressive renal disease is associated with the development of VDs in IgAN, renal vascular changes signify an elevated risk of VDs in this form of the disease.

The present findings showed renal insufficiency to have developed in about one third and hypertension in half of IgMN patients. Some IgMN patients develop FSGS, which is closely associated with ESRD. IgMN patients with only hematuria (HU) appeared to be mainly female and to have a lower progression rate. Elevation of serum C3 correlated with clinical and histopathological factors in IgMN indicating severe renal disease. Serum C3 was associated with progressive renal disease in IgMN patients.

In conclusion, all significant clinical risk factors for the progression of IgAN correlated with morphological changes in renal tissue. Of these, serum uric acid correlated most strongly with tubulointerstitial changes, which has been proved to be the most potent histopathological risk factor for progression. Inflammation of tubulointerstitial tissue clearly carries a poor prognosis in IgAN. Vascular diseases are more common in IgAN patients than in the general population regardless of renal function, but risk factor profiles for VDs are similar. IgMN is a more severe disease than has previously been suggested. It may be divided into two distinct subgroups with similar renal histology but different sex distribution

and clinical outcome. Serum and intrarenal immunoglobulin and complement measurements may be of prognostic significance in IgMN.

ABBREVIATIONS

ACE	Angiotensin-converting enzyme
BMI	Body mass index
BP	Blood pressure
C1q	Complement component C1q
C3	Complement component C3
C4	Complement component C4
CeVD	Cerebrovascular disease
CHD	Coronary heart disease
CS	Corticosteroids
CVD	Cardiovascular disease
ESRD	End-stage renal disease
F	Female
FSGS	Focal and segmental glomerulosclerosis
GFR	Glomerular filtration rate
H2000	Health 2000 Survey
HE	Hematoxylin and eosin
HU	Hematuria
IF	Immunofluorescence
IgA	Immunoglobulin A
IgAN	IgA nephropathy
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IgMN	IgM nephropathy
IL-1	Interleukin-1
IL-1β	Interleukin-1β
IL-10	Interleukin-10
LCA	Leukocyte common antigen
LDL	Low density lipoprotein
LM	Light microscopy
M	Male
MAC	Membrane attack complex
MAP	Mean arterial pressure
MCD	Minimal change disease
NS	Nephrotic syndrome
OR	Odds ratio
p	Probability value
PAS	Periodic acid-Schiff
p-group	Subgroup of patients with progressive renal disease
PU	Proteinuria
PUHU	Proteinuria and hematuria
r	Correlation coefficient
SIADH	Syndrome of inappropriate antidiuretic hormone secretion
s-group	Subgroup of patients with stable renal disease
SD	Standard deviation
UPE	Urinary protein excretion
VDs	Vascular diseases

LIST OF ORIGINAL COMMUNICATIONS

This thesis is based on the following original communications, referred to in the text by their roman numerals (I-V). In addition, some unpublished data are presented.

I.   Myllymäki J, Honkanen T, Syrjänen J, Helin H, Rantala I, Pasternack A, Mustonen J (2005). Uric acid correlates with the severity of histopathological parameters in IgA nephropathy. *Nephrology Dialysis Transplantation*, 20: 89-95.

II.   Myllymäki J, Honkanen T, Syrjänen J, Helin H, Rantala I, Pasternack A, Mustonen J (2007). The severity of tubulointerstitial inflammation predicts the prognosis in IgA nephropathy. *Kidney International* 71: 343-348.

III.   Myllymäki J, Syrjänen J, Helin H, Pasternack A, Kattainen A, Mustonen J (2006). Vascular diseases and their risk factors in IgA nephropathy. *Nephrology Dialysis Transplantation*, 21: 1876-1882.

IV.   Myllymäki J, Saha H, Mustonen J, Helin H, Pasternack A (2003). IgM nephropathy: clinical picture and long-term prognosis. *American Journal of Kidney Diseases*, 41: 343-350.

V.   Myllymäki J, Saha H, Helin H, Pasternack A, Mustonen J (2006). High serum C3 predicts poor outcome in IgM nephropathy. *Nephron Clinical Practice,* 102: 122-127.

1. INTRODUCTION

IgA nephropathy (IgAN) and IgM nephropathy (IgMN) are primary idiopathic glomerulonephrites with characteristic immunoglobulin depositions in the glomeruli. Diagnostic for IgAN are predominant mesangial IgA deposits, while in IgMN mesangial IgM are typically seen in renal biopsy specimens. Though the etiologies of both diseases have remained unresolved, it is believed that some type of immune response acts as a mediator in their course. It is conceivable that some infection in the mucosal area causes an abnormal immunological series of events inducing elevated levels of atypical IgA molecules, this leading to clinical manifestation of IgAN. Far fewer studies have been undertaken concerning the pathogenesis of IgMN. However, it is possible that abnormalities in immunoglobulin production constitute an underlying factor in IgMN. In spite of clear evidence of abnormal serum immunoglobulin (IgA or IgM) levels in both diseases, their role in the clinical picture or prognosis of IgAN or IgMN has not been properly studied.

Primary IgAN or IgMN may appear at any age. The onset of IgAN occurs mostly in adulthood, while IgMN commonly manifests itself in children. Male predominance is usually reported in the context of both diseases. Macroscopic HU occurring after respiratory tract infection is a classical manifestation of IgAN. The majority of IgMN patients have nephrotic syndrome (NS) or asymptomatic PU, while the clinical manifestation in IgAN patients is commonly microscopic HU and low-grade PU. As in many other renal diseases, the prevalence of hypertension is high among patients with IgAN or IgMN. It has also been proved that high blood pressure (BP) significantly influences the prognosis of IgAN.

A wide range of studies concerning the prognosis and prognostic factors for impaired renal function in IgAN have been made since 1968 when IgAN was first described. The varying indications for renal biopsy in different countries may affect incidences and prognostic figures in IgAN in different investigations. According to the Finnish Registry for Kidney Diseases, glomerulonephritis was after diabetes mellitus the second most common indication for renal replacement therapy in Finland in 2003. Worldwide IgAN is the most common form of glomerulonephritis. The incidence of IgMN in Finland seems to be about one fourth of that of IgAN. Accurate estimation of the worldwide incidence of IgMN based on previous studies is difficult due to the inconsistency of inclusion criteria. Generally speaking, patients with either renal disease usually have a fairly favourable course of renal disease, especially when they have only microscopic HU or low-grade PU. In addition to high-grade PU,

hypertension, high serum creatinine level, severe glomerulosclerosis and damage in the tubulointerstitium seen in renal biopsy specimens predicts progression of renal disease in IgAN. It has recently been found that hyperuricemia and hypertriglyceridemia are also independently associated with a poor course of IgAN. Little information is so far available regarding risk factors for the progression of IgMN. Most previous studies have focused on the significance of mesangial IgM depositions in the clinical picture of mesangioproliferative glomerulonephritis or minimal change disease (MCD).

Vascular diseases (VDs) are the most common cause of death in Western countries, also in patients with renal diseases. In evaluating the overall survival of renal patients in contexts apart from renal failure, VDs should also be taken into account. It has been clearly demonstrated that patients with chronic renal insufficiency have a substantially higher incidence of VDs compared to the general population. However, it is not clear whether renal patients with stable renal disease carry an elevated risk of VDs.

In the present study the clinical picture and histopathological changes in IgMN were further clarified. Supplementary data on prognosis and risk factors for the progression of IgAN and IgMN were established, taking into account serum and glomerular immunoglobulin and complement findings. Especially the role of hyperurichemia and tubulointerstitial inflammation in the natural course of IgAN was a focus of attention. The epidemiological factors associated with VDs were investigated in IgAN patients versus controls representing the general population.

2. REVIEW OF THE LITERATURE

2.1. Etiology and pathogenesis

Despite intensive research the etiology of IgAN remains unresolved. A manifestation or exacerbations of IgAN often arise concurrently or after clinical infection in the mucosal area, especially in the upper respiratory tract. For this reason it is assumed that some kind of mucosal antigen contact triggers an abnormal immunological response which leads to the development of IgAN. The possible pathogenetic mechanisms envisaged are summarized in Figure 1.

Immunoglobulin A (IgA) exists in three molecular forms: secretory, monomeric and polymeric. In polymeric IgA monomers are connected to each other by a J-chain. Secretory IgA is a dimeric form linked to a secretory component and produced in secretory organs. Normally, only a very small amount of secretory IgA passes into the systemic circulation. A second possible location for IgA synthesis is the epithelium in mucosal sites such as respiratory or gastrointestinal tract. A third IgA production site comprises non-mucosal lymphoid tissues such as spleen, lymph nodes and bone marrow. Human IgA has two subclasses, IgA1 and IgA2, evincing structural differences mainly in the hinge region, this leading to differences in their metabolism and defence mechanisms. Plasma cells producing IgA1 are distributed mainly in non-mucosal tissue, while the number of IgA2 cells is relatively greater in the gastrointestinal tract.

Mechanisms involved in IgAN pathogenesis
(in permissive genetic background)

Upper respiratory tract infections

Virus infections / bacterial products?
Cytokines?
Other factors?

Aberrant glycosylation of pIgA1

Increased pIgA1 with
Gal-deficient O-glycans

Anti-glycan antibodies

O-glycan- or hinge region
glycopeptide-crossreactive
IgG or IgA1

Formation of circulating immune complexes

Reduced clearance

Mesangial deposition

Initiation of glomerular inflammation

Activation of mesangial cells
(proliferation, matrix expansion)

Figure 1. Proposed mechanisms in the pathogenesis of IgAN. pIgA1= Polymeric immunoglobulin A type 1 (Julian BA and Novak J 2004)

A wide variety of antigens, microbial or nutritional, have been associated with the pathogenesis of IgAN. Such antigen stimulations may occur naturally in both the respiratory and the gastrointestinal tract. Especially tonsillar lymphocytes have been assumed to be a source of IgA, which accumulates in the glomeruli (Horie et al. 2003, Hiki et al. 2004). Previous studies have suggested the presence of subclinical intestinal inflammation in IgAN patients. There are elevated numbers of inflammatory cells in the small bowel (Rantala et al. 1999). Other markers reflecting inflammation, for example HLA class II antigen expression, stress protein production and activation of COX-2, have also been found (Rantala et al. 1999, Honkanen et al. 2005). In IgAN mesangial IgA seems to be of the polymeric IgA1 subclass. However, it has been shown that polymeric IgA production is down-regulated in the duodenum and up-regulated in the bone marrow (van den Wall Bake et al. 1989, Harper et al. 1994, Harper et al. 1996). On the basis of these findings it seems obvious that antigen stimulation causes mucosal hyperactivity, which triggers the mucosa-bone marrow axis, ending in elevated production of polymeric IgA1. However, this overproduced IgA1 seems to evince structural abnormalities. A defective galactosylation of the hinge region may cause reduced hepatic clearance of IgA1 (Roccatello et al. 1993). It is also evident that undergalactosylated IgA1 avidly forms complexes with other molecules such as IgG and binds to mesangial cells (Tomana et al. 1997, Hiki et al. 1999, Sano et al. 2002). This accumulation of IgA1 in the mesangium associated with inflammation in the renal tissue via a wide range of inflammatory mediators. In order to set in, IgAN needs the aforementioned mechanisms, but also a favourable combination of genetic factors (Hsu et al. 2000, Syrjänen et al. 2002).

Much less is known regarding the pathogenesis of IgMN. In fact, there is no unanimous conception regarding the status of IgMN as an independent disease, since the extremities of IgMN closely resemble two earlier described renal diseases: minimal change disease (MCD) and focal and segmental glomerulosclerosis (FSGS) (Border 1988, Korbet 1998, Fogo 2001). However, many researchers believe that IgMN is an independent form of glomerulonephritis with its own characteristic features, its diagnosis being based on the presence of diffuse immunoglobulin M (IgM) deposits in the mesangial area of glomeruli (Cohen et al. 1978, Border 1988, Saha et al. 1989, O'Donoghue et al. 1991). The origin and mechanisms of this accumulation of IgM are unknown.

Immunoglobulin M (IgM) is a pentameric molecule produced mainly at the early stage of immunization. Under the influence of cytokines secreted by T-cells, IgM expression

normally gradually fades and immunoglobulin G (IgG) synthesis increases (Purkerson and Isakson 1992, Snapper and Mond 1993). In the context of IgMN some abnormalities in IgM production have been brought out. Genetic factors may influence these pathological mechanisms (Scolari et al. 1990). Kishimoto and Arakawa (1999) found IgMN patients to have elevated serum IgM levels compared to patients with mesangial glomerulonephritis without IgM deposits (Kishimoto and Arakawa 1999). High concentrations of IgM are associated with considerable development of immunocomplexes consisting IgM (Helin et al. 1982, Lin and Chu 1986, Disciullo et al. 1988). The elevated IgM and decreased IgG levels may be caused by immunoglobulin class switch disturbance in IgMN with nephrotic syndrome (NS) (Lin et al. 1989). A similar assumption has in fact been made even earlier in children, in the case of NS without knowing the exact histopathological diagnosis (Giangiacomo et al. 1975). The aforementioned abnormal immunological mechanisms may promote the accumulation of IgM and/or IgM immunocomplexes to the mesangium, inducing local inflammation and clinical manifestation of IgMN.

2.2. Clinical presentation

Like many other renal diseases, IgAN and IgMN are also more frequent among males. In IgAN the male: female ratio ranging from 2:1 to 6:1 (Donadio and Grande 2002), in IgMN from 1:1 to 2:1 (Saha et al. 1989, O'Donoghue et al. 1991, Al-Eisa et al. 1996, Little et al. 2000, Zeis et al. 2001). The clinical picture in both IgAN and IgMN is variable. In IgAN the classical manifestation is macroscopic HU occurring after some infection. Patients with IgMN often suffer from symptoms caused by NS. However, it is not unusual for these diseases to be found in examinations made due to asymptomatic HU or proteinuria (PU) found during routine screening or investigation of another condition. The initial manifestation may be anything between microscopic HU and nephrotic-range PU with renal insufficiency in both diseases. The prevalence of hypertension is high among these patients, and it becomes still higher during the progression of renal disease. Even malignant hypertension has been reported in the case of IgAN.

2.2.1. Clinical renal findings

There are different clinical practices in performing renal biopsies. In some countries (e.g. the United States) it is not customary to perform renal biopsy in patients with only minor urinary abnormalities. In Finland, again, specimens have also been taken from the kidneys of patients with asymptomatic low-grade PU or HU (Saha et al. 1989, Wirta et al. 2008). It is uncommon to perform biopsies from pediatric patients who evince no nephrotic syndrome. Local attitudes toward urine testing are also highly variable. These differences affect prevalence figures for the different manifestations of IgAN and IgMN in different research materials. (Wirta et al. 2008)

In young IgAN patients (<40 years of age) the aforementioned manifestation with one or more episodes of macroscopic HU is common. Over 80% of pediatric IgAN patients in Europe and the United States have macroscopic HU as an initial presentation (Lau et al. 2004, Yoshikawa et al. 1999). In Japan the proportion of such patients is much lower, by reason of school screening finding abundant asymptomatic urinary abnormalities (Yoshikawa et al. 1999). Recurrent macroscopic HU is traditionally regarded as a hallmark of childhood IgAN (Yoshikawa et al. 1999). Loin or abdominal pain often accompanies HU visualized as tea-coloured urine (Donadio and Grande 2002). Macroscopic HU may coincide with infection in mucosal areas, usually in the upper respiratory or gastrointestinal tracts. In adults macroscopic HU is highly unusual and is almost never seen in patients over 40 years of age (Donadio and Grande 1997, Floege and Feehally 2000). Asymptomatic microscopic HU with or without PU is the presentation in 30-50% of patients in most series (Floege and Feehally 2000). In a Finnish material consisting more or less exclusively adults 80 % had microscopic HU and PU, and 13% had HU alone (Syrjänen et al. 2000). In addition to HU, white blood cells and casts are often seen in centrifuged urine microscopy (Ibels and Gyory 1994, Ibels et al. 1998).

Nephrotic syndrome (NS) is an unusual presentation of IgAN. In Finland it seems to occur in 3-5 % of patients (Mustonen et al. 1985, Syrjänen et al. 2000), while worldwide about 5% have NS, this more frequently among children and adolescents than in adults (Galla 1995). Acute renal failure is an infrequent initial manifestation in IgAN, occurring in 1 to 10% of patients. On the other hand, IgAN or HSP is diagnosed in about 4% of cases of acute renal insufficiency (Haas et al. 2000). Acute renal failure in IgAN seems to be associated with macroscopic HU and red blood cell casts seen in renal tubules (Delclaux et al. 1993,

Packham et al. 1994). Acute nephritic syndrome with a sudden appearance of edema, HU, PU, and hypertension may occur in isolated cases of IgAN (Mustonen et al. 1985).

In IgMN the clinical variants are similar, but their proportional representations diverge. The disease is often found when investigating nephrotic patients. Most studies concerning IgMN have been made with pediatric populations. Mainly due to renal biopsy practices a majority of patients are nephrotic in most studies. In one Greek pediatric material there were also patients with minor urinary abnormalities (Zeis et al. 2001). Of this patient population 31% was nephrotic. O'Donoghue and colleagues (1991) investigated 54 patients 14-69 years of age (median 31 y, and found the proportion of NS to be as high as 57%, this possibly because 19 patients with segmental sclerosis were included. Among adult IgMN patients in Finland the occurrence of NS as an initial manifestation has been 22% (Saha et al. 1989). In two other main studies with NS and minor urinary abnormalities figures for children and adults were not separated. In the studies in question the proportions of NS patients were 34% and 41% (Little et al. 2000, Vangelista et al. 1981).

Despite the high prevalence of NS, glomerular findings resembling those in IgMN were first described by van de Putte and associates in 1974 in patients with HU. Later Pardo and colleagues (1979) reported some IgMN patients with HU, with very good prognosis. Half of their patients were women. Further investigations have shown that a notable proportion of IgMN patients have HU with or without asymptomatic PU as their initial manifestation (Vangelista et al. 1981, Saha et al. 1989, O'Donoghue et al. 1991, Little et al. 2000, Zeis et al. 2001). In this subgroup of patients a female predominance may be observed (Saha et al. 1989). A third subgroup includes patients with asymptomatic PU without HU with a proportion of approximately 20%, ranging from 9 to 39% (Tejani and Nicastri 1983, Saha et al. 1989, O'Donoghue et al. 1991, Little et al. 2000, Zeis et al. 2001). Acute renal failure is virtually never recorded at the time of diagnosis of IgMN. The prevalence of impaired renal function at the time of renal biopsy appears to range from 2 to 6% (Saha et al. 1989, Al-Eisa et al. 1996, Little et al. 2000). In the above-mentioned study by O'Donoghue and colleagues 28% of patients had initially elevated serum creatinine level (O'Donoghue et al. 1991). However, as noted, in that study there were also patients with segmental glomerulosclerosis seen in their renal biopsy specimen.

2.2.2. Hypertension

A blood pressure (BP) value consistently over 140/90 mmHg is diagnostic for systemic hypertension (HT) in healthy persons. However, since the diagnostic criteria for HT have changed considerably during past decades, the definitive limits of high BP are higher in earlier studies. This affects the prevalence of HT as reported in various studies. One recent study showed that the age- and sex- adjusted prevalence of HT was 28% in North America and 44% in Europe (Wolf-Maier et al. 2003). As previously confirmed, the prevalence of HT correlates strongly with cardiovascular and stroke mortality. In patients with renal disease HT is particularly frequent. In the Spanish population its prevalence is from 5 to 93% according to type of nephropathy (Ridao et al. 2001). Among patients with glomerulonephritis the prevalence is 54%. Of Brazilian patients with some glomerulonephritis 63% were hypertensive (Corpa and Soares 2002). The highest reported HT prevalence in the context of chronic glomerulonephritis is 80% (Martinez-Maldonado 2001). When renal function remains normal despite the presence of renal disease, HT seems to be much more infrequent (Johnston and Davison 1993). Evidence shows that HT is a strong risk factor for chronic renal failure independent of the precise diagnosis. On the other hand, the prevalence of HT increases markedly as renal failure progresses (Martinez-Maldonado 2001). For these reasons the BP limits for initiation of antihypertensive medication are lower in renal patients than in the general population (Anonymous 1997).

The prevalence of HT in IgAN patients and in different materials from Europe, the United States, Japan and Australia has been from 9% to 47% at the time of renal biopsy (Donadio and Grande 1997). Among Finnish IgAN patients, as many as, 52% had HT (Syrjänen et al. 2000). Single cases with malignant HT have been reported in IgAN materials (Perez-Fontan et al. 1986, Subias et al. 1987). In the context of IgAN hypertension is among the most reliable risk factors for a poor course of renal disease (Mustonen et al. 2001). Hypertension seems to accelerate the development of both glomerular and renal vascular sclerosis, which may precede the impairment of renal function (Katafuchi et al. 1988). However, adequate control of HT may make the prognosis of IgAN patients with HT as good as that of normotensives (Payton et al. 1988).

The prevalence of hypertension in IgMN is according to previous studies very difficult to estimate as definitions of HT have varied considerably. In three major studies with significant proportions of adult patients and also patients with minor renal abnormalities the prevalence ranged from 21% to 28% (Saha et al. 1989, O'Donoghue et al. 1991, Little et al.

2000). Saha and O'Donoghue with their collaborations defined as hypertensive patients with a BP of ≥160/100 mmHg and/or usage of antihypertensive medication. In the third study the need for antihypertensive drug treatment sufficed for a diagnosis of HT. If these investigators had used the current criteria for HT (≥ 140/90 mmHg) in their investigations, the prevalence would have been much higher, probably near that reported in the case of glomerulonephritis generally.

2.3. Biochemical laboratory findings

2.3.1. Serum immunoglobulins and complement particles

Serum immunoglobulins and complement particles are often routinely measured for differential diagnostics in renal patients. Even though both IgAN and IgMN are conceived to be immunomediated diseases, the role of immunoglobulins in their natural course of has not hitherto been intensively investigated.

An abnormal immunological response to some external or internal antigen stimulus typically causes elevated serum IgA levels in IgAN. Mustonen and colleagues (1981) found 58% of IgAN patients to have elevated serum IgA and in general 50 to 70% of IgAN patients seem to have elevated levels (Rantala et al. 2001). In a Japanese population serum IgA concentrations appeared to be higher in IgAN patients than in patients with non-IgAN glomerulonephritis and healthy controls (Tomino et al. 2000). In view of these findings, serum IgA more than 350 mg/dl in adults is defined as a diagnostic criterion in Japan (Sakai et al. 1995). Elevated IgA levels may be associated with progressive IgAN (Komatsu et al. 2004).

It is a generally known fact that in idiopathic NS serum total IgG decreases during the nephrotic period. The phenomenon is traditionally thought to develop solely due to increased excretion of IgG into the urine with other protein molecules (Kaysen and al Bander 1990). However, the production of IgG may also be altered. Of IgG subclasses, IgG_1 and IgG_2 are typically significantly decreased during NS relapse, while IgG_4 is usually normal at any stage (Kemper et al. 2002, Warshaw and Check 1989). Likewise many other high-molecular-weight proteins derived from the liver are concentrated in NS (Kaysen and al Bander 1990). Serum IgE may also be elevated, while IgA is often within normal range (Chan et al. 1987). However, especially in pediatric NS patients, serum IgA may also be elevated (Giangiacomo

et al. 1975). These abnormalities typically revert to normal when a remission has been reached. On the other hand, IgG_2 may also be decreased during remission (Kemper et al. 2002). Nephrotic patients with a high IgG_1/IgM ratio (>3) may respond better to corticosteroid (CS) treatment compared to those with IgG_1/IgM <3 (Fu et al. 1998, Wang et al. 1997). Interestingly, high serum IgE may be associated with a poor clinical response to CS, also in IgMN (Chan et al. 1987, Shu et al. 1988).

In the aforementioned reports IgMN had not been studied separate from other idiopathic renal diseases causing NS. There are, however some investigations in which immunoglobulin changes characteristic of IgMN are reported separate from other causes of NS. Lin and Chu studied nephrotic children with IgMN versus normal healthy children (Lin and Chu 1986), and found IgMN patients to have higher serum IgM levels, while serum IgA or IgG were slightly but not significantly decreased. Serum IgM seems to be more elevated in IgMN than in other NS types such as MCD (Disciullo et al. 1988, Lin et al. 1989). Serum IgM is also usually elevated in IgMN patients without NS (Helin et al. 1982, Kishimoto and Arakawa 1999). However, the IgM level seems to correlate with UPE rate (Kishimoto and Arakawa 1999). Total IgM elevation may be explained by a significant elevation in circulating heavy IgM, probably in the form of circulating immune complexes (Helin et al. 1982, Disciullo et al. 1988).

The complement system is crucial in protection against micro-organisms. However, activation of complement is associated with the development of tissue injury such as damage to the glomeruli and renal tubulointerstitium (Nangaku 2003). Even though IgA is not a very effective complement activator, alternative pathway components C3, properdin and the membrane attack complex (MAC) are often found in the mesangial depots in IgAN, while the classical pathway components C1q and C4 are usually absent (Wyatt and Julian 1988). Mesangial C3 is also often present in IgMN. Levels of complement components are usually normal in IgAN and IgMN (Helin et al. 1982, Julian et al. 1983). Serum C3 concentrations are similar in patients with IgMN or non-IgM glomerulonephritis (Kishimoto and Arakawa 1999), whereas in the case of NS there are often high serum C3 concentrations probably reflecting increased hepatic protein synthesis (Chan et al. 1987). High C3 may exist in the form of circulating immunocomplexes (Lin and Chu 1986). There is some evidence to indicate local intraglomerular synthesis of C3 in IgAN (Abe et al. 2001). No such activation was found in MCD or normal controls. However, C3-containing circulating immunocomplexes are often present in the serum of IgAN patient, which may reflect

systemic activation of the complement system (Mustonen et al. 1981). The systemic complement activation theory in IgAN is further supported by the finding that levels of activation products C3 and C3a are significantly higher in patients with IgAN as compared with healthy controls or patients with non-immune renal disease (Janssen et al. 2000).

2.3.2. Uric acid in renal diseases

Uric acid is an insoluble and toxic end product of purine metabolism (Maesaka and Fishbane 1998). States of enhanced purine catabolism increase the uric acid load. The primary causes of rapidly increased purine metabolism are states involving accelerated cell turnover or lysis, for example leukemias, lymphomas and conditions induced by chemotherapy or radiation therapy (Conger 1990). The degradation of purine nucleotides occurs mainly in the liver. In the last step of this process xantine oxidase enzyme converts xantine to uric acid. The catalytic activity of xantine oxidase is inhibited by allopurinol, commonly used to lower uric acid levels in gout. In adults approximately 30% of uric acid is excreted through the biliary and gastrointestinal tract, and the kidneys excrete the remaining 70% (Maesaka and Fishbane 1998).

The renal handling of uric acid most probably occurs in a four–component system; filtration, reabsorption, secretion and postsecretory reabsorption (Maesaka and Fishbane 1998), the proportions of these different components remaining as yet incompletely resolved. The mechanisms of the renal handling of uric acid are summarized in Figures 2 and 3. Uric acid is filtered practically freely into primary urine at the glomerulus. It is generally accepted that its reabsorption occurs mainly in the proximal tubule and predominantly in the first (S_1) segment. Active uric acid secretion appears to occur in the S_2 segment and postsecretory reabsorption in the S_3 segment of the proximal or early distal tubule. As the reabsorption of uric acid is indirectly coupled to sodium transport, the sodium balance may also affect its excretion. Recently four different uric acid transport proteins have been defined and localized in the renal proximal tubules (Rafey et al. 2003). Under normal conditions, less than 10% of the filtered uric acid is excreted in the urine. However, in many diseases such as cirrhosis, many malignancies, hypoparathyroidism and SIADH, the uric acid excretion rate may be raised (Maesaka and Fishbane 1998). In addition to those states associated with increased excretion, also conditions or medications which reduce uric acid production may cause hypouricemia. Allopurinol treatment leads to underproduction of uric

acid, while the angiotensin II receptor antagonist losartan increases its excretion (Burnier et al. 1996).
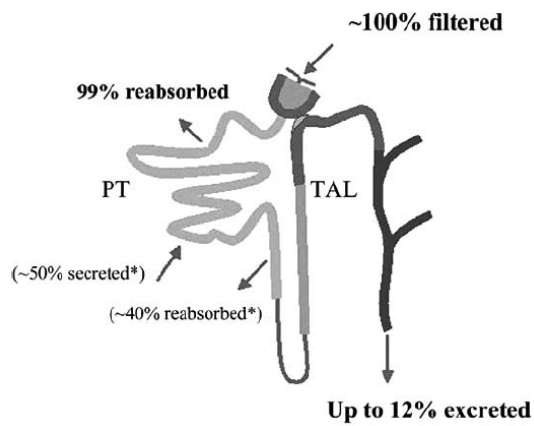

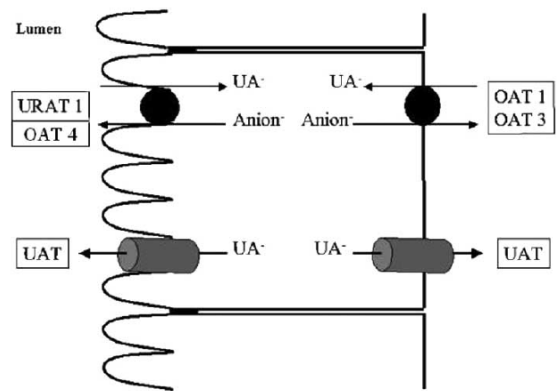Figure 2. Uric acid handling by the nephron (Capasso et al. 2005).

Figure 3. A cell model of molecular mechanisms underlying transepithelial uric acid transport in the proximal tubule. OAT= Organic anion transporter, UA$^-$= Uric acid anion, UAT= Voltage sensitive urate transporter, URAT= Urate-anion exchanger (Capasso et al. 2005).

Hyperuricemia is traditionally related to gout, which may lead to the formation of uric acid calculi in the renal pelvis, and to gouty nephropathy with typical tophus formation (Nickeleit and Mihatsch 1997). However, hyperuricemia has more recently been included in the metabolic syndrome (Vuorinen-Markkola and Yki-Jarvinen 1994, Daskalopoulou et al. 2004). As chronic low-grade hyperuricemia is strongly associated with traditional risk factors for cardiovascular diseases, especially HT and age, its independent etiological role in the development of atherosclerotic complications is still unresolved.

Persistent hyperuricemia is usually caused by a chronic defect in renal uric acid clearance. Glomerular filtration may be decreased in renal diseases such as glomerulonephritis, owing to damage to functional glomeruli. Tubular reabsorption increases in the setting of volume depletion, i.e. in association with treatment by diuretics (Johnson et al. 1999). Secretion may be inhibited by lactate or ketoacids. Hypertension has been held to induce hyperuricemia by causing microvascular injury, this leading to tissue hypoxia and lactate generation, which in turn may affect uric acid clearance. Tissue hypoxia, again, might lead to uric acid generation (Johnson et al. 1999). Hyperuricemia may be present in up to 25% of untreated hypertensives (Cannon et al. 1966). This condition is assumed to be a consequence of early renal involvement (nephrosclerosis) in essential HT (Messerli et al. 1980). Some forms of salt-dependent hypertension can result from renal microvascular injury with local ischemia and tubular damage, which might in turn impair uric acid clearance (Johnson et al. 1999).

It seems obvious that both glomerular and tubulointerstitial damage may cause high uric acid levels. There is also some evidence that chronic hyperurichemia without gout may induce renal injury, but the independent role of uric acid in this process has remained unresolved. The clearest evidence that hyperuricemia itself may induce renal damage has been reported in experimental studies. Hyperuricemia can be induced in rats by oxonic acid, which inhibits uricase, leading to increased metabolism of purines to uric acid instead of allantoin (Mazzali et al. 2001). Mild hyperuricemia without uric acid crystals may induce hypertension, PU, higher serum creatinine levels, glomerular and renal hypertrophy, glomerulosclerosis, interstitial fibrosis and renal vasculopathy (Mazzali et al. 2001, Kang et al. 2002, Mazzali et al. 2002, Nakagawa et al. 2003). These changes may be associated with increased activity of the renin-angiotensin system, expression of COX-2 and inhibition of neuronal nitric oxide synthase (Mazzali et al. 2001, Kang et al. 2002). Allopurinol significantly reduced uric acid levels and blocks renal functional and histopathological

changes (Kang et al. 2002). An angiotensin-converting enzyme (ACE) inhibitor has partly prevented glomerular hypertrophy in hyperuricemic rats (Nakagawa et al. 2003). Increased activation of the intrarenal renin-angiotensin system has also been indirectly shown also in hyperuricemic humans (Perlstein et al. 2004).

The concentration of serum uric acid correlates positively with UPE, occurrences of HT, the severity of diffuse proliferative glomerulonephritis and tubulointerstitial damage in IgAN (Ohno et al. 2001). A notable proportion (30%) of Finnish IgAN patients are hyperuricemic (Syrjänen et al. 2000). A negative correlation has been found between serum uric acid level and creatinine clearance. Miura and colleagues found IgAN patients with macroscopic HU to have significantly lower serum levels of uric acid and enhanced uric acid clearance compared to other IgAN patients (Miura et al. 1990).

2.4. Renal histopathology, immunohistochemistry and diagnostics

Study of renal tissue samples is a sine qua non in diagnostic evaluation and choice of adequate medical therapy in renal diseases. The diagnostics of IgAN and IgMN is based on IF. Some IF findings may also be valuable in determining the prognosis. Light microscopy (LM) is used in differential diagnostics. The risk factor profile evaluation in IgAN and IgMN is partly based on LM findings. Immunohistochemistry makes it possible to quantitatively measure many dynamic changes in renal tissue, for example cell proliferation and inflammation. These methods have been somewhat infrequently used in risk factor analysis. Also electron microscopy is sometimes employed, but this approach has a rather limited role in diagnostics or predicting the natural course in both diseases.

Typical, but not specific, light-microscopic (LM) findings in IgAN are focal or diffuse expansion of mesangial regions with cells and matrix (Donadio and Grande 2002). However, a wide variety of glomerular lesions may be seen, including endocapillary proliferation, segmental or global sclerosis with hyalinosis, segmental necrosis, membranoproliferative lesions, and crescent formation (Donadio and Grande 1997, Donadio and Grande 2002). From this it follows that the typical pattern of mesangial proliferative IgAN, moderate uniform global mesangial hypercellularity and proportionate matrix expansion, is seen in only 20% of patients (Emancipator 1994). The changes may range from minimal to segmental or diffuse hypercellularity resembling FSGS (Floege and Feehally 2000).

Endocapillary proliferation may be seen in segmental manner, involving ≤ 50% of glomeruli in 15%, and in diffuse global, diffuse segmental or focal global patterns in ≤ 5% of patients (Emancipator 1994). In relevant specimens leukocyte infiltrations are sometimes seen, usually by immunohistology. Membranous and membranoproliferative lesions are very rare. Crescents, typically small, partial and cellular, are observed in approximately 20% of IgAN patients, usually those evincing severe mesangial and/or endocapillary proliferation, or other renal histopathological lesions (Emancipator 1994, Tang et al. 2002).

A variety of tubulointerstitial and vascular changes may also be identified in patients with IgAN. Medial hypertrophy and/or hyaline arteriolosclerosis are present in 54% of IgAN patients, who are often hypertensive (Emancipator 1994). Vascular changes may be more frequent in patients with IgAN than in those with other forms of glomerulonephritis (Wu et al. 2005). A number of different histopathological grading systems have been used in previous IgAN studies (Lee 1997). Those are usually semiquantitatively performed by LM. The classifications of Lee and associates (1982), revised in 2005 and those of Haas (1997) are the most common procedures.

Generally similar histopathological LM findings are also seen in renal biopsy specimens from IgMN patients, the only difference being that patients with focal and segmental glomerulosclerosis are excluded. The proportions of patients with different morphological lesions have varied considerably, accounting for the different diagnostic criteria used in studies of IgMN. Usually mild mesangial hypercellularity and/or glomerular matrix expansion are seen in LM. However, normal glomeruli or no more than minimal changes are common findings in IgMN (Tejani and Nicastri 1983, Saha et al. 1989, Zeis et al. 2001). Severe-stage mesangial proliferation/hypercellularity is rarely seen (Little et al. 2000). Mild tubulointerstitial lesions are sometimes present in IgMN (Saha et al. 1989). Arteriolosclerosis of varying degree is fairly frequently (30-40 %) observed (Kishimoto and Arakawa 1999, Saha et al. 1989).

Different protein molecules may be localized and quantified in tissue samples by immunohistochemical methods. A tissue specimen is incubated by antibodies against molecules under study. To these antibodies are attached different labels, which can be observed by light or immunofluorescence microscopy (IF). As previously mentioned, the diagnosis of IgAN and IgMN is based on IF study. Evaluation of renal biopsy by IF is also a crucial method in the context of overall nephropathology (Bonsib 2002). Immunohistochemical methods were also employed in the present study to localize

proliferative cells, but the main focus was on inflammatory cells and mediators in the renal tissue of IgAN patients.

2.4.1 Diagnostic methods and criteria

Immunofluorescence (IF) microscopy is a first-line method in diagnosing primary glomerulonephritis such as IgAN and IgMN. The diagnosis of these disorders can be confirmed when characteristic IF findings are registered and diseases inducing secondary renal disease have been excluded by other sufficient methods.

Diffuse IgA distributed mainly in the mesangial areas is a diagnostic finding in IgAN. Glomerular IgA must be the sole or predominant IF finding in the renal biopsy specimen (Pettersson 1997, Hunley and Kon 1999). IgA deposits can also extend beyond the mesangium and to peripheral glomerular capillaries. Measuring serum IgA and complement 3 (C3) may be of help in identifying IgAN when a renal biopsy specimen is not available (Ishiguro et al. 2002). Other laboratory measurements have also been studied, without striking success, in an effort to formulate serological diagnostic tools for IgAN (Galla 1995).

In IgMN solely or predominantly IgM deposits are seen in the mesangium in the renal tissue specimen. These deposits are typically globally, diffusely and granularly distributed. In addition to IF study light-microscopic (LM) evaluation should be performed to exclude focal and segmental glomerulosclerosis (FSGS) (Border 1988).

2.4.2. Differential diagnosis

A wide range of diseases are associated with IgAN (Mustonen 1984). The most common being Henoch-Schönlein purpura (HSP), believed to be a systemic form of the same disease with similar IF findings and histopathological changes in the glomeruli (Rai et al. 1999). Hence HSP is categorized as a primary cause of glomerular IgA deposition (Donadio and Grande 2002). The presence of systemic symptoms, including purpuric rash, arthralgias and abdominal pain distinguishes HSP from IgAN. Post-infectious glomerulonephritis also induces macroscopic HU, but the onset of HU occurs 7 to 14 days after, not at the time of the infection as in IgAN (Galla 1995). Of other diseases causing renal pathology resembling IgAN (secondary IgAN) the best characterized are rheumatoid arthritis, ankylosing spondylitis, systemic lupus erythematosus, Reiter's syndrome, celiac disease and dermatitis herpetiformis, chronic liver disease, and viral diseases such as HIV and hepatitis B infections

(Pettersson 1997, Floege and Feehally 2000). Many other associations have been reported, albeit with low grade evidence.

The diagnostic criteria for IgMN are not entirely clear. The typical IF finding is required, but there is no strict consensus on LM findings falling under the term IgMN. When Cohen and Bhasin with their colleagues first described IgMN in 1978 they included solely patients with a significant extent of mesangial cell proliferation seen at renal biopsy (Bhasin et al. 1978, Cohen et al. 1978). The same definitive criteria have been followed in some later investigations (Vangelista et al. 1981, Hirszel et al. 1984, Disciullo et al. 1988, Little et al. 2000, Zeis et al. 2001). Even though histological changes resembling FSGS have usually been defined as histopathological changes exclusive to IgMN, some studies also include patients with the histological changes indicated above (Pardo et al. 1984, Kopolovic et al. 1987, Lawler et al. 1980, O'Donoghue et al. 1991). In cases of normal morphology or minimal histopathological changes in the glomeruli accompanying clinical renal findings, the disease is occasionally defined as MCD even if a significant extent of IgM is seen in IF study (Vilches et al. 1982). It was considered that IgM would be a non-specific finding in MCD. Nonetheless, in many previous investigations the term IgMN has been used in the aforementioned situations (Mampaso et al. 1981, Helin et al. 1982, Pardo et al. 1984, Gonzalo et al. 1985, Lin and Chu 1986, Saha et al. 1989, Donia et al. 2000). It would appear to be established that in MCD there are normal glomeruli or at least minimal histopathological changes with negative or no more than trace immunoglobulin findings under IF (Border 1988, Fogo 2001). In FSGS the clinical picture may be similar to that in IgMN, but the histopathological picture is typical and if there are IgM depositions, they are distributed in the sclerotic areas (Border 1988, Fogo 2001). Both MCD and FSGS must be taken into account in the differential diagnostics of IgMN.

Due to afore mentioned reason it has been suggested that MCD, IgMN and FSGS may be parts of the one-spectrum disease (Hirszel et al. 1984, Cho et al. 2007). However, it seems clear that a response to steroid therapy and maybe also prognosis are better in MCD than in IgMN with mesangial hypercellularity or in FSGS (Alexopoulos et al. 2000, Cho et al. 2007). Some authors believe that glomerular hypertrophy and mesangial hypercellularity may be the first warning signs predicting MCD to progress to FSGS (Cho et al. 2007). Tejani and colleagues reported significant number of MCD cases converting to IgMN or FSGS in secondary biopsy (Tejani 1985). However, a transition to FSGS has been found to be more common in patients with diffuse mesangial hypercellularity with mesangial IgM than in

those without mesangial IgM (Zeis et al. 2001). IgM would appear to be an independent risk factor for ESRD and poor response to therapy in context of diffuse mesangial hypercellularity and NS (Alexopoulos et al. 2000). IgMN has often been considered as a separate entity due to fact that patients with minimal changes and IgM depositions show a poorer response to corticosteroids. However the opposite findings have also been reported (Habib et al. 1988, Al-Eisa et al. 2006). So, even though the independence of IgMN remains controversial, IgM itself seems to be an important component in the suggested transition of MCD to FSGS.

No diseases unambiguously causing secondary IgMN have been reported. There are however systemic diseases which promote the development of renal lesions, which may give rise to diagnostic problems. Hence patients with diseases such as systemic lupus erythematosus, vasculitis, Alport's syndrome, rheumatoid arthritis and diabetes mellitus are often excluded from studies concerning IgMN. Many rheumatoid arthritis patients with HU and/or PU have mesangial glomerulonephritis as the renal finding. Sole or predominant mesangial IgM seems to be a frequent finding in these cases (Korpela et al. 1997). There are some case reports in which IgMN have been associated with diseases like psoriasis, type II thin basement membrane nephropathy, familial Mediterranean disease, Behcet's syndrome, Kimura's disease and spondylarthropathy (Steinsson et al. 1983, Maffei et al. 1990, Said and Hamzeh 1990, Chan et al. 1991, Hamuryudan et al. 1991, Said et al. 1992). Penicillamine medication is also reported to be associated with IgMN (Rehan and Johnson 1986). The clinical significance of the last-mentioned associations is somewhat limited.

2.4.3. Pathogenesis and pathophysiology of tubulointerstitial damage

Even though investigation of glomeruli is essential in the diagnostics of IgAN and IgMN, tubulointerstitial lesions may be the most significant histopathological finding predicting the course in these diseases. It is essential to identify pathogenetic mechanisms of tubulointerstitial injury.

Activation of proximal tubular cells inducing tubular inflammation is a common final pathway in many chronic renal diseases (Lai et al. 2005). This may occur through multifactorial mechanisms. As mentioned in section 2.4.1., uric acid in high concentrations probably itself induces tubulointerstitial injury. However, an increasing body of evidence points to the role of PU in the development of tubulointerstitial damage. In MCD PU is thought to be caused by dysfunction of small-charge sensitive pores in the glomerular

capillary walls. Hence in this case urine protein consists more or less exclusively of albumin. Albuminuria thus reflects only minor defect in glomerular function, not significant morphological changes. In glomerulonephritis, IgAN or IgMN, circulating immunocomplexes precipitated in glomeruli may induce inflammation by cytokines or other mediators secreted by mesangial cells.

Significant inflammation may "open" larger pores in the capillary wall. These will allow non-specific high-molecular-weight protein transportation into primary urine, and some of these plasma proteins may be directly toxic to renal tubular cells (Matsuo et al. 1998). The proportion of high-molecular-weight proteins in the urine can be measured by selectivity indices (Tencer et al. 1998, Tencer et al. 2000, Bakoush et al. 2001a, Bakoush et al. 2001b). These are usually based on IgG, haptoglobin, cerruloplasmin, and α_2-macroglobulin (Tishkov et al. 1978). However, a selectivity index for IgM has recently been found useful in differentiating glomerular diseases or predicting survival in them (Tencer et al. 1998, Bakoush et al. 2001b). As IgM is a fairly large protein, its presence in urine reflects the development of very large pores or shunts in the glomerular capillary walls. Through these shunts many potentially harmful circulating macromolecules such as cytokines or complement particles may leak into the tubular lumen. Under the influence of the immunoglobulin influx mesangial cells secrete proinflammatory mediators, which may flow into the tubular lumen and activate inflammation in proximal tubular cells (Lai et al. 2005).

The complement system is held to have a potential role in inducing tubulointerstitial damage in proteinuric patients (Matsuo et al. 1998). Even if C3 levels are usually normal in IgAN or IgMN, circulating fragments generated by the activation of C3 are often detected in IgAN (Wyatt and Julian 1988). When large capillary pores are open, in addition to C3, these fragments may accumulate in the mesangium or leak into the urine. However, intraglomerular synthesis of C3 and it activation products such as MAC have also been detected by Abe and colleagues (Abe et al. 2001). There is evidence that filtered C3 may be activated through amidation or the effects of damaged renal cells (Nath 1985). A common end product of this activation is MAC. Both filtrated and intraluminally produced MAC is able to trigger apoptosis in tubular cells or induce a variety of proinflammatory mediators, which may contribute to tubulointerstitial damage (Biancone et al 1994). However, in an experimental model of glomerulonephritis, less severe tubulointerstitial damage was seen when the complement components were pharmacologically depleted or when activation of the complement cascade was blocked (Morita et al. 1997, Nomura et al. 1997). In the light of

experimental findings and clinical data, it is clear that activated complement is an important causative factor in PU-associated tubulointerstitial injury.

2.4.4. Intrarenal immunoglobulins and complement particles

Previous studies have focused on intrarenal immunoglobulins and complement particles in necropsies representing the general population in the same residential area. In a study from Singapore the authors investigated renal tissue specimens from 200 patients who died of traumatic injuries, without history of kidney disease. Predominant mesangial IgA was found in 4% and IgM in 1% of cases (Sinniah 1983). An analogous Finnish material comprised 756 subjects (Varis et al. 1993). Renal deposits of some immunoglobulin, almost exclusively granular, diffuse and mesangial, were found in 12% of specimens. Some of the patients involved had yielded clinical renal findings at term of life. The predominant IF finding was mesangial IgA in 6.9% of cases. It was an isolated finding in 4.5% of cases. Predominant mesangial IgM was found in 2.5% of specimens. Also predominant IgG was occasionally found. Disease with predominant mesangial IgG together with clinical renal findings may be termed IgG glomerulonephritis (Fakhouri et al. 2002). However, IgA-IgM primary glomerulonephritis, with prominent mesangial deposits of both IgA and IgM, has also been described (Mustonen et al. 1991); some authors have indeed suggested that a primary glomerulonephritis IgA-IgG nephropathy also exists. The frequency of mesangial C1q deposits was about 1% in both of the aforementioned autopsy studies (Sinniah 1983, Varis et al. 1993). Sinniah and colleagues (1983) found only arterial, but no mesangial, C3 deposits in 4.5% of specimen. In another study predominant C3b was present in 0.3% of samples and colabelling of C3b in 0.5% of samples (Varis et al. 1993). Clinical renal findings together with C1q or C3 depositions may be definitive for two other primary mesangial glomerulonephritis; C1q nephropathy and C3 mesangial glomerulonephritis (Jennette and Hipp 1985, Calls Ginesta et al. 1995).

Predominant mesangial IgA depositions, found by IF, are definitive for IgAN. In some cases (15%-20%) IgA is also present in capillary walls (Emancipator 1994). As mentioned in the context of pathogenesis, the IgA1 subclass predominates over IgA2 in glomerular depositions. There are no IgAN studies reporting significant correlations between the intensity of IgA immunostaining and prognosis. However, other immunoglobulin findings are often present in renal tissue specimens from IgAN patients. Mesangial IgG and/or IgM of lower intensity are found in some cases (Jennette 1988, Emancipator 1994). A majority of

the IgG depositions seems to comprise subclasses IgG1 and IgG3 (Aucouturier et al. 1989). It would appear that mesangial IgG co-deposition is not correlated with the severity of histopathological damage, but may be a risk factor for decreased renal survival in IgAN (Jennette 1988, Nieuwhof et al. 1998). Mesangial labelling of IgM seems to be correlated with the level of UPE, but not with progression in pediatric IgAN patients (Welch and McAdams 1998).

Mesangial C3 deposits are seen in a clear majority of IgAN specimens. In some studies positive mesangial staining for C3 has been one of the inclusion criteria (Nieuwhof et al. 1998). The origin of mesangial C3 may be in the systemic circulation by passive accumulation, but the evidence for intraglomerular C3 synthesis is strong (Abe et al. 2001). Classical pathway components such as C1q are rarely seen in renal tissue specimens from IgAN patients. They are often correlated with co-deposits of IgG or IgM (Emancipator 1994).

Characteristic of IgMN is predominant diffuse, granular and mesangial IgM deposits found by IF. The glomeruli of IgMN patients are often co-labelled by complement particles. The most frequent complement finding is C3, observed in approximately 30-50% of samples Co-depositions of IgA are also sometimes seen (Saha et al. 1989, Al-Eisa et al. 1996, Kishimoto and Arakawa 1999, Zeis et al. 2001). Deposits of C3 have been detected more frequently in IgMN than in IgM-negative mesangioproliferative glomerulonephritis when IgAN was excluded. Also C1q and/or C4 are more frequent in IgMN patients (Kishimoto and Arakawa 1999). Noteworthy is also the fact that IgMN patients also have significantly higher UPE than those with IgM-negative mesangioproliferative glomerulonephritis. The frequency of C1q depositions in IgMN specimens seems to be approximately 10%-20% (Saha et al. 1989, Kishimoto and Arakawa 1999, Zeis et al. 2001). O'Donoghue and colleagues found no correlation between the intensity and frequency of IgM, IgA, IgG or C3 depositions and renal survival in IgMN (O'Donoghue et al. 1991).

2.4.5. Intrarenal leukocyte infiltrations

Inflammatory cell infiltrations are fairly frequently seen in IgAN. Intraglomerular monocytes and /or macrophages are sometimes found in IgAN patients with focal-segmental histological changes (Emancipator 1994). However, the most prominent leukocyte infiltrations are usually seen in extraglomerular foci. Lymphocytes and macrophages are occasionally seen mostly around glomeruli, in small arteries/arterioles, degenerative changes

in the tubulointerstitium being usually present in the same area (Emancipator 1994). The amounts and types of these inflammatory cells may be evaluated by immunohistochemical methods.

Leukocyte common antigen (LCA, CD45) is a hemopoietic cell-specific tyrosine phosphatase expressed on all leukocytes. It is has an important role in regulating signalling in lymphocytes and is essential for the activation of T-cells. However, CD45 is expressed in several different isoforms depending on the subpopulation, maturation and activation state of cells (Altin and Sloan 1997, Tchilian and Beverley 2006). Simplified, naive human T-cells carry a CD45RA isoform, but after activation CD45RO is expressed. A gene polymorphism and genetic modifiers may alter CD45 expression, which leads to alterations in the threshold of T-cell receptor activation, the regulation of cytokine responses, and lymphocyte survival (Tchilian and Beverley 2006). An altered expression of CD45 has been reported in other diseases such as in systemic lupus erythematosus (Tchilian and Beverley 2006). However, the function of the individual isoforms remains unclear, and on the other hand T-cell functions are related more to the level of CD45 than to which isoforms are present (Tchilian and Beverley 2006).

Leukocytes are also present in normal renal tissue. In glomeruli there are usually more monocytes than other infiltrating cells. T-cells are very rare. Both T-cells and monocytes are present in the interstitium of the normal kidney (Hooke et al.1987). The number of interstitial T-cells, but not glomerular inflammatory cells, is increased in non-proliferative forms of glomerulonephritis (Hooke et al. 1987). Only in aggressive forms of proliferative glomerulonephritis has an increase of glomerular inflammatory cell infiltrations (monocytes and granulocytes) been found (Hooke et al. 1987). Lee and colleagues (1996) observed interstitial infiltration of CD45+ (CD45RA+ and CD45RO+) cells to correlate with serum creatinine concentration at renal biopsy and at the end of follow-up, and with MAP in IgAN. No such correlations were found between glomerular inflammatory cell infiltrations and these clinical factors. Positive CD45 cells were mainly macrophages in glomeruli and lymphocytes in the interstitium. The CD45RO+/CD45RA+ cell ratio was lower in IgAN or in membranous glomerulonephritis than in the normal kidney. As a conclusion to the aforementioned study, it would appear that only the presence of interstitial inflammatory cell infiltrations is associated with impaired renal function in IgAN.

T-lymphocytes typically express CD3 antigen. The CD3 complex, constructed by multiple chains (γ, δ, ε, ζ and η), is linked to the T-cell antigen receptors αβ or γδ. This complex is

essential in signal transduction from actual antigen receptor to the intracellular space. By immunohistochemically evaluated CD3 expression, the amount and localization of T-cells can be investigated. The T-cell appears to be the predominant inflammatory cell type in the tubulointerstitium in all forms of glomerulonephritis (Markovic-Lipkovski et al. 1990). However some numbers of T-cells may also be found in normal renal tissue (Hooke et al. 1987). Interstitial infiltration of CD3+ cells correlates with the occurrence of hypertension and elevated serum creatinine during follow-up in IgAN (Lee et al.1996, Arrizabalaga et al. 2003). Falk and colleagues (1995) found the amount of CD3+ cells to be increased in IgAN child patients with progressive disease. Especially the rate of T-cell proliferation seems to be associated with severe renal tissue damage and poor prognosis in primary glomerulonephritis (Yang et al. 1998, Segerer et al. 1999).

Expression of CD68 is present mainly in macrophages, but also in activated thrombocytes. Hence immunohistochemically evaluated CD68 has been used to localize and quantify macrophages in tissue specimen. Macrophages, with different names in different tissues, are capable of effectively phagocytising foreign material in the human body. Significant glomerular and/or interstitial macrophage infiltrations are seen in most forms of glomerulonephritis (Nikolic-Paterson and Atkins 2001). The role of glomerular macrophages remains unresolved, but interstitial macrophage infiltrations are clearly associated with renal dysfunction at the time of renal biopsy at least in lupus nephritis and IgAN (Nikolic-Paterson and Atkins 2001). Recent evidence shows that macrophages may be the main effectors in T-cell-dependent renal injury. Macrophages may also cause renal injury irrespective of T-cells. Activated macrophages can secrete a wide range of molecules (proinflammatory cytokines, reactive oxygen species etc.) potentially causing renal injury (Nikolic-Paterson and Atkins 2001).

Tubular cells are active components of interstitial inflammation in damaged renal tissue. Tubules are the principal producers of cytokines, chemokines and adhesion molecules, which promote macrophage infiltration and tubulointerstitial damage (Nikolic-Paterson and Atkins 2001). There are occasionally CD68+ macrophages inside the tubules. These may adhere to the leukocyte adhesion molecules on the luminal side of tubular walls. The same cells are also detected in the urine of patients with glomerulonephritis. High amounts of urine macrophages seem to be correlated with interstitial CD68+ cell infiltrations and may indicate the activity and progression of renal disease (Oda et al. 1998, Hotta et al. 2000, Maruhashi et al. 2004). Intrinsic macrophage proliferation found in glomeruli or the tubulointerstitium

may indicate poor prognosis in human glomerulonephritis, for example in IgAN (Yang et al. 1998). The respiratory tract has been proposed to be one of the main foci in initiating abnormal immune response in IgAN. Abe and colleagues found lowering of CD68 expression in IgAN patients after tonsillectomy (Abe et al. 2004). However, the number of interstitial CD68+ cells seems to be equal in IgAN and in non-IgA mesangial glomerulonephritis (Wagrowska-Danilewicz and Danilevicz 2002).

2.4.6. Intrarenal cytokines

Cytokines are protein molecules secreted by different cells to activate or inactivate other cells. The requisite information transfers to a cytoplasm of receiver cells by specific cytokine receptors. The effect of cytokines is not organ-specific, but may influence in any tissue. The term cytokine includes many subgroups such as interleukins, interferons, tumour necrosis factors, different growth factors and chemokines. Cytokines affect with a wide range of actions the mechanisms and symptoms of immune reactions and inflammation at many levels. Some cytokines, so-called proinflammatory cytokines, promote inflammation, while others, so-called anti-inflammatory cytokines, act against inflammation. The present work focused on two significant cytokines, interleukins 1β and 10 (IL-1β and IL-10).

Interleukin-1 (IL-1) is a highly proinflammatory cytokine secreted by many different cell types, for example macrophages, monocytes, T-cells and epithelial cells. The margin between clinical benefit and toxicity is thus particularly narrow (Dinarello 1996). There is an IL-1 gene common to all three members of the IL-1 family; IL-1α, IL-1β and IL-1 receptor antagonist (IL-1Ra). Both IL-1α and IL-1β are synthesized as precursor molecules, and are later activated by specific cellular proteases (Dinarello 1996). Two types of receptors bind IL-1, one which transmits signals (type I) and one inactive (type II), which acts as a sink for IL-1β (Dinarello 1996). The portions of these receptors vary in different states of health and disease, acting as natural buffers for IL-1 effects. In addition to IL-1Ra and the aforementioned buffers, the production and activity of IL-1 is also suppressed by several other cytokines, among them IL-10 (Dinarello 1996). The biological effects of IL-1α and IL-1β are closely similar. However, there are significant differences between these two molecules in the regulation of gene expression and secretion. Also the affinity to receptors is different. It is evident that IL-1α acts primarily as a mediator of local inflammation and regulator of intracellular events, whereas IL-1β is intended to be released from cells and has systemic effects (Dinarello 1996).

Experimental studies have documented IL-1β expression in glomerular endothelial and cortical tubular cells in the normal rat kidney (Tesch et al. 1997a). In experimental anti-GBM glomerulonephritis the expression of IL-1β has been found to be increased in podocytes, mesangial cells, infiltrating macrophages and crescents. However, the most significant increase in IL-1β expression was found in the tubular epithelium due to local IL-1β production (Tesch et al. 1997a). In experimental mesangial glomerulonephritis (anti-Thy nephritis), IL-1β is evidently produced by mesangial cells (Tesch et al. 1997b). Treatment with IL-1 receptor antagonist (IL-1Ra) has reduced mesangial cell proliferation and glomerular accumulation of macrophages, also reducing significant PU. In human IgAN, intrarenal IL-1β gene transcripts were higher in patients with marked PU than in other IgAN patients (Lim et al. 2003). Podocytes appear to be one of the main sites of IL-1β secretion in human glomerulonephritis (Niemir et al. 1997). In human extracapillary glomerulonephritis with anti-neutrophil cytoplasmic antibodies IL-1β+ mononuclear cells have been detected both in glomeruli and in the interstitium, while in IgAN IL-1β was absent in glomeruli (Waldherr et al. 1993). In IgMN, IL-1 gene expression has been at a higher level than in the normal kidney in glomeruli, but also in tubulointerstitial tissue (Chen and Lin 1994). Yoshioka and colleagues also found interstitial expression of IL-1β in IgAN (Yoshioka et al. 1993). Even though increased IL-1β expression seems to correlate with high-grade histopathological lesions such as tubular atrophy or crescent formation, in glomerulonephritis in general there is no clear evidence that intrarenal IL-1β is associated with any clinical or histopathological factors in IgAN (Taniguchi et al. 1996, Lim et al. 2003).

Interleukin-10 (IL-10) is an anti-inflammatory cytokine traditionally secreted by T-helper cells, B-cells, monocytes and macrophages. The main function of IL-10 is to limit or terminate inflammation by inhibiting a wide range of immune parameters such as cytokine production, antigen presentation, T-cell proliferation and inflammation mediated by proinflammatory cytokines (Moore et al. 2001). It also has multifunctional role in growth and differentiation on most hemopoetic cells, dendritic cells, keratinocytes and endothelial cells (Moore et al. 2001).

In the normal kidney IL-10 expression (mRNA) seems to be present in constitutive amounts in glomerular tufts, in capsular and tubular epithelial cells, smooth muscle cells of the arteries and in the interstitium (Niemir et al. 1998). However, IL-10 molecules have been found present only in trace amounts in renal arteries and tubular epithelial cells. Marked

glomerular proliferation in IgAN and acute vascular lesions in microscopic polyangitis are related to a marked up-regulation of IL-10. High-grade PU correlates with increased expression of tubulointerstitial IL-10 in human glomerulonephritis (Niemir et al. 1998). It seems that the principal producers of IL-10 in human glomerulonephritis are resident renal cells. Lim and colleagues found the level of IL-10 gene transcripts to be associated with the presence of tubular atrophy and interstitial fibrosis in IgAN, IL-10 expression being seen mainly in tubules (Lim et al. 2003).

2.5. Clinical course

2.5.1. Prognosis

Traditionally IgAN has been regarded as a benign disease. However, a substantial proportion of IgAN patients end up with terminal renal failure and permanent renal replacement therapy and/or renal transplantation (Figure 4).

The overall prognosis of IgAN and IgMN reported in different studies from different countries depends significantly on the diagnostic approach adopted. In some countries no renal biopsies are taken in cases with only microscopic HU and/or low grade PU. In Finland biopsies have also been performed in HU patients. In Japan, routine urinalysis screening of school children is in use and brings out a large population with asymptomatic urinary abnormalities. Many patients can be found with mild disease and good prognosis. For these reasons an accurate assessment of worldwide prognosis of IgAN and IgMN is impossible.

According to recent review articles, 20 to 30% of IgAN patients reach ESRD within 20-25 years (Floege and Feehally 2000, Barratt and Feehally 2005). Altogether about 15 to 40% of IgAN patients may eventually have ESRD (Donadio and Grande 2002). On the other hand actuarial renal survival has been suggested to be 80 to 85% at ten years. In Finland the ESRD rate seems to be slower (5%), possibly due to the local diagnostic procedures (Mustonen et al. 2001). In childhood IgAN renal survival has reported to be 90% in 15 years and 87% in 20 years of follow-up (Ronkainen et al. 2006). Renal insufficiency is usually chronic and progresses slowly (Donadio and Grande 2002).
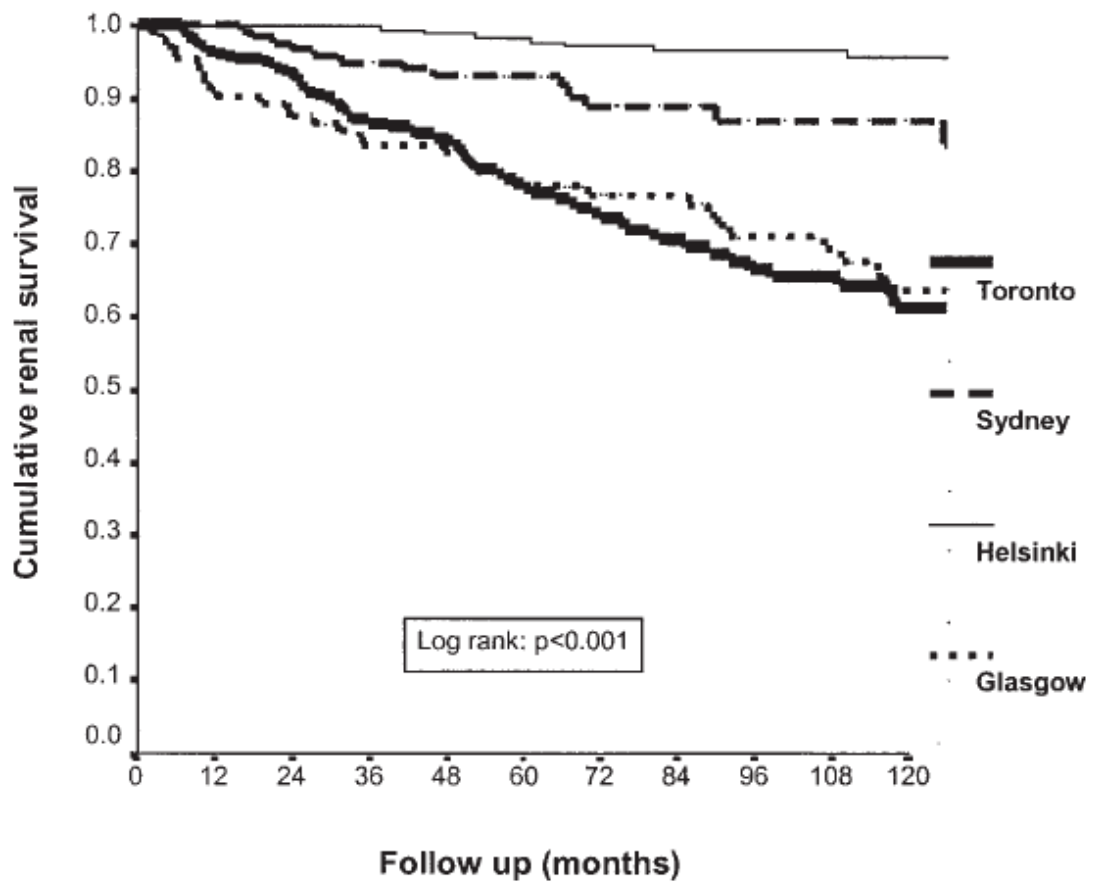
Figure 4. Comparison of renal survival between four geographically distinct centres (Geddes et al. 2003).

In the case of IgMN there are even more variable diagnostic approaches than in IgAN. Even the diagnostic criteria vary in different studies. We lack proper IgMN studies with sufficient material for an evaluation of the incidence of terminal renal failure. Saha and colleagues (1989) found 9/54 (17%) IgMN patients to have renal insufficiency and 3/54 (6%) have ESRD at the end of five years' post-biopsy follow-up. In a British IgMN population, including patients with segmental sclerosis, 20% reached ESRD in five years and 36% in ten years (O'Donoghue et al. 1991). Among Irish IgMN patients the prevalence of

ESRD seems to be 7% after 7.4 years' follow-up (Little et al. 2000). Noteworthy is that the age of patients has varied somewhat in these studies.

2.5.2. Risk factors for progression in renal disease

During recent years a number of papers have been published reporting factors indicating poor prognosis in IgAN. Many clinical, familial, histopathological, immunological and genetic risk factors have been found to predict the progression of renal impairment or terminal uremia. However, many of these parameters have been investigated by simple univariate analysis, and the independence of these factors has not been established. It has commonly been accepted that older age, hypertension, high glomerular histopathological scores, severe tubulointerstitial changes, high-grade PU, persistent microscopic HU and impaired renal function at the time of renal biopsy are strong risk factors for the progression of IgAN (Ibels et al. 1998, Mustonen et al. 2001, Donadio and Grande 2002). D'Amico graded elevated serum creatinine level, severe PU, marked glomerular and/or tubulointerstitial lesions and high classes in histopathological grading systems to be the strongest risk factors for progression in IgAN (D'Amico 2000). Crescentic IgAN usually shows rapid progression correlating with severe changes in glomeruli, the tubulointerstitium and blood vessels, and high-grade UPE (Tang et al. 2002, Bitencourt-Dias et al. 2004, Barratt and Feehally 2005). Many studies have shown a high renal survival rate in IgAN patients with recurrent periods of macroscopic HU (Ibels et al. 1998).

Among clinical factors hypertension has been proved to be the strongest and apparently independent risk factor for progression in IgAN. The relationship between renal diseases and hypertension was noted in section 2.3.2. The progression seems to be rapid when malignant hypertension exists (Subias et al. 1987). Also so-called "white coat hypertension", or the absence of diurnal blood pressure variation, measured by ambulatory blood pressure monitoring, may accelerate progression (Csiky et al. 1999). Further, a family history of hypertension may be sufficient to elevate the risk (Li et al. 2002). In any case, antihypertensive medication seems to improve the renal prognosis of hypertensive IgAN patients (Payton et al. 1988, Woo et al. 1988, Kanno et al. 2000).

Biochemical factors associated with the metabolic or insulin resistance syndrome and the development of atherosclerosis and cardiovascular diseases have recently been a focus of intensive research in renal diseases such as IgAN. Serum lipid levels, with high total and LDL cholesterol and often high triglyceride and low HDL cholesterol, are usually abnormal

in nephrotic patients (Wheeler 2001). There are some clues to indicate that both medical and dietary interventions improving lipid levels also reduce PU in NS (D'Amico and Gentile 1992, Stenvinkel et al. 2000, Muso et al. 2001, Vaziri and Liang 2004). A substantial proportion of patients with IgAN have elevated cholesterol and triglyceride levels (Ibels and Gyory 1994, Ibels et al. 1998, Syrjänen et al. 2000).

Body mass index (BMI) is markedly associated with the level of blood pressure in humans. Recently a study of the role of elevated BMI in predicting a poor outcome in IgAN has been published (Bonnet et al. 2001). Previous to this Hall and colleagues brought out mechanisms by which obesity may induce the development of impaired renal function (Hall et al. 2003a, Hall et al. 2003b). Insulin resistance occurs in most patients with long-term obesity, and insulin resistance correlates independently with hypertension in IgAN (Eiro et al. 2003). Recently Kaartinen and colleagues found that insulin resistance seems to be a risk factor for progression in IgAN (Kaartinen et al. 2007).

In addition to elevated BMI, insulin resistance and hypertension, hyperuricemia and hyperlipidemia, especially high serum triglyceride levels, have been connected to the metabolic syndrome. Two recent studies reported that elevated serum cholesterol could affect adversely the prognosis of IgAN (Li et al. 2002, Rauta et al. 2002). These findings were not confirmed in a study by Syrjänen and colleagues, where an elevated total serum cholesterol level did not correlate significantly with progression, whereas hypertriglyceridemia did (Syrjänen et al. 2000). High triglycerides as well as low high-density lipoprotein levels seem to aggravate declining renal function in the general population without elevated serum creatinine at baseline (Muntner et al. 2000). It would also appear that lipid-lowering therapy with statins may have an antiproteinuric effect in IgAN (Buemi et al. 2000, Nakamura et al. 2002). However, the positive effect of statins on long-term survival of renal function in NS remains unproven.

Hyperuricemia is often present in association with the metabolic syndrome. An elevated serum uric acid concentration is commonly assumed to be a result of decreased uric acid clearance through a damaged kidney. However, uric acid may itself, even in lower concentrations, promote impairment of renal function. Some of the possible mechanisms were presented above in section 2.4.1. Three recent studies have shown that elevated uric acid levels may have role as an independent risk factor for progression in IgAN (Syrjänen et al. 2000, Ohno et al. 2001, Rauta et al. 2002). However, no investigations have been made concerning the effect of uric acid-lowering therapy specifically on the natural course of

IgAN or other forms of glomerulonephritis. Nonetheless, hyperuricemic patients with mild- or moderate-degree chronic renal disease may benefit from allopurinol treatment (Siu et al. 2006).

Individual studies have reported that male gender (Frimat et al. 1997, Rauta et al. 2002), duration of preceding symptoms and age (Barratt and Feehally 2005), diabetes (Syrjänen et al. 2000), number of casts in the urine and elevated serum β-globulins seen by electrophoresis (Ibels et al. 1998) are associated with poor prognosis in IgAN. A wide range of genetic factors have been connected to the development of IgAN and its prognosis. Especially polymorphisms of angiotensin-converting enzyme, angiotensin II receptor type 1, uteroglobin, neuropeptide Y, T-cell receptor, IL-1Ra, tumour necrosis factor α and HLA molecules have been under study. The present knowledge of these correlations has been summarized in recent review articles (Hsu et al. 2000, Mustonen et al. 2001).

Even though the serum IgA level is usually elevated in IgAN, it may not have role as an independent risk factor for progression. However, recent studies have shown that serum IgA in unison with serum C3 may be of prognostic relevance. An elevated serum IgA/C3 ratio correlated significantly with a prognostic grading system in IgAN (Ishiguro et al. 2002, Maeda et al. 2003, Komatsu et al. 2004). The studies concerned did not examine the direct effect of IgA/C3 ratio on the long-term prognosis. Ibels and colleagues found serum C4 to be associated with a poor natural course of IgAN (Ibels et al. 1998). The presence or intensity of glomerular IgA, C3, IgM and IgG has in some studies been found to predict outcome in IgAN (Ibels et al. 1998, Nieuwhof et al. 1998).

Even though expressions of cytokines and leukocyte infiltrations have been found to correlate significantly with a variety of clinical, biochemical or histopathological risk factors, their direct effect on progression has not be fully proven. Two studies have focused on the presence of marked glomerular and tubulointerstitial cell proliferation approximated by MIB-1 expression (Nabokov et al. 1997, Radford et al. 1997). Especially tubulointerstitial MIB-1 expression, present mainly in tubular epithelial and infiltrating cells, correlated with serum creatinine, interstitial fibrosis and clinical progression in IgAN. On the other hand, cell proliferation may be caused by exposure of renal cells to activating cytokines or conversely may lead to enhanced cytokine production.

Many different light-microscopy grading systems have been used in evaluating histopathological risk factors for progression in IgAN. Some investigators have graded many single histological changes in renal biopsy specimens, while others have combined

histological changes prior to activity or localization. Probably the most widely used individual grading systems are those of Haas (1997) and Lee (1982), where many different histopathological changes have been combined into five classes. As a conclusion to all studies, severe lesions would appear to be markers of poor prognosis in IgAN regardless of their localization. Some authors have found, prior to other histopathological or clinical factors, severity of tubulointerstitial changes to be the most powerful histopathological predictor of outcome in IgAN (Shu et al. 1999, Daniel et al. 2000, Mera et al. 2000).

It is of note that some patients with only minimal urinary abnormalities and initially normal renal function may evince severe renal histopathological changes indicating poorer prognosis (Shu et al. 1999). On the other hand, certain patients with at most minimal histological changes seen in renal biopsy may reach ESRD (Koyama et al.1997). It is necessary to evaluate both clinical and histopathological risk factors to determine the prognosis of individual patients.

Very little is known regarding risk factors for progression of ESRD in IgMN. IgMN patients with nephrotic syndrome as their initial manifestation appear to have more frequently a poor prognosis (Little et al. 2000). O'Donoghue and colleagues found persistent HU, UPE, serum creatinine, the amount of global sclerosis, segmental glomerulosclerosis, mesangial proliferation and the nature of mesangial content to correlate with renal survival in univariate analysis (O'Donoghue et al. 1991). In multivariate analysis only microscopic HU, marked mesangial proliferation and excessive global sclerosis emerged as independent risk factors for developing ESRD in IgMN.

2.6. Vascular diseases

2.6.1. Epidemiology of vascular diseases in the general Finnish population

Like in other western countries cardiovascular diseases are the most common cause of death in Finnish population. According to Statistics Finland ischemic heart disease or cerebrovascular disease was the main cause of death in 21% among working-aged Finnish males. The proportion of vascular disease is even bigger in the older age groups. According to Statistics Finland an age-standardized death rate by Diseases of the circulatory system has been about 330/100000 per year in the whole population during 2000-2005. According to National Public Health institute of Finland the age-standardized cumulative incidence of

cerebrovascular attacks or deaths was 509/100000 in males and 312/100000 in females in 2004. The age-standardized cumulative incidence of ischemic heart disease or cardiovascular death was 1093/100000 in males and 405/100000 in females (Statistical database of Finnish National Cardiovascular Diseases Register).

2.6.2. Risk factors for vascular diseases in the general population

It is widely accepted that older age, male gender, hyperlipidemia, high blood pressure, diabetes mellitus (DM) and smoking are risk factors for vascular diseases in the general population. Lipid lowering and antihypertensive medication as well as strict glucose control in DM will reduce this risk. More questionable has been the role of uric acid in vascular diseases. In many population-based studies elevated serum uric acid has been found to be independently associated with cardiovascular mortality and morbidity (Alderman 2002). The association seemed to be more significant among women. Culleton and colleagues (1999) obtained no such results when attention was limited to white females or males in the Framingham study. However, the populations in these studies were not identical (Alderman 2002). The principal studies concerning uric acid as a risk factor for cardiovascular disease are summarized in a review by Michael W Rich (2000). In all those studies elevated uric acid correlated with vascular diseases in univariate analysis, but in some the relationship was not independent (Rich 2000). High uric acid levels may reflect subclinical renal insufficiency.

Certain anatomical or physiological abnormalities such as large-artery atherosclerosis, mechanical heart valve, arterial dissection, patent foramen ovale or atrial fibrillation involve an elevated risk for ischemic stroke. In addition to the aforementioned traditional risk factors also elevated serum homocysteine, C-reactive protein or lipoprotein-associated phospholipase A2 levels may be associated with an increased risk of stroke (Gupta and Krieger 2005). The role of uric acid in the context of stroke is less clear. Nevertheless, in some studies hyperuricemia has been found to be an independent predictor of stroke morbidity and mortality (Becker and Jolly 2006). Elevated C-reactive protein, homocysteine as well as lipoprotein(a) and sitosterol may also constitute risk factors for coronary heart disease (Wilson 2004).

2.6.3. Vascular diseases in patients with renal disease

Patients with chronic renal insufficiency are at high risk of cardiovascular diseases (CVDs). They are more likely to die of CVDs than to develop terminal kidney failure

(Sarnak et al. 2000). In 1996-2000 cardio- and cerebrovascular disease was the cause of death in 44-60% of Finnish dialysis patients (Finnish Registry for Kidney Diseases). Infections were also fairly common, but kidney disease itself was registered as the cause of death in only 2-10%. Cardiovascular morbidity is also particularly prevalent in renal patients. Angina pectoris was present in 21% of all Finnish patients at the onset of renal replacement therapy in 2000-2001; 26-29% had left ventricular hypertrophy and 10-11% heart failure. Over one fifth of renal patients over 65 years of age had myocardial infarction in their history when initiating dialysis. Previous cerebral infarction or hemorrhage was present in 10% of Finnish renal patients at the onset of renal replacement therapy in 2000-2001.

Patients with renal insufficiency frequently have both small-vessel atherosclerosis and arteriolosclerosis or remodeling in larger arteries (Sarnak et al. 2000). Anatomical and pathophysiological cardiovascular changes found in chronic renal failure are summarized in Figure 5.

The risk of cardiovascular diseases correlates strongly with the level of renal dysfunction (Manjunath et al. 2003). Dialysis patients have a 10 to 30 times higher CVD mortality rate than the general population (Sarnak et al. 2006). This may be caused by an elevated frequency of traditional cardiovascular risk factors in patients with renal diseases (Muntner et al. 2004). Almost all patients have hypertension at the onset of dialysis therapy, 81-86% in the Finnish population (Finnish Registry for Kidney Diseases). A total of 47-52% of Finnish renal patients had hyperlipidemia at the time of initiation of renal replacement therapy. Diabetes mellitus was clearly the most common individual reason for initiating dialysis in Finland in 2000-2005 (Finnish Registry for Kidney Diseases). However, uremia may itself cause insulin resistance (Fliser et al. 1998). It also seems evident that good control of these risk factors with adequate medical therapy may improve the prognosis.
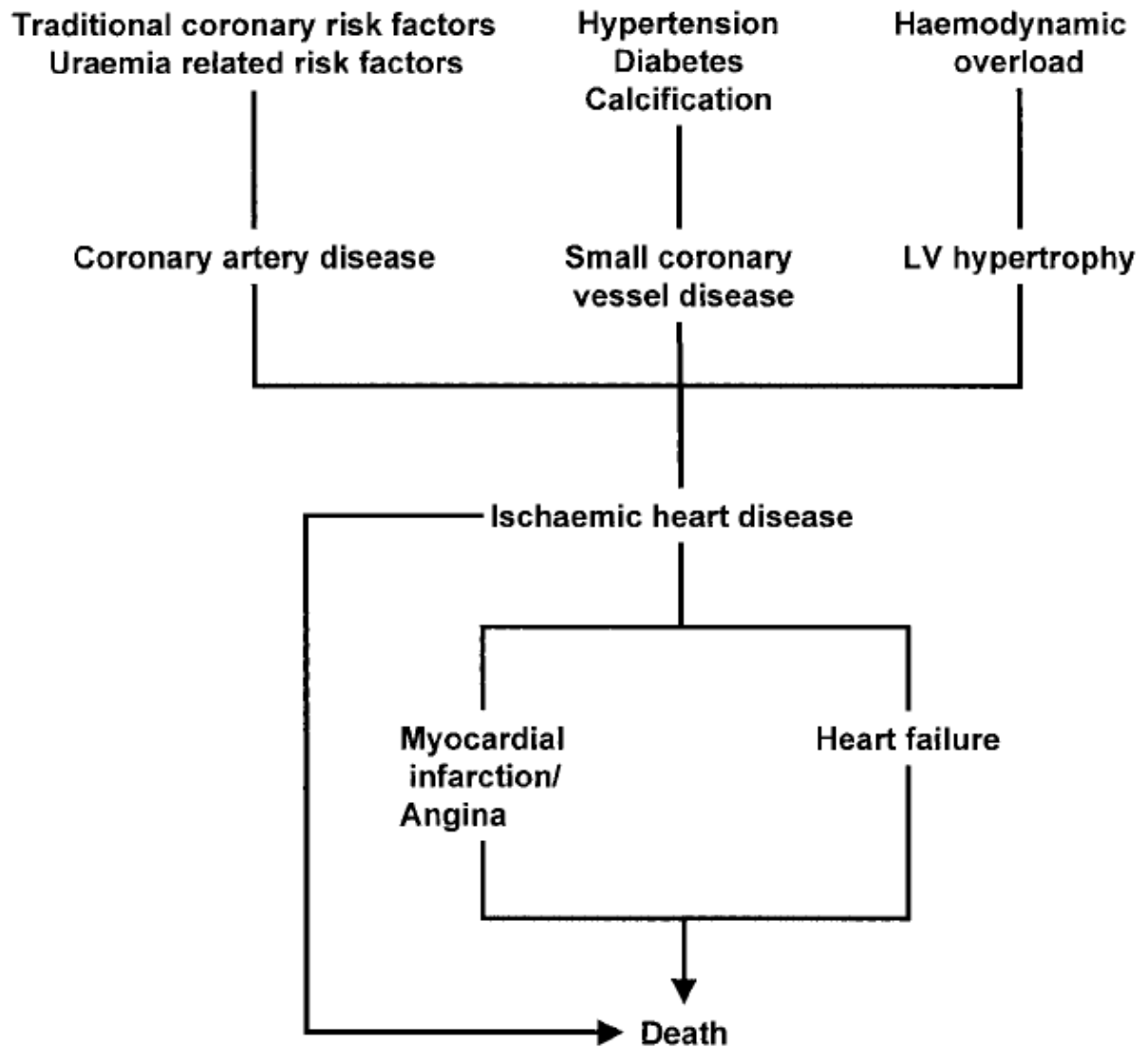
Figure 5. Development of ischemic heart disease in chronic renal failure (Parfrey 2000)

Certain nontraditional CVD risk factors are also encountered among patients with chronic kidney disease (Sarnak et al. 2000). Especially albuminuria, anemia, abnormal calcium/phosphate metabolism, extracellular fluid overload, electrolyte imbalance, thrombogenic factors and inflammation are often found in persons with clinical kidney disease. Likewise patients with minor renal dysfunction seem to have an elevated risk of CVD (Ritz 2003, Zhang et al. 2006). It is not, however, clear whether patients with diagnosed kidney disease without clinical renal dysfunction are at greater risk of CVD in comparison with the normal population. Certain pathophysiological changes seem to be

present even when GFR is still normal (Ritz and McClellan 2004). For example, early activation of the sympathetic nervous system, oxidative stress and microinflammation, abnormal apolipoprotein patterns, abnormal concentrations of an inhibitor of nitric oxide synthase and left ventricular dysfunction have been documented in patients with kidney disease when GFR was normal (Ritz and McClellan 2004). Through these abnormalities patients with subclinical renal disease may also frequently suffer from CVD manifestations.

Serum cystatin C approximates direct measures of GFR (Filler et al. 2005). Shlipak and colleagues (2006) found cystatin C to correlate significantly with the incidence of CVD manifestations in elderly subjects without kidney dysfunction as detected by serum creatinine or estimated GFR measurements. It is striking that about half of nephrons are damaged when serum creatinine exceeds the upper normal limit or estimated GFR decreases. The overall renal parenchymal metabolic function may have been markedly decreased far before that limit. This phenomenon may be detected by serum cystatin C measurement. There are no reported data on the CVD risk in the context of different primary kidney diseases, especially glomerulonephritis. Based on the aforementioned risk factors, it seems clear that glomerulonephritis causing NS with hypertension, hyperlipidemia and peripheral edema, involves an increased risk of CVD, as is also the case in glomerulonephritis caused by vasculitis with systemic inflammation. Far less unambiguous is the CVD risk in patients with primary forms of glomerulonephritis such as IgAN without high grade PU or renal insufficiency.

3. AIMS OF THE STUDY

The purpose of the present study was to obtain further information on the clinical picture and prognosis of two forms of primary glomerulonephritis, IgA and IgM nephropathy. The main effort was focused on histopathological and immunological changes in renal tissue and their relations to the key clinical parameters. In addition to factors affecting the natural course of renal function, it was also sought to clarify the overall survival of IgA nephropathy patients by investigating the epidemiology of cardiovascular disease in this population. The specific aims were to study:

1. the associations of clinical risk factors with histopathological findings in IgAN and IgMN **(I, IV)**

2. the significance of immunological parameters reflecting interstitial inflammation for renal survival in IgAN **(II)**

3. the occurrence of vascular diseases and their risk factors in IgAN and compare it with that in the general population **(III)**

4. the clinical and histopathological features and risk factors for progression in IgMN **(IV)**

5. the role of serum and intrarenal complement particles and immunoglobulins in the clinical course IgMN and to compare this with IgAN **(V)**

4. SUBJECTS AND METHODS

4.1. Subjects and data collection

4.1.1. Patients with IgAN (I-III)

The original study population comprised all 223 IgAN patients diagnosed in Tampere University Hospital during a period of 11 years from January 1980 to December 1990. All subjects were Caucasians. Tampere University Hospital is the only center where renal biopsies were performed in the residential area of Pirkanmaa, Finland, with about 440 000 inhabitants. IgAN was diagnosed when there was IgA as the sole or predominant glomerular IF finding in renal biopsy. Of all IgAN cases 131 (64%) were men and 73 (36%) women. Their median age was 41 years (range 16-78) at the time of renal biopsy; 166 of them had normal serum creatinine level. None had systemic lupus erythematosus or liver cirrhosis, but 11 had one or more signs or symptoms suggestive of Henoch Schonlein purpura. A summary of the baseline data is presented in Table 1. In the original studies I and II 221 adult IgAN patients were studied. In comparing the prevalence of vascular diseases between IgAN patients and the general population, 203 patients ≥30 of age at the end of follow-up were included (III).

4.1.2. Patients with IgMN (IV-V)

The present material comprised all IgMN patients whose renal biopsies had been evaluated in Tampere University Hospital during a period of 21 years from October 1977 to July 1998. Of the 2217 biopsies 110 met the criteria for IgMN. The diagnostic criteria are presented in section 2.2.1. All subjects were Caucasians. Patients with systemic disease (SLE, rheumatoid arthritis, diabetes mellitus and paraproteinemia) were excluded. Of the patients studied 63 (57 %) were males and 47 (43 %) females. The mean age was 29 years, ranging from 1 to 75; 36 (33 %) cases were children (<16 years). A summary of the baseline data is presented in Table 1.

Table 1. Clinical findings in IgAN and IgMN patients at time of renal biopsy.

Finding	No. of patients (%)	
	IgAN	IgMN
Age ≥ 41 years	112 (51)	34 (31)
Male gender	140 (63)	63 (57)
Hypertension*	116 (53)	49 (45)
Nephrotic syndrome	6 (3)	50 (46)
Proteinuria ≥ 1g/24 h	65 (29)	63 (57)
Microscopic HU	205 (93)	33 (32)
Macroscopic HU in history	62 (28)	-
Renal insufficiency	42 (19)	16 (15)
Hyperuricemia[a]	56 (30)	-
Hypertriglyceridemia[b]	69 (36)	-
Hypercholesterolemia[c]	113 (61)	-
BMI ≥ 27 kg/m²	65 (29)	-
Smoking**	93 (42)	-
Diabetes mellitus	10 (5)	0

HU= hematuria, BMI= body mass index. [a]Measured in 189 pts; [b]Measured in 191 pts; [c]Measured in 188 pts. *Blood pressure ≥140/90 or usage of antihypertensive medication, **Ex-smoker or current smoker, - = no data.

4.1.3. Controls (III)

A control group, comprising 203 persons ≥30 years of age, was collected in the same residential area to compare the prevalence of certain vascular diseases between IgAN patients and the general population. The control group matched for age (at the end of follow-up), sex and residential area was constructed from the Health 2000 Survey (H2000), a large cross-sectional health examination survey carried out by the National Public Health Institute in 2000-2001. The implementation of the survey is described in detail elsewhere (Aromaa et al. 2004).

4.1.4. Clinical data collection and follow-up

The baseline clinical data on both IgAN and IgMN patients were collected from patient records. IgAN patients were prospectively studied. The follow-up of IgAN patients ended at the control visit, including comprehensive clinical examination and adequate laboratory measurements during the years 1996-1997, or if the patient died. Of all IgAN patients 178 (80%) attended the control assessment. Fifteen had moved away from the area. The median follow-up time after renal biopsy was 10 years (range, 0.2-17). One hundred and ninety seven patients were followed for at least 5 years, 119 for at least 10 years and 46 for at least 15 years. Causes of death were confirmed from patient records and death certificates kept by Statistics Finland. Thirty patients (14%) died during the follow-up, six of them due to myocardial and three due to cerebral infarction. The data on VD morbidity in patients with IgAN were based on patient records and careful clinical examination at the control visit or on the last available check in the case of those who died during the follow-up or were not available for the control visit. CHD included a history of myocardial infarction or angina pectoris, CeVD a history of transient ischemic attack or cerebral infarction and any VD occurrence of CHD and/or CeVD in IgAN All VDs were clinically diagnosed by a physician. In H2000 information on CHD and CeVD was elicited in the home health interview. Subjects were asked whether a doctor had diagnosed myocardial infarction, angina pectoris or stroke (cerebral hemorrhage or thrombosis).

The mean post-biopsy follow-up time for IgMN patients was 8 years (range, 0-20). One hundred and six patients were followed for at least one year, 64 for at least 5 and 44 for at least 10 years, and in 22 cases the follow-up continued for 15 years or more. Also the clinical retrospective follow-up of IgMN patients was based on the medical records of Tampere University Hospital, other hospitals or health care centers.

4.2. Methods

4.2.1. Clinical definitions and laboratory measurements

Serum creatinine values ≤ 125 μmol/L in men or ≤ 105 μmol/L in women were considered normal and in pediatric patients $> +2$ SD of the mean of healthy children of corresponding age. Progression of kidney disease was defined as an elevation of serum creatinine value above the normal limit and more than 20% from baseline. Based on these definitions, the

patient population was divided into those with stable renal disease (s-group) and those with progressive course (p-group) **(III)**. End-stage renal disease (ESRD) was in question when the patient needed chronic renal replacement therapy.

Blood pressure was measured by sphygmomanometer after rest. In IgAN studies **(I-III)** hypertension was defined as systolic blood pressure over 140 mmHg and/or diastolic blood pressure over 90 mmHg or use of antihypertensive medication in both IgAN patients and controls. An IgMN patient was regarded as hypertensive if the resting blood pressure was ≥145/95 mmHg in adults **(IV, V)**. In children, classification of hypertension was made taking age and sex into account (Anonymous 1996) **(IV, V)**. Mean arterial pressure (MAP) was calculated by the formula: diastolic blood pressure + 1/3 * (systolic blood pressure − diastolic blood pressure).

Determination of body mass index (BMI) was based on measured height and weight. It was calculated as weight in kilograms divided by the square of height in meters. Elevated BMI was defined as > 27 kg/m².

Patients with IgAN were divided into two groups according to their smoking habits: patients who had never smoked, i.e. non-smokers, and patients who had smoked earlier or were smokers at that time, i.e. ex-smokers or current smokers.

Measurement of urine protein excretion (UPE) was based on 24 h urine collection. The nephrotic syndrome (NS) was defined as UPE =3.5 g/24 hours, decreased serum level of total protein <60 g/L or/and albumin <30 g/L, and edema. Proteinuria was present when UPE was >0.15g/24 h. This definition was used to classify IgMN patients into the different groups according to their clinical manifestation. In evaluating the role of PU as a risk factor for progression or vascular diseases, the amount of urine protein excretion 1g/24h or more was used as a definition of significant PU. The definition of hematuria (HU) was three or more erythrocytes per high-power field (hpf) of urinary sediment.

Serum uric acid was measured in 189 (85%) IgAN patients. Hyperuricemia was defined as serum uric acid >0.45 mmol/l in men and >0.34 mmol/l in women. Two IgAN patients were using allopurinol for gout at the time of biopsy.

Serum cholesterol and triglyceride concentrations were measured enzymatically after overnight fast at the time of biopsy. Serum triglyceride values were studied in 191 (86%) and cholesterol in 188 (84%) of all IgAN patients. Hypertriglyceridemia was defined as a serum triglyceride concentration >1.7 mmol/l, and hypercholesterolemia as a serum cholesterol

concentration >5.0 mmol/l. None of the IgAN patients was using lipid-lowering medication at time of biopsy. In H2000 measurements of cholesterol and triglyceride levels and BMI were performed during programmed health examinations after a fast of at least four hours. For more strict definitions of the methods of H2000, see the baseline results of H2000 (Aromaa et al. 2004)

Concentrations of serum IgA (SIgA), IgG (SIgG), IgM (SIgM), C3 (SC3) and C4 (SC4) were measured by single radial immunodiffusion using commercial equipment (Behring AG) or using a Behring Laser-Nephelometer (Hoechst AG, Frankfurt, Germany) according to the manufacturer's recommendations, the results obtained by these methods being almost equal at least in measuring serum IgA concentrations (Mustonen et al. 1981). SIgA and SIgG were measured in 89, SIgM in 87, SC3 in 90 and SC4 in 87 patients. The normal range of serum immunoglobulins and complement was defined as the mean ± 2 SD of the values of healthy persons of corresponding age and gender. Serum sampling occurred at the time of renal biopsy.

4.2.2. Grading of histopathological changes by light microscopy

A renal biopsy specimen was considered representative for LM and IF examination if it contained four or more glomeruli. Of IgAN cases 202 and all 110 IgMN patients fulfilled this criterion. Paraffin sections for LM were stained by the hematoxylin-eosin (HE), periodic acid-Schiff reaction (PAS), Masson's trichrome and periodic acid-silver methenamine methods. Histopathological evaluation has been made by one investigator without knowledge of the clinical data. Mesangial cellularity, glomerulosclerosis, tubular atrophy, interstitial fibrosis, interstitial inflammation, hyaline arteriolosclerosis and arterial intimafibrosis were evaluated (Figures 6-7).
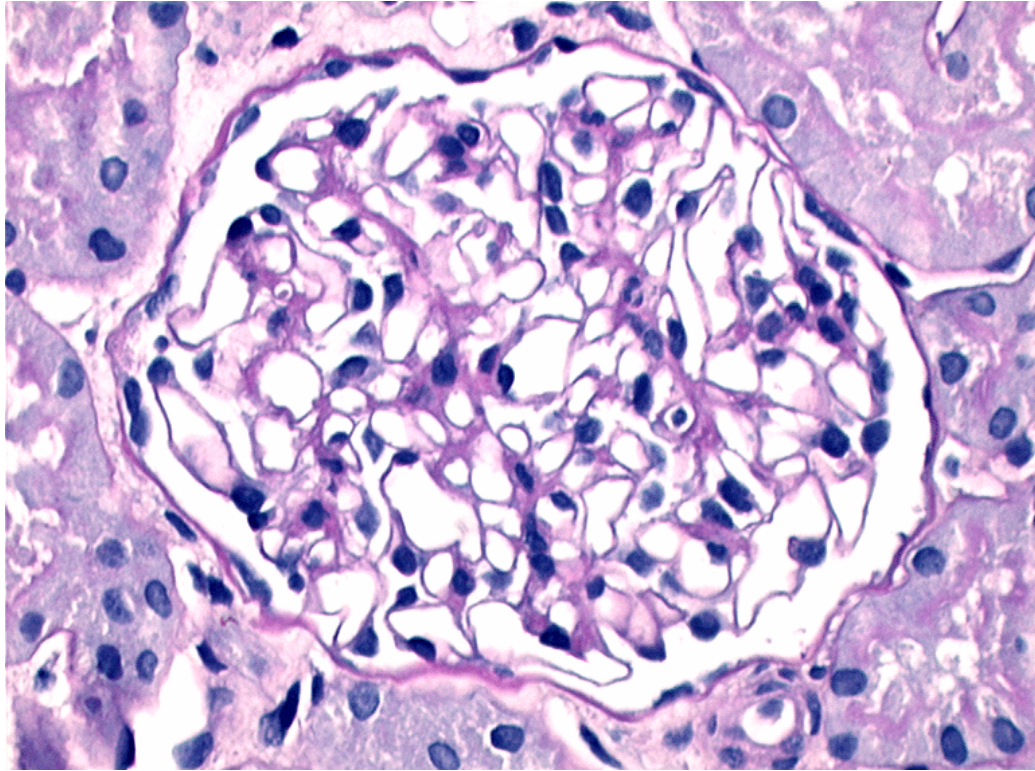
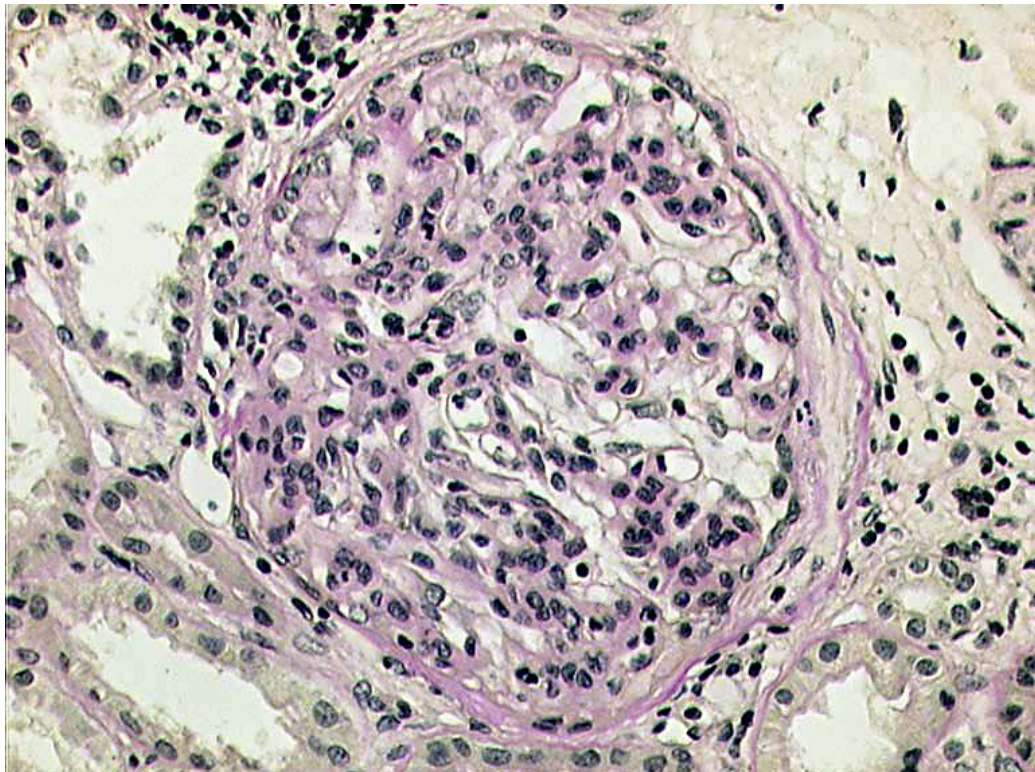Figure 6. Glomerulus with normal morphology (IgMN). PAS-HE staining, original magnification, x400.

Figure 7. Glomerulus with marked mesangial hypercellularity and mild glomerulosclerosis (IgAN). PAS-HE staining, original magnification, x400

In IgMN the histological parameters mentioned were semiquantitatively graded into four groups as normal, mild, moderate and marked **(IV)**. In view of the rareness of the marked-grade histopathological changes, in further studies **(I-III, V)** moderate and marked classes were combined into one. Thereafter the grouping was normal, mild and marked. The grading was mainly modified after the revised Banff 97 classification for renal allograft pathology (Racusen et al. 1999). The histopathological grading scheme used in the present study is presented in Table 2.

Table 2. Histopathological classification used in the present study (Reprinted with permission from: Myllymäki et al. *Nephrol Dial Transplant*, 20: 89-95. © ERA-EDTA)

Histopathological change	Definition
Mesangial cellularity[a]	
Normal	Under four cells per mesangial zone
Mild hypercellularity	Mesangial cellularity 1–2× normal cellularity
Marked hypercellularity	Mesangial cellularity >2× normal cellularity
Glomerulosclerosis[a]	
Normal	Thickness of mesangial matrix around the mesangial cell nucleus is less than one diameter of nucleus
Mild	Thickness of mesangial matrix is 1–2.5× diameter of nucleus
Marked	Thickness of mesangial matrix is >2.5× diameter of nucleus
Tubular atrophy	
Normal	No significant tubular atrophy
Mild	Tubular atrophy in up to 25% of cortical tubules
Marked	Tubular atrophy in >25% of cortical tubules
Interstitial inflammation	
Normal	No significant infiltrations
Mild	Up to three local infiltrations involving up to 25% of interstitium
Marked	Many local infiltrations involving >25% of interstitium or diffuse infiltration
Interstitial fibrosis	
Normal	Interstitial fibrosis in up to 5% of cortical area
Mild	Interstitial fibrosis in 6 to 25% of cortical area
Marked	Interstitial fibrosis in >25% of cortical area
Hyaline arteriolosclerosis	
Normal	No hyaline thickening
Mild	Mild to moderate PAS-positive hyaline thickening in at least one arteriole
Marked	Moderate or severe PAS-positive hyaline thickening in more than one arteriole
Arterial intimafibrosis	
Normal	No changes in intima
Mild	Arterial narrowing of ≤25% lumenal area by intimafibrosis[b]
Marked	>25% Narrowing of vascular lumenal area[b]

[a]Average of all glomeruli seen in biopsy.
[b]In most severely affected vessel.
[c]Periodic acid Schiff.

For multivariate analysis, the aforementioned histopathological parameters were further graded into two groups; normal and abnormal (mild or marked) (**I**). The relative number of glomeruli with extracapillary proliferation was also observed in IgAN, and totally obliterated glomeruli were studied in both diseases. Extracapillary proliferation was very rare in the context of IgMN, and was not systematically investigated. The histopathological evaluation was performed without knowledge of patients' clinical data of the patient in question.

4.2.3. Immunofluorescent (IF) study

The data on IF procedure were collected from forms routinely used in the diagnostics of kidney diseases in Tampere University Hospital. For direct IF studies, monospecific antisera against the heavy chains of human immunoglobulins (IgG, IgM, IgA), complement (C3, C1q), fibrinogen, light chains (kappa, lambda) and albumin (Behring AG 1977-1987 and DakoCytomation Denmark A/S, Glostrup, Denmark 1987-1998) were used. Glomerular IF findings were graded as -, +/-, +, ++, +++ in study **IV** and negative or positive in study **V**.

4.2.4. Immunohistochemical methods (I, II)

Tissue specimens were available for immunohistochemistry from 176 patients, 146 of them having initially normal renal function. Renal tissue was available from 163 patients for IL-1β and IL-10 studies. For light-microscopic immunoperoxidase staining, 3-μm paraffin sections were cut onto ChemMate^{TM} capillary gap microscope slides (DakoCytomation Denmark A/S). Proliferation activity in the mesangial areas was investigated by staining the Ki-67 antigen (anti-Ki-67, clone MIB-1, DakoCytomation Denmark A/S) (1:100) identifying cells in all but the G0-phase of the cell cycle. Inflammatory cells were investigated by staining the CD45 (DakoCytomation Denmark A/S, leucocyte common antigen, LCA, clone 2B11+PD7/26) (1:2000), CD3 (Novocastra Laboratories Ltd, clone NCL-CD3-PS1) (1:100) and CD68 (DakoCytomation Denmark A/S, clone PG-M1) (1:150) antigens identifying lymphocytes and macrophages, respectively. Monoclonal mouse antibodies to IL-1β (Abcam, Cambridge, UK, clone 11E5) (1:200) and IL-10 (Serotec, Oxford, UK, clone B-S10) (1:100) were used to study the intrarenal expression of these cytokines.

Antigen retrieval was performed on re-hydrated sections in a microwave oven at 850 W for two 7-minute cycles using Tris-EDTA buffer (pH 9.0) as retrieval solution with CD45, CD3 and CD68 antibodies. Enzymatic digestion with 0.01% trypsin (Sigma, T-8253) in

phosphate-buffered saline (pH 7.4) was used with IL-1β and IL-10 antibodies for 10 minutes at 37ºC. Immunostaining was carried out in a TechMate™ 500 Immunostainer (DakoCytomation Denmark A/S) using the EnVision™ polymer technique (DakoCytomation Denmark A/S). Diaminobenzidine (DAB) was used as chromogen and hematoxylin as nuclear stain. The specificity of immunohistochemistry was controlled by omitting the primary antibodies or replacing them with irrelevant antisera. Known positive tissue samples were also used to confirm staining reliability of all separate staining batches.

Immunoperoxidase staining results were investigated at 400x magnification with an ocular grid (0.0625 mm^2). In the glomeruli, MIB-1- and LCA-positive cells were counted as the number of cells per mm^2 of glomerular area. The number of glomerular CD68$^+$ cells was graded into five groups from 1 (negative) to 5 (strong positive), because their staining pattern made it impossible to obtain exact cell numbers. All glomeruli were counted. The area of the glomeruli and tubulointerstitium was determined by point counting using a 100-point square lattice in the eyepiece. Tubulointerstitial LCA-, CD3- and CD68-positive cells per mm^2 were counted from the cortical area in 10 adjacent fields (Figure 8). Fields presenting cortical scarring were excluded.

The expression of IL-1β and IL-10 was graded from 0 to 5 according to the following scale: 0 = no immunoreactivity / no positive cells; 1 = faint immunoreactivity in single positive cells; 2 = clear immunoreactivity in single positive cells; 3 = scattered moderately intense reactivity/ numerous positive cells; 4 = dense intense immunoreactivity / focal clusters of positive cells; 5 = dense intense immunoreactivity / numerous clusters of positive cells (Figure 9).
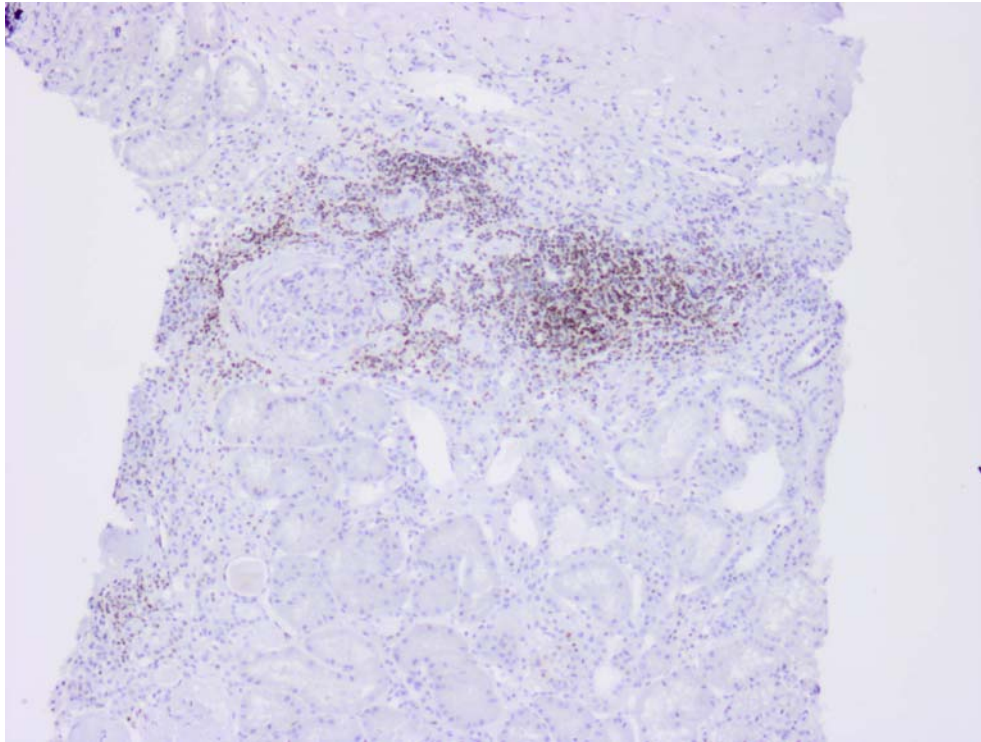
Figure 8. Immunohistochemistry performed on section from biopsy with IgAN with a monoclonal antibody against CD3 (brown color). Original magnification x200.
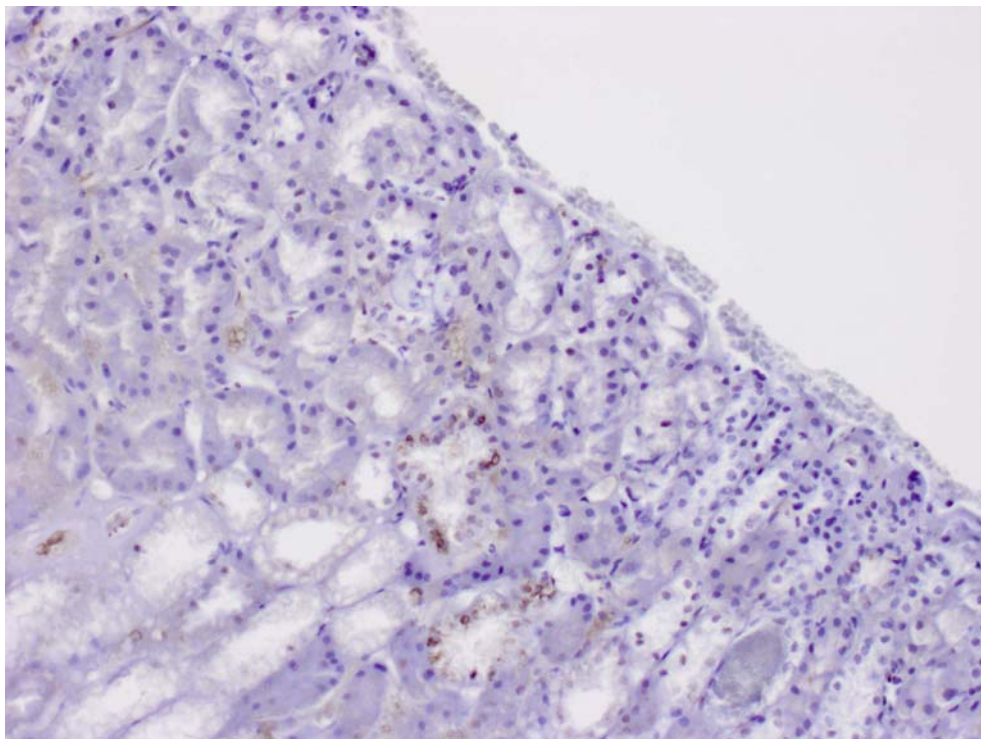
Figure 9. Immunohistochemistry performed on section from biopsy with IgAN with a monoclonal antibody against IL-1β. Grade 4 (brown color). Original magnification x400.

4.2.5. Statistics

Comparison of non-normally distributed continuous variables between two or three groups was made by Mann Whitney U-test or Kruskall-Wallis test when appropriate. Normally distributed variables were compared by ANOVA (analysis of variance), when there were more than two groups. Differences between categorical variables were tested by χ^2-test or Fisher's exact test when appropriate. Spearman correlation coefficients were calculated for statistically significant correlations between continuous non-normally distributed variables. Differences in occurrences of VDs between patients with IgAN and the general population were tested by McNemar's test. Multivariate analysis was made by stepwise logistic regression analysis or stepwise Cox proportional hazard regression analysis when appropriate. P-values less than 0.05 were considered significant for all tests. The SPSS for Windows 9.0 (SPSS Inc., Chicago, IL, USA) statistical package was used for analysis.

5. RESULTS

5.1. Clinical features and laboratory measurements

5.1.1. Urinary abnormalities and nephrotic syndrome

In the present IgAN patient group comprising almost entirely of adults, the prevalence of NS was 3%. Only 6% had isolated PU, 73% had PU and HU (PUHU) and 21% had isolated HU.

The majority of IgMN patients had PU, PUHU or NS as their initial manifestation. The median daily UPE was 1.4g (range, 0.05-39.1). Of adult patients 24% had NS, 45% had PU, 7% had PUHU and 24% isolated HU. Of the pediatric patients 89% had NS and the others had high-grade asymptomatic PU. In Tampere University Hospital renal biopsies are not performed on children with only HU or low-grade PU. One patient (4%) with HU and 16 (15%) of all had elevated serum creatinine levels at the time of renal biopsy **(IV)**.

5.1.2. Gender

In IgAN there was a male majority in all subgroups; in all 63% were men.

Male gender also predominated in IgMN, 57% of all patients being males. However, of patients with HU or PUHU 65% were females. The male predominance was more obvious in children, 75% being boys, this possibly because there were no HU patients in the subgroup of pediatric patients. Gender did not correlate with serum creatinine level at the time of renal biopsy **(IV)**.

5.1.3. Hypertension

Of all IgAN patients 53% were hypertensive at the time of renal biopsy and 73% at the end of a median 10 years' follow-up. The mean MAP was 103±14 mmHg. In age- and sex-matched controls representing the normal population the prevalence of hypertension was 41%.

At the time of renal biopsy hypertension was present in 35% or 45% of all IgMN patients depending on the definition used. Correspondingly, the prevalence of hypertension at the end of follow-up was 48% or 55% (Figure 10). When children were excluded, the percentages

were 49% or 56% and 59% or 69%, respectively. The mean arterial pressure (MAP) was
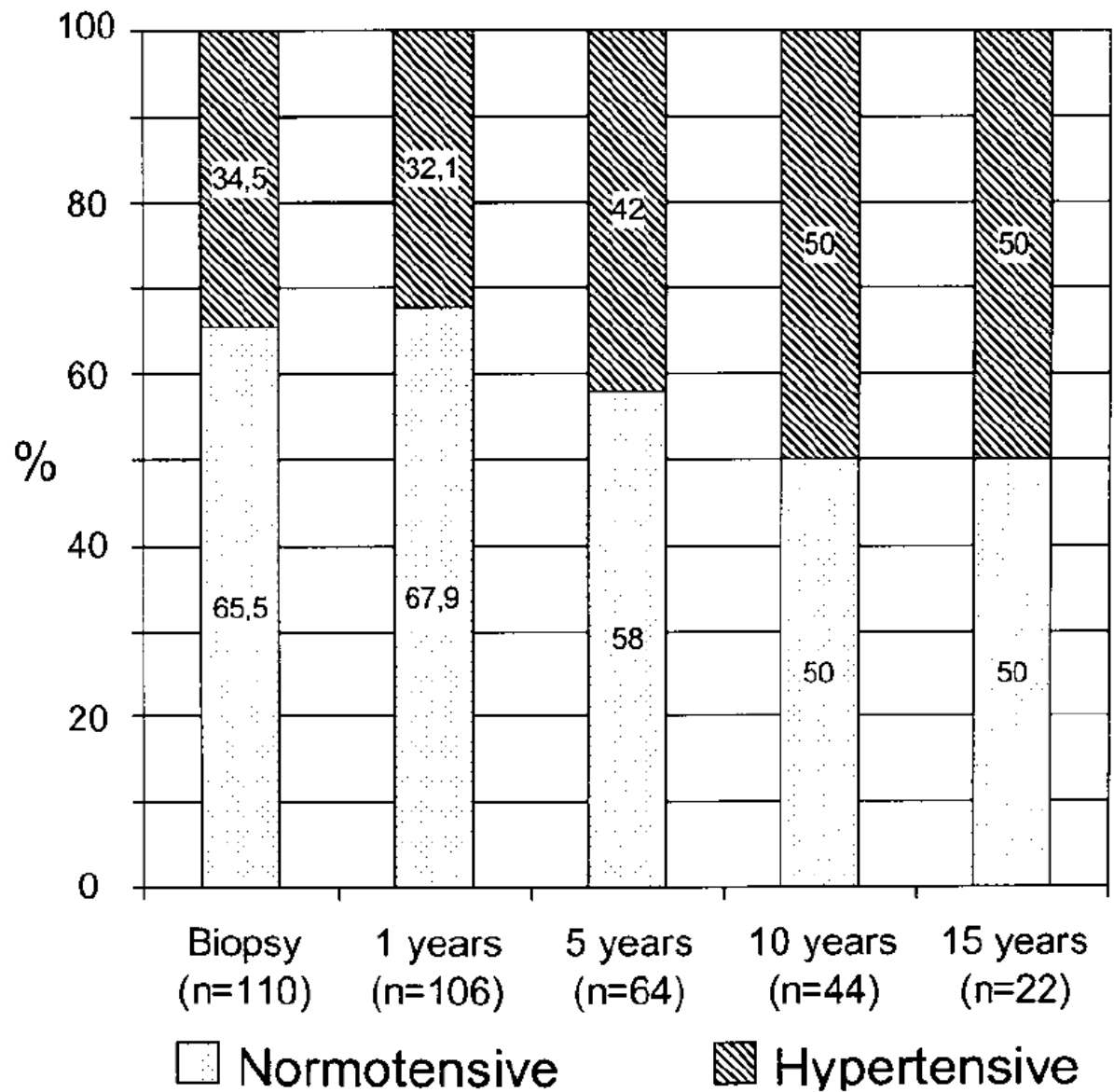107±35 mmHg in adult IgMN patients **(IV)**.

Figure 10. Hypertension in patients with IgM nephropathy at time of biopsy and in
postbiopsy follow-up. Reprinted with permission from Myllymäki J, et al (2003). *Am J
Kidney Dis*, 41: 343-350. © the National Kidney Foundation, Inc

5.2. Serum immunoglobulins and complement: associations with clinical and biochemical parameters

The concentrations of serum immunoglobulins (IgA, IgG, and IgM) and complement components (C3, C4) at the time of renal biopsy in adult patients with IgAN and IgMN are presented in Table 3. Patients with IgMN, compared to IgAN patients, had significantly higher IgM levels and lower IgA levels in serum. Similar findings were made when excluding patients with daily UPE >1g. In the subgroup of IgAN patients without substantial PU, serum C3 concentrations were also significantly higher than in similar IgMN patients.

Table 3. Serum immunoglobulins and complement components in adult IgAN and IgMN patients (Unpublished data)

Serum immunoglobulins and complement components	IgAN patients, n=201		IgMN patients, n=64	
	All patients	Patients with UPE <1g/d	All patients	Patients with UPE <1g/d
IgA, (mean ± 2SD)	3.37 ± 2.90 **	3.49 ± 2.92 **	2.16 ± 1.87	2.06 ± 1.66
IgG, (mean ± 2SD)	12.4 ± 7.34	13.0 ± 7.10	11.4 ± 8.60	12.9 ± 6.52
IgM, (mean ± 2SD)	1.25 ± 1.48**	1.28 ± 1.52 *	1.96 ± 2.31	1.70 ± 2.11
C3, (mean ± 2SD)	1.10 ± 0.55	1.10 ± 0.54 *	1.05 ± 0.60	0.95 ± 0.45
C4, (mean ± 2SD)	0.30 ± 0.29	0.29 ± 0.27	0.32 ± 0.56	0.26 ± 0.32

UPE = Urinary protein excretion. * $p<0.01$, ** $p<0.001$, IgAN compared to IgMN

In the IgMN population, more than half of the patients with NS, as against only one patient with PU and/or HU, had decreased serum IgG levels. Serum C3 was also more frequently elevated in nephrotic IgMN patients (Figure 11). None of the IgMN patients had decreased serum IgM concentration. There were no statistically significant differences in IgA or C4 levels between IgMN patients with NS, PU or HU **(V)**.
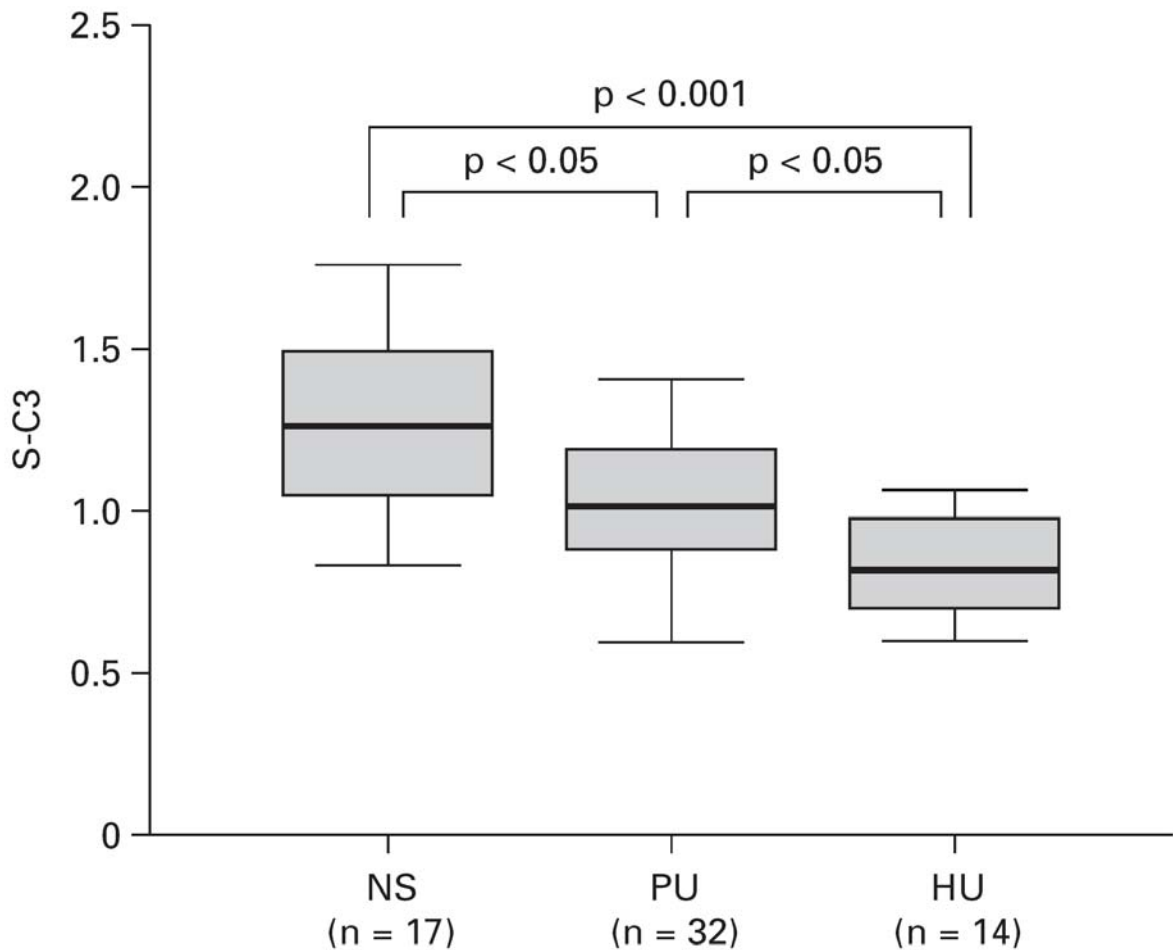
Figure 11. Serum C3 levels in adult IgMN patients with nephrotic syndrome (NS), asymptomatic proteinuria (PU) or hematuria (HU). Reprinted with permission from Myllymäki J, et al. *Nephron Clin Pract,* 102: 122-127, 2006. © S.Karger AG, Basel.

The serum IgG concentration correlated with serum total protein level and inversely with UPE in IgMN. Serum IgM and C3 correlated inversely with serum protein concentration. Serum C3 correlated with UPE in IgMN ($r=0.32$, $p<0.01$). No such statistically significant associations were found in the subgroup of IgMN patients without NS. Neither serum IgA nor C4 correlated with any biochemical parameter evaluated. The initial serum creatinine concentration was not associated with the serum level of any immunoglobulin or complement component measured in IgMN **(V)**.

5.3. Histopathological findings

5.3.1. Light microscopy

Light-microscopic findings in IgAN and IgMN are summarized in Table 4. Marked histopathological findings were more frequent in IgAN. Normal glomerular morphology or no more than minimal changes were found in 6% of IgAN and in 35% of IgMN patients. Glomerulosclerosis was the most frequently noted glomerular histological change; found in up to 88% of IgAN and 36% of IgMN patients (**I, IV**). Totally obliterated glomeruli were seen principally immediately beneath the renal capsule in 36% of IgAN and in 15% of IgMN specimens.

Table 4. Histopathological findings in 202 IgAN and 110 IgMN patients. (Modified from original studies I and IV, partly unpublished data).

	Patients, %		
Histological finding	IgAN	IgMN (all)	IgMN (adults)
Glomeruli			
Normal or minimal lesions	5	35	23
Mesangial hypercellularity			
Mild	43	32	38
Marked	21	2	6
Glomerulosclerosis			
Mild	62	35	38
Marked	26	1	6
Tubulo-interstitial tissue			
Normal morphology	46	75	68
Tubular atrophy*	40	20	27
Interstitial fibrosis*	30	7	11
Interstitial inflammation*	26	6	7
Vascular tissue			
Normal morphology	40	75	62
Hyaline arteriolosclerosis*	42	19	30
Arterial intimafibrosis*	41	6	10

*Mild and marked combined

Extracapillary proliferation was seen in 10% of IgAN patients. Normal morphology of tubulointerstitial tissue was found in 46% of IgAN and 75% of IgMN specimens. Tubular atrophy was the commonest tubulointerstitial lesion present in 40% of IgAN and in 20% of IgMN patients. Vascular changes were seen in 60% of IgAN and 25% of IgMN specimens. Hyaline arteriolosclerosis was the most prominent vascular lesion in IgMN (**I, IV**).

5.3.2. Immunofluorescent findings

Glomerular IgG was present in 17% and IgM in 38% of IgAN specimens. Glomerular C3 was present in 94% of IgAN patients. The prevalence of arteriolar C3 in IgAN was the same (63%) as in IgMN.

In concord with the definition of disease, mesangial IgM was the predominant immunofluorescent microscopy (IF) finding in IgMN. A further positive glomerular IF finding was that of IgG in 7%, C3 in 32%, C1q in 19% and fibrinogen in 5% of IgMN specimens. IgA depositions were found no more than minimal degree in glomeruli of 11% IgMN specimens, usually segmentally distributed. Positive staining for C3 was present in arterioles in 63% cases. Mesangial IgA was associated with higher serum IgA concentrations in IgMN ($p < 0.05$) (**IV**).

The degree of mesangial C3 correlated inversely with serum C3 level in both IgMN ($r=-0.33$, $p < 0.01$) and IgAN ($r=-0.20$, $p < 0.01$). Mesangial C1q was inversely correlated to higher serum creatinine concentration at the time of biopsy in both IgMN ($p < 0.05$) and IgAN ($p < 0.05$). Mesangial C3 was associated with higher creatinine in IgMN ($p < 0.05$), but not in IgAN (**IV**).

5.3.3. Immunohistochemically evaluated proliferation and inflammatory markers

Glomerular MIB-1 did not correlate with any central biochemical parameter. Glomerular LCA correlated significantly with the degree of UPE ($r=0.18$, $p < 0.05$), glomerular CD68 with UPE ($r=0.34$, $p > 0.001$) and serum uric acid concentration ($r=0.19$, $p < 0.05$), tubulointerstitial LCA with UPE ($r=0.22$, $p < 0.01$), tubulointerstitial CD3 with UPE ($r=0.32$, $p < 0.001$), serum uric acid ($r=0.21$, $p < 0.05$) and initial serum creatinine concentration ($r=0.26$, $p < 0.001$) and tubulointerstitial CD68 with UPE ($r=0.32$, $p < 0.001$) and serum creatinine ($r=0.26$, $p < 0.01$). All aforementioned tubulointerstitial leukocyte markers correlated statistically significantly with the severity of light-microscopically evaluated

interstitial inflammation (LCA: r=0.19, p<0.05; CD3: r=0.30, p<0.001; CD68: r=0.24, p<0.01). Tubulointerstitial CD3 expression also correlated with interstitial fibrosis (r=0.18, p<0.001) (I, II).

The expression of anti-inflammatory IL-10 in tubulointerstitial tissue was more prominent than IL-1β expression. There was a strong correlation between expressions of these two cytokines (r=0.53, p<0.001). Both IL-1β and IL-10 correlated with CD3 (r=0.37, p<0.001 and r=0.23, p<0.01) and CD68 (r=0.49, p<0.001 and r=0.36, p<0.001) expressions. Immunoreactivity of IL-1β also correlated with the number of tubulointerstitial LCA+ cells (r=0.17, p<0.05). The initial serum creatinine concentration was the only biochemical parameter which correlated statistically significantly with IL-1β expression (r=0.21, p<0.01). Immunoreactivity for IL-1β, but not for IL-10, correlated significantly with interstitial fibrosis (r=0.20, p<0.05) and almost significantly with interstitial inflammation (r=0.15, p=0.056) (II).

5.4. Clinicopathological correlation

5.4.1 Serum uric acid and renal histopathological changes in patients with IgAN

The mean serum uric acid concentration in IgAN patients was 0.36 ± 0.21 ($\pm 2SD$) mmol/l. Hyperuricemia was present in 30% of patients. Serum uric acid levels correlated with those of serum cholesterol (r=0.35, p<0.001), serum triglycerides (r=0.49, p<0.001), serum creatinine (r=0.62, p<0.001), UPE (r=0.31, p<0.001) and MAP (r=0.42, p<0.001) (I).

The severity of glomerulosclerosis (r=0.20, p<0.05), tubular atrophy (r=0.39, p<0.001) (Figure 12), interstitial fibrosis (r=0.42, p<0.001), interstitial inflammation (r=0.13, p<0.05), hyaline arteriolosclerosis (r=0.24, p<0.001) and arterial intimafibrosis (r=0.22, p<0.01) correlated with serum uric acid concentration (I).

Of all biochemical and clinical parameters evaluated, serum uric acid and age (r=0.40, p<0.001) correlated most strongly with tubular atrophy. Serum uric acid was found to correlate independently with the presence of tubular atrophy (p<0.01) and interstitial fibrosis (p<0.05) when studied by multivariate analysis in a model with clinical

and biochemical factors significantly correlating with these histopathological changes in univariable analysis **(I)**.
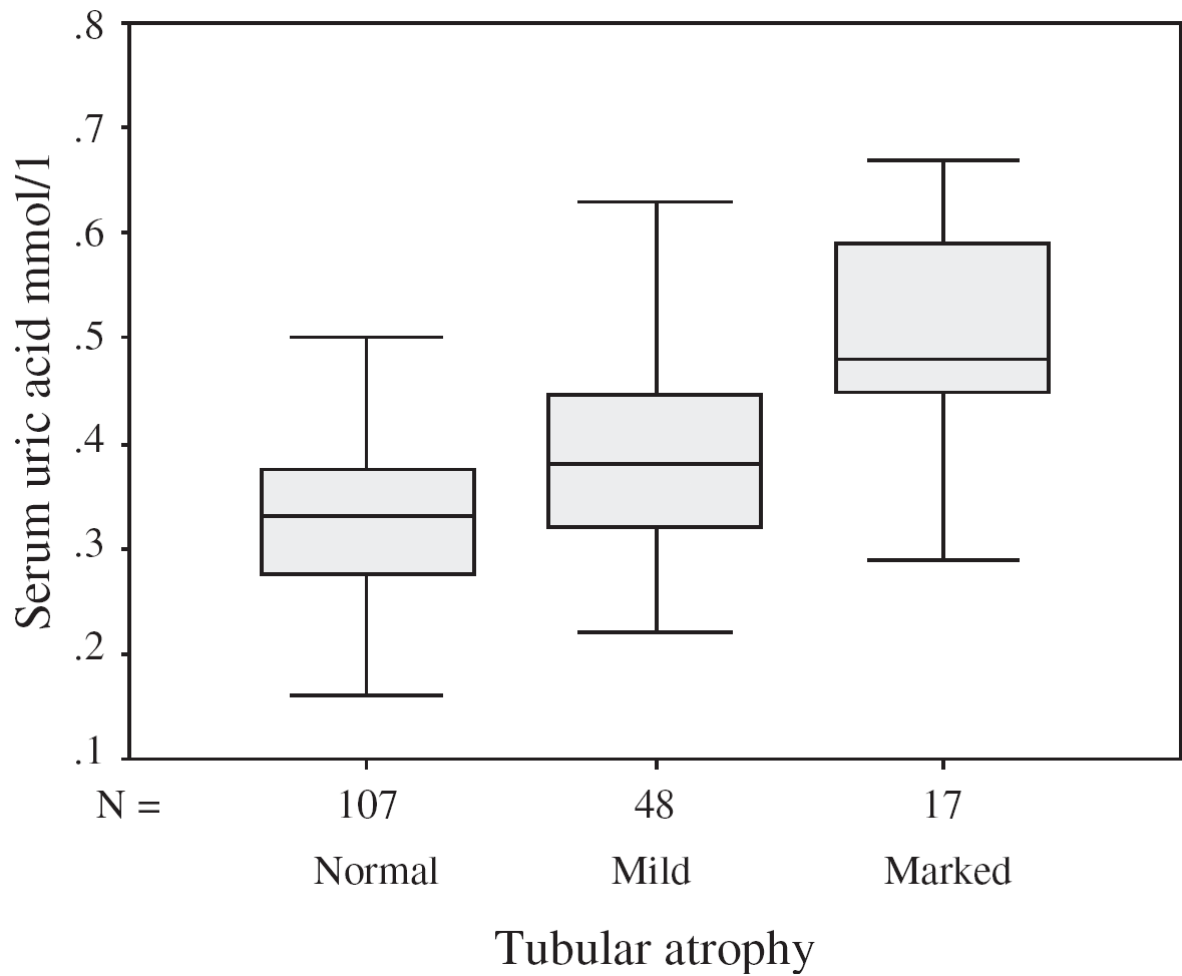

Figure 12. Serum uric acid levels in IgAN patients with different severity of tubular atrophy. Reprinted with permission from Myllymäki J, et al (2005). *Nephrol Dial Transplant*, 20: 89-95. © ERA-EDTA.

5.4.2. Hypertension and renal histopathological changes

In IgAN systolic blood pressure (BP) as well as diastolic BP and MAP correlated with glomerulosclerosis (r=0.28, p<0.001; r=0.23, p<0.01; r=0.27, p<0.01), tubular atrophy (r=0.33, p<0.001; r=0.31, p<0.001; r=0.36, p<0.001), interstitial fibrosis (r=0.32, p<0.001; r=0.27, p<0.01; r=0.32, p<0.001) and hyaline arteriolosclerosis (r=0.42, p<0.001; r=0.34, p<0.001; r=0.40, p<0.001). High systolic BP and MAP were associated with severe arterial intimafibrosis (r=0.23, p<0.01; r=0.18, p<0.05). When investigating the association of BP with histopathological lesions by multivariate analysis, MAP had independent correlations with glomerulosclerosis (p<0.05) and hyaline arteriolosclerosis (p<0.05) **(I).**

In IgMN closely similar findings were found. MAP correlated significantly with tubular atrophy (p<0.001), interstitial fibrosis (p<0.05), hyaline arteriolosclerosis (p<0.05) and arterial intimafibrosis (p<0.05). High systolic BP was associated almost significantly with severe glomerulosclerosis (p=0.111).

5.4.3. Other clinicopathological correlations

Age correlated with all histopathological changes evaluated, and independently with tubular atrophy (p<0.001), interstitial fibrosis (p<0.01) and both vascular changes in IgAN. The correlation between age and mesangial cellularity was inverse (r=-0.14, p<0.05). Congruent results were found between age and histopathology in IgMN, though correlations between age and mesangial cellularity (p=0.133) or glomerulosclerosis (p=0.096) were not statistically significant. The total serum cholesterol concentration did not correlate with any histopathological parameter, but serum triglycerides correlated significantly but not independently with interstitial fibrosis (r=0.20, p<0.05) and arteriolosclerosis (r=0.18, p<0.05) in IgAN. High BMI in IgAN patients was associated with tubulointerstitial and vascular changes. Serum C3 concentration correlated significantly with the severity of glomerulosclerosis in IgMN (Figure 13) **(I, V).**
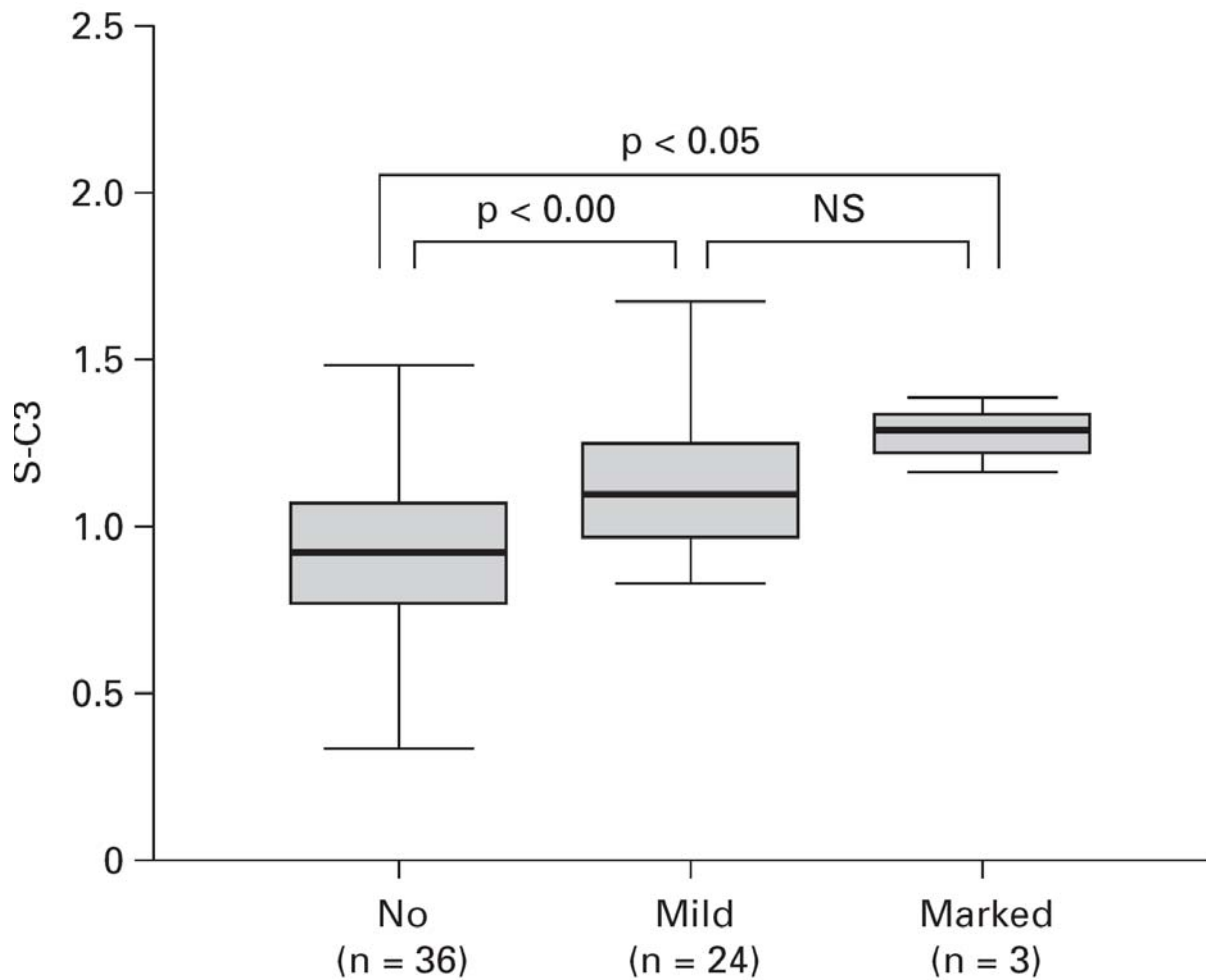
Figure 13. Serum C3 in adult IgMN patients with different levels of glomerulosclerosis. Reprinted with permission from Myllymäki J, et al. *Nephron Clin Pract,* 102: 122-127, 2006. © S.Karger AG, Basel.

5.5. Prognosis of renal function

5.5.1. Post-biopsy follow-up and development of renal insufficiency

In 28% of IgAN patients serum creatinine was above normal limits at the end of follow-up. Progression occurred in 18% of IgAN cases and 8% reached ESRD **(I, II)**.

Forty-four patients were followed for at least 10 years and 22 patients at least 15 years. In 19% of IgMN patients, serum creatinine was above normal limits at the end of follow-up. All but one patient with HU, without NS had normal creatinine concentrations at the end of follow-up. Progression of renal insufficiency was found in 12% of cases. Six (5%) patients,

five with NS, reached ESRD. In the subgroup of IgMN patients followed for up to 15 years, the occurrence of progression was 36% and ESRD was 23% (Figure 14) **(IV, V)**.
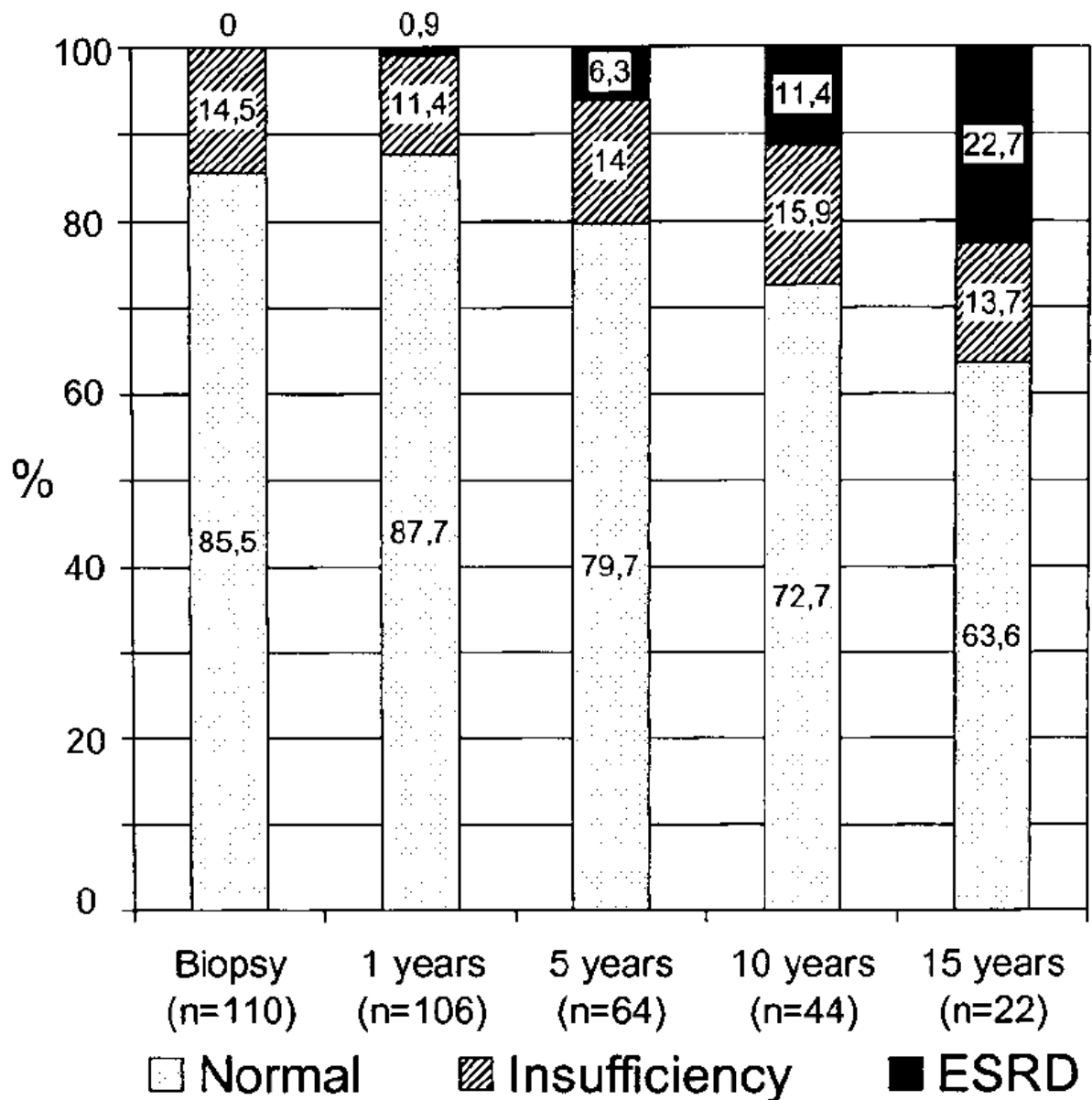
Figure 14. Renal insufficiency and end-stage renal disease (ESRD) in patients with IgMN at time of biopsy and in post-biopsy follow-up. (Reprinted with permission from Myllymäki J, et al (2003). *Am J Kidney Dis*, 41: 343-350. © the National Kidney Foundation, Inc.)

Table 5. Correlations of tubulointerstitial expressions of CD3, CD68, LCA, IL-1β, and IL-10 with progressive disease in patients with IgAN. Reprinted with permission from Myllymäki J, et al (2007). *Kidney Int* 71: 343-348. © International Society of Nephrology

Histopathological parameter	All patients, *n*=176		Patients with initially normal serum creatinine, *n*=146	
	Progressive disease, *n*=34	Stable disease, *n*=142	Progressive disease, *n*=24	Stable disease, *n*=122
CD3, mean (2 s.d.)	99.5 (208.8)	34.4 (132.8)***	86.7 (200.5)	22.1 (55.2)***
CD68, mean (2 s.d.)	290.2 (351.1)	195.7 (334.3)**	288.0 (360.4)	164.1 (230.9)**
LCA, mean (2 s.d.)	18.5 (34.3)	8.3 (17.1)***	20.6 (38.7)	7.30 (13.8)***
IL-1β, mean (2 s.d.)	1.62 (2.70)	0.95 (1.90)**	1.52 (2.72)	0.83 (1.72)*
IL-10, mean (2 s.d.)	2.74 (3.28)	2.26 (2.56)	2.80 (3.10)	2.19 (2.52)

IgAN, IgA nephropathy; IL, interleukin; LCA, leukocyte common antigen; s.d., standard deviation.
*P<0.05, **P<0.01, ***P<0.001.

5.5.2. Risk factors for progression in IgAN

Glomerulosclerosis (p<0.01), tubular atrophy (p<0.01), interstitial inflammation (p<0.01), interstitial fibrosis (p<0.001) and hyaline arteriolosclerosis (p<0.001) were histopathological parameters correlating significantly with progression in all IgAN patients **(II)**. All of these factors except interstitial fibrosis were also associated with progression in the subgroup of IgAN patients with normal serum creatinine at the time of renal biopsy. The amounts of tubulointerstitial CD3+, CD68+ and LCA+ cells correlated with progression in IgAN, also in the subgroup of patients with initially normal serum creatinine (Table 5) **(II)**. Tubulointerstitial proinflammatory IL-1β expression, but not anti-inflammatory IL-10, correlated significantly with progression in IgAN (Table 5) **(II)**.

To find the strongest risk factors for progression in IgAN, all aforementioned risk factors were placed in the same model of multivariate analysis **(II)**. Glomerulosclerosis (p<0.05), interstitial fibrosis (p<0.01) and tubulointerstitial CD3 (p<0.001) were found to be most strongly associated with progression in all IgAN patients and hyaline arteriolosclerosis (p<0.05), tubulointerstitial CD3 (p<0.001) and IL-1β (p<0.5) expressions in the subgroup of patients with initially normal serum creatinine. The aforementioned histopathological and immunohistochemical factors found to be most strongly associated with progression were studied by the multivariate analysis model with the biochemical and clinical parameters. Hypertriglyceridemia (p<0.05), elevated serum creatinine (p<0.05), interstitial fibrosis (p<0.05) and tubulointerstitial CD3 expression (p<0.001) were independently associated with progression in all patients IgAN, and tubulointerstitial CD3 expression (p<0.001) and hyaline arteriolosclerosis (p<0.05) in the subgroup of patients with initially normal serum creatinine.

5.5.3. Risk factors for progression in IgMN

Of the parameters evaluated at the time of renal biopsy, age ($p<0.01$), hypertension ($p<0.001$), serum creatinine ($p<0.001$) and severity of tubular atrophy ($p<0.05$), interstitial fibrosis ($p<0.05$) and hyaline arteriolosclerosis ($p<0.05$) correlated significantly with the prevalence of elevated serum creatinine at the end of follow-up in IgMN **(IV)**. The correlation between hypertension and elevated serum creatinine was found by the multivariate analysis to be independent ($p<0.05$). In addition to the above factors, serum C3 and low serum IgG/C3 ratio were associated with progression of IgMN in adult patients with or without NS (Table 6) **(V)**.

When studying by the multivariate analysis model with hypertension, UPE >1 g/d and initially elevated serum creatinine, serum C3 correlated independently with progression in the subgroup of adult IgMN patients without NS ($p<0.05$). Hypertension, UPE, serum total protein concentration and occurrence of glomerular C1q were related to the risk of reaching ESRD in IgMN **(V)**.

Table 6. Serum IgG, IgM and C3 concentrations and IgG/IgM and IgG/C3 ratios in all adult IgMN patients and in adult IgMN patients without NS with progressive or stable renal disease. Reprinted with permission from Myllymäki J, et al. *Nephron Clin Pract,* 102: 122-127, 2006. © S.Karger AG, Basel.

| | Renal function at the end of follow-up | | | |
| | all patients | | patients without NS | |
	progression	stable	progression	stable
IgG, mean ± 2 SD (g/l)	9.0±11.8	11.9±7.8	11.2±3.8	12.8±6.3
IgM, mean ± 2 SD (g/l)	1.9±1.8	2.0±2.4	1.9±2.6	1.8±2.1
C3, mean ± 2 SD (g/l)	1.2±0.6*	1.0±0.6	1.4±0.8**	0.9±0.5
IgG/C3, mean ± 2 SD	7.0±11.0*	12.8±13.7	8.4±6.0*	14.3±12.9

NS = Nephrotic syndrome.
* $p < 0.05$, ** $p < 0.01$ (patients with progressive disease vs. patients with stable disease).

5.5.4. Development of FSGS in IgMN

Eleven IgMN patients whose renal function deteriorated, UPE markedly increased and/or evinced poor response to pharmacotherapy had a repeat renal biopsy. Five of the repeat samples showed histology typical of FSGS, in five samples minimal histological lesions were seen with mesangial IgM in four and with negative IF finding in one. One patient developed typical lupus nephritis. Four FSGS patients had elevated serum creatinine and three had reached ESRD at close of follow-up. All patients with minimal lesions had normal serum creatinine. The known duration of disease was similar in patients with FSGS and those with minimal lesions. There was no clinical or morphological parameter predicting the development of FSGS **(IV)**.

5.6. Vascular diseases in IgAN

5.6.1. Prevalence

Controls had significantly higher serum cholesterol and triglyceride levels compared to IgAN patients, but hypertension was less frequent (Table 7). Other clinical parameters studied were equal between these two groups. Prevalence of vascular diseases (VDs) in 203 IgAN patients ≥ 30 years of age was compared with age- and sex-matched controls representing the general population in the same residential area. The data are summarized in Table 8 **(III)**.

Of IgAN patients 25% and of controls 9% had some VD at the end of follow-up. Of patients with stable renal function 17% had some VD. Also CHD and CeVD were significantly more frequent in patients with IgAN with or without progressive renal disease (Table 8). Patients with progressive renal disease had significantly more frequently some VD or CHD (Table 8). Also CeVD occurred somewhat more frequently in IgAN patients with progressive disease than in those with stable disease (p=0.064) **(III)**.

Table 7. Central clinical parameters in controls and IgAN patient ≥30 years of age at the end of follow-up Reprinted with permission from Myllymäki J, et al (2006). *Nephrol Dial Transplant*, 21: 1876-1882. © ERA-EDTA.

Parameter	Controls, $n = 203$	IgAN patients, $n = 203$	P-value
Age, mean (2SD), years	53.5 (29.1)	53.5 (29.1)	NS
Serum total cholesterol, mean (2SD), mmol/l	6.0 (2.3)	5.4 (2.0)	<0.001
Serum LDL cholesterol, mean (2SD), mmol/l	3.9 (2.4)	3.4 (1.8)	<0.001
Serum triglycerides, mean (2SD), mmol/l	1.6 (2.0)	1.7 (2.3)	<0.001
BMI, mean (2SD), kg/m^2	26.8 (9.3)	26.8 (9.4)	NS
Hypertension, n (%)	83 (41)	114 (73)	<0.001
Diabetes mellitus, n (%)	14 (7)	21 (10)	NS
Smoking[a], n (%)	111 (56)	72 (45)	NS

NS, non-significant.
[a]Ex-smoker or current smoker.

Table 8. Vascular diseases in controls and IgAN patients ≥30 years of age. Reprinted with permission from Myllymäki J, et al (2006). *Nephrol Dial Transplant*, 21: 1876-1882. © ERA-EDTA.

Manifestation	Patients		
	s-group ($n = 180$)	p-group ($n = 41$)	All patients ($n = 221$)
CHD, n (%)	19 (11)	17 (42)***	36 (16)
CeVD, n (%)	19 (11)	9 (22)	28 (13)
Some VD, n (%)	30 (17)	22 (54)***	52 (24)

CHD, coronary heart disease; CeVD, cerebrovascular disease; VD, vascular disease; s-group, patients with stable renal disease; p-group, patients with progressive renal disease (defined by serum creatinine concentration). $P<0.05$, **$P<0.01$, ***$P<0.001$, s-group compared with p-group.

5.6.2. Risk factors

Significant correlations were found between all clinical parameters evaluated and VDs in all IgAN patients (Table 9). CeVD did not correlate with male gender, PU, hypercholesterolemia and hypertriglyceridemia. All the aforementioned clinical parameters were included in the same multivariate analysis model. Among all IgAN patients, initial renal insufficiency ($p<0.05$) and smoking ($p<0.01$) were found to be independent risk factors for VDs, male gender for CHD ($p<0.01$) and hypertension for CeVD ($p<0.05$) **(III)**.

A similar multivariate analysis was performed separately for patients with normal serum creatinine concentration at the time of renal biopsy. Male gender was an independent risk factor for VDs ($p<0.05$), hypertriglyceridemia for CHD ($p<0.05$). In this subgroup analysis brought out no parameter associated with the occurrence of CeVD. The severity of glomerulosclerosis ($p<0.01$), tubular atrophy ($p<0.05$), interstitial fibrosis ($p<0.05$) and hyaline arteriolosclerosis ($p<0.01$) was associated with the prevalence of VDs. Of patients with CeVD, 42%, as against only 17% of those without, had marked arteriolosclerosis. All histopathological parameters were included in the multivariate analysis model. The severity of hyaline arteriolosclerosis seen in renal biopsy was independently associated with VDs ($p<0.05$) and CeVD ($p<0.05$) and arterial intimafibrosis with CHD ($p<0.05$) **(III)**.

Table 9. Correlations of clinical and biochemical parameters with VDs in IgAN patients in univariate analysis. Reprinted with permission from Myllymäki J, et al (2006). *Nephrol Dial Transplant*, 21: 1876-1882. © ERA-EDTA.

Parameter	Some VD			CHD			CeVD		
	No/Yes	OR (95% CI)	P-value	No/Yes	OR (95% CI)	P-value	No/Yes	OR (95% CI)	P-value
Age >41 years (%)	37/94	28.0 (8.38–93.6)	<0.001	43/92	15.0 (4.45–50.8)	<0.001	44/96	34.9 (4.65–262)	<0.001
Male gender (%)	58/81	3.06 (1.44–6.49)	<0.01	58/89	5.73 (1.95–16.8)	<0.001	62/75	1.88 (0.76–4.62)	0.21
Hypertension (%)	43/85	7.56 (3.36–17.0)	<0.001	47/81	4.76 (1.99–11.4)	<0.001	47/93	15.1 (3.50–65.7)	<0.001
Proteinuria (%)	24/46	2.55 (1.34–4.87)	<0.01	25/53	3.24 (1.56–6.73)	<0.01	29/36	1.35 (0.59–3.09)	0.51
Renal insufficiency (%)[a]	12/42	5.54 (2.69–11.4)	<0.001	15/42	4.23 (1.94–9.21)	<0.01	16/39	3.42 (1.48–8.03)	<0.01
Ccr <90 ml/min/1.73 m² (%)	40/85	8.13 (3.60–18.3)	<0.001	44/83	6.27 (2.49–15.8)	<0.001	46/86	7.14 (2.39–21.4)	<0.001
Hyperuricaemia (%)	23/49	3.17 (1.59–6.32)	<0.01	26/46	2.73 (1.26–5.90)	<0.05	25/58	4.06 (1.73–9.54)	<0.01
Hypercholesterolaemia (%)	56/79	2.86 (1.28–6.41)	<0.01	57/80	2.95 (1.14–7.60)	<0.05	59/74	1.94 (0.73–5.17)	0.25
Hypertriglyceridaemia (%)	30/58	3.18 (1.58–6.40)	<0.01	32/59	2.91 (1.25–6.54)	<0.05	34/52	2.07 (0.86–4.98)	0.11
Diabetes mellitus (%)	1/15	15.4 (3.15–74.9)	<0.001	3/14	5.87 (1.61–21.5)	<0.05	3/18	8.26 (2.22–30.7)	<0.01
Smoking (%)	36/64	3.21 (1.68–6.13)	<0.01	38/61	2.57 (1.23–5.34)	<0.05	38/68	3.45 (1.48–8.03)	<0.01
BMI >27 (%)	23/50	3.39 (1.77–6.49)	<0.001	27/42	1.96 (0.94–4.09)	0.11	25/61	4.73 (2.07–10.8)	<0.001

CHD, coronary heart disease; CeVD, cerebrovascular disease; VDs, vascular diseases; NS, Non-significant.
[a] Renal insufficiency defined by serum creatinine concentration.

6. DISCUSSION

6.1. Advantages and weaknesses of the study design

The main advantage of the present study was the large number of cases. This is partly attributable to a liberal renal biopsy policy. The present study included patients with PU and NS, but also patients with minor urinary abnormalities such as microscopic HU. In many reports dealing with IgMN, patients with only HU as their initial manifestation have been excluded and the focus has been mainly on nephrotic patients. Also pediatric patients, patients under 16 years of age, were included in certain sections of this study. These features may be counted as advantages of the present study. In the context of IgMN the study offers the largest patient group and the longest follow-up time so far reported. Also the IgAN group was notably large when compared to earlier studies concerning IgAN. One of the advantages of the IgAN section of the study is the long follow-up time. The proportion of IgAN patients participating in invited control visit was encouraging. Tampere University Hospital is the only unit in the district of Pirkanmaa, Finland, where renal biopsies are evaluated. The criteria for histopathological changes are quite clear and histopathological evaluation has been made by one investigator without knowledge of the clinical data. Also expert knowledge of clinical nephrology is centralized in Tampere University Hospital. These aspects may for their part diminish the probability of any significant selection bias and facilitated the long follow-up periods. The large material of the T2000 population comprehensive study is unique. The control group was eminently well composed of age- and sex-matched subjects from the same residential area. The essential data, chiefly data on vascular diseases, were collected at almost the same time from IgAN patients and controls. However, the diagnostic and laboratory methods in Tampere University Hospital and in H2000 study may vary and that way bring on a possibility for bias.

The kidney diseases studied in the present series are somewhat rare, as are also other forms of glomerulonephritis. A purely prospective collection of a sufficient number of cases would take a very long time. Like almost all previous studies of its kind the present study was performed primarily retrospectively. The part of the study evaluating the prognosis and risk factors for progression was longitudinal and retrospective. The initial feature of the study cohort was kidney disease and the end-point the development of renal insufficiency or progression of disease. Relationships between clinical parameters and histopathological

changes were studied by cross-sectional method. A pure case-control design was used when correlating the prevalence of vascular diseases between patients and the general population. Part of the study is descriptive, in clarifying the clinical picture of IgMN. The portion of the study concerning IgAN is partly prospective, involving invited control visits. However, the retrospective design brought out some serious problems. The diagnostic methods or even the criteria for certain additional diseases may vary. The locations for the diagnostic procedures may vary, and laboratory methods may also change. The significance of this may be considerable in the present case due to the long period over which the patients were collected.

6.2. Clinical features

6.2.1. Gender

Male majority has normally been found in all forms of glomerulonephritis (Korbet et al. 1996). In a Danish population during the period 1985-1997, 41% of patients with biopsy-proven mesangioproliferative glomerulonephritis were female (Heaf et al. 1889). Among all glomerulonephritis only in focal glomerulonephritis and haemolytic uraemic syndrome and thrombotic thrombocytopenic purpura was there a slight female predominance. In Australia lupus nephritis is more common in females, but in all primary forms males predominated (Briganti et al. 2001). The incidence of IgAN was 5.7 per 100000 per year in males and 2.9 per 100000 per year in females. However, the incidence peak came earlier in females (25-34 vs. 55-64 years) (Briganti et al. 2001). Rivera and colleagues (2002) found a male/female ratio of 1.2 in children and 1.5 in adults with any glomerulonephritis in Spain in 1994-1999. Similar findings regarding gender distribution among the various forms of glomerulonephritis have also been reported at least from France, Brazil, Macedonia, USA and Italy (Stratta et al. 1996, Wyatt et al. 1998, Polenakovic et al. 2003, Simon et al. 2004, Malafronte et al. 2006). According to previous reviews the male: female ratio seems to be between 2:1 and 6:1 depending on race and place of study.

Some studies of IgMN have reported a male predominance (Bhasin et al. 1978, Lawler et al. 1980, Vangelista et al. 1981, Vilches et al. 1982, O'Donoghue et al. 1991, Al-Eisa et al. 1996), whereas others have found more females (Cohen et al. 1978, Cavallo and Johnson

1981, Helin et al. 1982, Gonzalo et al. 1985, Kopolovic et al. 1987, Saha et al. 1989). In the present study the whole material was dominated by males, but there were significantly more females among IgMN patients with HU. HU would appear to be more frequent finding in women regardless of the specific diagnosis (Yun et al. 2004). Similar findings have been reported in urine screening studies (Topham et al. 2004, Park et al. 2005). However, the difference is evidently fairly small. Clear male predominance was also found in the present IgAN material. A recent meta-analysis reported that male gender has a negative prognostic value in non-diabetic chronic renal diseases such as IgAN (Neugarten et al. 2000). In the present study, no statistically significant difference was found between males and females in the prognosis of IgAN and IgMN.

In summary, the results of the present study are in agreement with earlier findings. Male gender predominated among both IgAN and IgMN patients. However, IgMN patients with HU were mainly women. Gender was not of prognostic significance for progression or development of renal insufficiency **(II, IV)**.

6.2.2. Clinical renal findings

In the present IgAN material, consisting almost entirely of adults, only 9% had NS or isolated PU as their initial manifestation. Most IgAN patients had PU with HU. These findings are congruent with those in earlier IgAN studies (Barratt and Feehally 2005).

In addition to the present work, there are only a few reports on patients with HU or asymptomatic PU as the initial clinical symptoms of IgMN. It would appear that at the time of renal biopsy the majority of adult IgMN patients have asymptomatic PU with or without microscopic HU. Due to our renal biopsy policy all pediatric IgMN patients had high-grade PU. According to the present and some previously published findings, patients with HU would appear to have a more favourable course of the disease compared to those with PU or NS (Cohen and Border 1982, Saha et al. 1989) **(IV)**.

6.2.3. Epidemiology of hypertension

High blood pressure was significantly more frequent in IgAN and IgMN patients than in the general population. Slightly different definitions of hypertension were used in various parts of the present study. The prevalence of hypertension in IgAN and IgMN patients was significantly high even at the time of biopsy. At the end of eight years' follow-up about half

of the IgMN patients had hypertension. This corresponds to what has been previously reported (Saha et al. 1989). The incidence of hypertension during follow-up was highest in the PU group, which may have had an influence on the clinical outcome of these patients. Due to the significantly different patient materials, comparison of hypertension prevalences in the present and previously published IgMN studies is not reasonable.

In IgAN the prevalence of hypertension was of the same magnitude. In various IgAN studies with various materials the prevalence of hypertension has been between 20% and 60%. When comparing the present results to those obtained in another recent study dealing with IgAN patients from the three continents, MAPs were almost the same (Geddes et al. 2003).

In summary, the present study confirms earlier findings. Hypertension is particularly common in IgAN and IgMN. The prevalence of hypertension in the present series was of the same magnitude as in earlier reports **(I, III, IV)**.

6.3. Immunoglobulins and complement

High serum IgA concentration has been proposed to offer a practical means of non-invasively differentiating IgAN from other forms of glomerulonephritis (Sakai et al.1995). Also in the present study adult IgAN patients had clearly higher serum IgA concentrations than in IgMN. Conversely, adult IgMN patients had substantially higher serum IgM levels than those with IgAN. Serum IgM is often elevated in association with NS (Akinsola et al. 1984, Chan et al. 1987). Because human IgM is a significantly larger molecule than IgG, it does not drift to the urine before there are large pores in the glomerular barrier (Bakoush et al. 2001b). There were more nephrotic IgMN patients than nephrotic IgAN patients in the present cohort, which may explain part of the differences in the serum IgM concentrations. The aforementioned disturbance in the immunoglobulin switching process may explain the remainder.

Even though IgMN is assumed to be an immune-mediated glomerulonephritis, the role of immunoglobulins and other inflammatory mediators in its clinical picture has not hitherto been studied.

C3 is a crucial component in both classical and alternative complement pathways. However, complement, as well as active C3, has many other important functions in the

immune system. Elevated C3 is usually seen in acute phase reactions, for example in bacterial infections and surgical operations, but also in rheumatoid arthritis. Inflammatory processes consume complement components, resulting in lowered C3 levels. Elevated levels of activated C3 may be associated with more severe renal disease in IgAN (Zwirner et al. 1997).

In the present study (V), serum C3 was associated with those clinical and histopathological factors which mainly determine the prognosis of IgMN. IgMN patients with high-grade PU and/or severe mesangial glomerulosclerosis had higher levels of serum C3. High serum C3 was especially common in patients with NS. A similar association between C3 and nephrotic level PU was noted over 20 years ago in Africans, Asians and Caucasians (Akinsola et al. 1984, Strife et al. 1986, Chan et al. 1987). Elevated C3 levels are proposed to reflect increased hepatic protein synthesis (Chan et al. 1987). This may occur due to non-selective depletion of C3 in the urine in NS. However, the pathophysiological background to this phenomenon is not fully resolved.

Total serum IgG levels are usually decreased in NS when nonselective PU is present. Some authors suggest that there may be a disturbance in the immunoglobulin-switching process from IgM to IgG in IgMN (Lin et al. 1989). There may thus be other pathogenetic factors other than nephrotic range PU which induce hypogammaglobulinemia in IgMN. In the present study over 50% of IgMN patients with NS had decreased serum IgG levels. In the other IgMN patients, a serum IgG level below the lower normal limit was very rare (V).

In summary, IgMN patients with high-grade PU and/or severe mesangial glomerulosclerosis had higher levels of serum C3. Serum IgG concentrations were low in nephrotic IgMN patients due to high-grade non-selective PU. Serum IgA levels were higher in IgAN than in IgMN and IgM levels were higher in IgMN than in IgAN.

6.4. Renal histopathology

6.4.1. Light-microscopic findings

Evaluating all IgAN and IgMN patients it would seem that normal histology or only minimal changes are more frequently seen in IgMN.   Extracapillary proliferation, which often reflects rapidly progressive disease, was seen in some IgAN samples, while in IgMN

it was very rare **(II, IV)**.Chronic histopathological changes such as tubular atrophy, interstitial fibrosis, glomerulosclerosis and arteriolosclerosis were more common findings in IgAN than in IgMN **(II, IV)**. This may be explained by the different age distribution in these patient groups. It is possible that IgAN is longer in the subclinical phase, possibly due to the larger number of patients with NS as this initial manifestation in IgMN. The histopathological differences between IgAN and IgMN may affect the prognostic differences in renal function in these diseases. However, more precise comparison of progression rates in IgMN and IgAN demands further studies.

6.4.2. Immunohistochemically evaluated findings

In addition to the predominant immunoglobulin, other less intensive glomerular immunofluorescence findings are fairly common in IgAN and IgMN. Almost all IgAN samples included mesangial C3 in addition to IgA. In IgMN mesangial IgA was correlated with serum IgA concentration **(V)**, which may bespeak passive migration of IgA molecules through mesangial area and accumulation to the mesangium. The relation of mesangial and serum C3 was inverse both in IgAN and in IgMN. The clinical significance of colabelling with other immunoglobulins or complement components in IgAN or IgMN is unknown.

Mesangial IgM or C1q may be associated with the level of PU in children with IgAN (Welch and McAdams 1998). Mesangial IgG deposition has been found to correlate with the development of hypertension and renal failure in IgAN (Nieuwhof et al. 1998). In the context of IgMN no similar findings have been reported. Without responding to the histopathological manifestation, C1q depositions have been associated with good prognosis in NS (Davenport et al. 1992). In the present study mesangial C1q and C3 correlated with an elevated serum creatinine concentration at the time of renal biopsy in IgMN **(V)**.

The presence of interstitial inflammatory cell infiltrates has previously been found to be a prognostic high-risk factor in IgAN (Freese et al. 1998). In previous studies, infiltrations of CD3- positive cells have been found to be associated with the occurrence of interstitial fibrosis in glomerulonephritis and elevation of serum creatinine concentration in IgAN (Segerer et al. 1999, Arrizabalaga et al. 2003). Infiltrations of macrophages (CD68 positive cells) seem to be more common in the aggressive type of glomerulonephritis (Yang et al. 1998). The level of CD68-positive interstitial infiltrations may correlate significantly with the activity of renal disease and UPE in children with IgAN (Maruhashi et al. 2004). In the

present study, all leukocyte markers evaluated, possibly reflecting active inflammation, correlated significantly with the degree of UPE in IgAN **(II)**. Glomerular CD68 and tubulointerstitial CD3 positivism was associated with elevated serum uric acid concentration **(I, II)**.

The amount of inflammatory cell infiltration evaluated by light microscopy correlated strictly with that studied by immunohistochemical methods **(II)**. Thus, the count of tubulointerstitial macrophages defines the level of active inflammation in renal tissue in IgAN. The number of urinary macrophages strongly correlates with that of interstitial macrophages (Hotta et al. 2000, Maruhashi et al. 2004). Intracellular adhesion molecule-1 (ICAM-1) is a major ligand in leukocyte trafficking (Arrizabalaga et al. 2001). Enhanced luminal expression of ICAM-1 has been found in tubular cells in IgAN (Arrizabalaga et al. 2001, Arrizabalaga et al. 2003). This induces the migration of leukocytes such as macrophages and CD3+ cells to the tubular region. Activated macrophages are usually the main source of IL-1β. However, in the present study IL-1β expression was found mainly in tubular epithelial cells. Tubular regions seem to be the main site of inflammation, which may be initiated by migrating macrophages or T-cells adhering to intraluminal ICAM-1. Tubulointerstitial IL-1β expression was significantly associated with high risk factors, interstitial fibrosis and serum creatinine concentration **(II)**. However, cytokine production of renal tubular epithelial cells may be intensively depressed by corticosteroid treatment (de Haij et al. 2002). Measurement of tubulointerstitial or preferably urinary macrophages as a noninvasive method may thus help in targeting medical therapy in IgAN.

In summary, mesangial C1q and C3 correlated with serum creatinine concentration in IgMN **(V)**. Tubulointerstitial CD3 and CD68 expressions correlated with UPE and the extent of tubulointerstitial cell infiltration reflecting active inflammation in renal tissue in IgAN **(II)**.

6.5. Clinicopathological correlations

6.5.1. Hypertension and renal vascular changes

Almost all renal disorders may induce hypertension. Conversely high BP may cause renal damage, possibly most traditionally hypertensive nephrosclerosis. Hypertension has been

proved to be one of the most significant risk factors for progression not only in IgAN, but also in other forms of glomerulonephritis. Recently, inflammatory cell infiltrations have been linked to the development of hypertension (Rodriguez-Iturbe and Johnson 2006). Inflammation induces reactive oxygen species, which in turn induces angiotensin II production and thus natrium retention and salt-sensitive hypertension (Rodriguez-Iturbe and Johnson 2006). The angiotensin II inhibitor losartan seems even to reduce the intrarenal cytokine expression in IgAN (Park et al. 2003). High BP may cause glomerular changes by elevating intraglomerular pressure, but the most substantial mechanism of hypertension may be the inducing of renal vascular damage. In the present study BP correlated with glomerular, tubulointerstitial and vascular histopathological changes in both IgAN and IgMN **(I)**. The associations between BP and arteriolosclerosis were the strongest and independent of other clinical factors in IgAN. Katafuchi and colleagues discovered by evaluating serial renal samples that both glomerular and vascular sclerotic lesions progressed more markedly in hypertensive IgAN patients (Katafuchi et al. 1988).

In summary, the results obtained in the present series are in line with earlier findings. Hypertension is strongly associated with renovascular lesions in IgAN and IgMN. Tubulointerstitial inflammation may for its part constitute a pathophysiological background factor in the development of hypertension in glomerulonephritis.

6.5.2. Uric acid and tubulointerstitial changes

In addition to other previously reported risk factors hyperuricemia has also recently been found to be associated with progression in IgAN. In an experimental study, hyperuricemia was associated with severe glomerular, vascular and interstitial changes, but antihyperuricemic medication seemed to reduce the histopathological damage (Kang et al. 2002). Some investigators suggest that hyperuricemia causes glomerular hypertension and cortical vasoconstriction and thus leads to glomerular damage and tubular ischemia (Heinig and Johnson 2006). Intrarenal macrophages may swallow uric acid crystals by endocytosis, this leading to increased expression of many inflammatory mediators (Cameron 2006, Heinig and Johnson 2006). In the present series serum uric acid concentration correlated with chronic tubulointerstitial changes, and also with interstitial inflammation and less strongly with glomerulosclerosis in IgAN **(I)**. The association between serum uric acid level and tubulointerstitial changes was independent of other clinical parameters investigated. It has

recently been found that uric acid-lowering therapy by allopurinol may slow the progression rate in chronic kidney disease (Siu et al. 2006). Tubulointerstitial damage is the strongest histopathological predictive risk factor in IgAN. Even though serum uric acid levels correlate strongly with those of serum creatinine, hyperuricemia was here found to be the only clinical factor independently associated with tubulointerstitial changes (**I**). These findings may justify treatment of hyperuricemic IgAN patients with allopurinol.

In summary, hyperurichemia associated independently with chronic tubulointerstitial lesions. This finding may facilitate decisions on the use of uric acid-lowering therapy.

6.5.3. BMI and lipids and renal histopathological changes

In the present case BMI was found to correlate, but not independently, with the severity of tubulointerstitial and vascular lesions in IgAN (**I**). Massive obesity has been traditionally linked to glomerular enlargement, glomerulomegaly (Cohen 1975). Another typical renal pathological lesion associated with obesity is focal segmental glomerulosclerosis (Praga et al. 2001). In an experimental study with obese Zucker rats, a high-fat diet aggravated macrophage-mediated tubulointerstitial lesions (Matsuda et al. 1999). Obese rats evinced more severe glomerular and tubulointerstitial changes than did lean rats (Matsuda et al. 1999). Obesity or elevated BMI may also be an independent risk factor for progression in IgAN (Bonnet et al. 2001). In this last-mentioned study BMI correlated significantly with glomerular, tubulointerstitial and vascular lesions in IgAN.

High serum triglyceride and/or cholesterol levels seem to predict a decline in renal function in IgAN (Mera et al. 2000, Syrjänen et al. 2000, Rauta et al. 2002). In an experimental study, both hypercholesterolemia and hypertriglyceridemia induced tubulointerstitial damage, but not mesangial changes in rat kidneys (Joles et al. 2000). In the present series, serum triglyceride concentrations correlated most markedly with interstitial fibrosis and hyaline arteriolosclerosis in IgAN, but there were no significant correlations between serum cholesterol and any histopathological parameters (**I**).

In summary, obesity or elevated BMI and high serum cholesterol and triglyceride levels were associated with tubulointerstitial and vascular lesions in IgAN (**I**).

6.5.4. Correlations between age and histopathology

Based on the present findings, age correlated significantly with chronic histopathological lesions such as tubular atrophy, interstitial fibrosis and renal vascular changes in IgAN (**I**). Similar renal histological changes have previously been found in persons without known renal disease. There is no doubt that age influences renal function in kidney diseases like IgAN. The renal blood flow decreases about 10% per decade after the age of 40 (Kasiske 1987). GFR decreases approximately nearly 1 ml/min per year after the age of 20. The amount of renovascular atherosclerosis and glomerulosclerosis increases with age (Kasiske 1987). Atherosclerosis may cause glomerular changes by reducing intrarenal blood flow, but it also seems possible that atherosclerosis and glomerulosclerosis may have common pathogenetic features (Kasiske 1987). Likewise the amount of interstitial tissue changes with age in the general population (Kappel and Olsen 1980). In IgAN, age appeared to correlate inversely with mesangial cellularity in the present study (**I**). It has recently been reported that pediatric IgAN patients have more commonly mesangial hypercellularity in renal biopsy at the onset of disease than have adult IgAN patients. (Ikezumi et al. 2006). Mesangial hypercellularity is regarded as a marker of more active disease (Suzuki and Joh 2004).

Similar results were obtained between histopathology and age in the context of IgMN in the present study.

Table 10. Prognostic clinical and histopathological markers at presentation in IgAN (Modified from Floege and Feehally 2000 and Barrat and Feehally 2005)

Clinical factors	Histopathological findings
Poor prognosis	*Poor prognosis*
Old age	Diffuse proliferative glomerulonephritis
Duration of symptoms	Extent of global glomerulosclerosis
Severe PU (>1g/d)	Interstitial sclerosis/fibrosis
Hypertension	Adhesions and crescents
Renal insufficiency	Tubule atrophy
Increased BMI	Vascular wall thickening
Male gender	Capillary-loop IgA deposits
Persistent microscopic HU	Mesangiolysis
	Glomerular basement membrane abnormalities
Good prognosis	*Good prognosis*
Recurrent macroscopic HU	Minimal LM abnormalities

6.6. Prognosis and prognostic factors in renal disease

6.6.1. Prevalence of renal insufficiency

The incidence or prevalence of renal insufficiency or ESRD reported in IgAN depends on differences in renal biopsy criteria. According to figures recently presented, 15-40% of IgAN patients reach ESRD during 25 years of post-biopsy follow-up. In the present study 8% of IgAN patients reached ESRD in ten years. In another IgAN material from Helsinki, Finland, the 10 years' renal survival was 96% (Geddes et al. 2003). The rate of development of ESRD in the Finnish population would thus appear to be among the lowest world wide. The liberal renal biopsy policy in Finland for its part influences these figures. Also genetic factors such as angiotensin-converting enzyme polymorphism may affect the prevalence differences seen in various IgAN populations. Nonetheless, Hall and colleagues found no significant racial differences in severity of IgAN (Hall et al. 2004). According to the latest reports from the Finnish Registry for Kidney Diseases 5% of patients in renal replacement therapy suffer from IgAN. Thus, despite the low incidence of ESRD, IgAN markedly burdens dialysis resources.

In the present material, including also children and patients with minimal urinary abnormalities, the prevalence of elevated serum creatinine and ESRD at the end of eight years' follow-up was 19% and 5% respectively in IgMN patients **(IV)**. Because of the small number of patients, short follow-up times and divergent inclusion criteria in previous studies, no accurate estimate of the incidence of renal insufficiency in IgMN is possible. For the same reasons the prevalences of renal insufficiency in the present and earlier studies cannot be compared with each other.

6.6.2. Prognostic factors

There are abundant studies concerning risk factors for progression, renal insufficiency or ESRD in IgAN during the four decades since its definition. Many different parameters indicating poor course of IgAN have been reported. The most significant previously reported risk factors for progression in IgAN are summarized in Table 10. In addition to these, hyperuricemia and hypertriglyceridemia have recently been suggested to be independent risk factors for progression in IgAN (Syrjänen et al. 2000). Possibly the most powerful risk factors are histological tubulointerstitial lesions. For this reason the present study focused on

the role of light-microscopically detected chronic tubulointerstitial changes and immunohistochemically evaluated intrarenal inflammatory cell and inflammation mediators in evaluating the long-term prognosis of IgAN (**II**).

In univariate analysis the numbers of tubulointerstitial leukocytes (LCA+), T-lymphocytes (CD3+) and macrophages (CD68+) all correlated significantly with progression of IgAN (**II**). Arrizabalaga and colleagues have also found a similar relationship between interstitial CD3+ cells and progression in IgAN (Arrizabalaga et al. 2001, Arrizabalaga et al. 2003). In a British IgAN material interstitial infiltration of CD68+ macrophages did not influence prognosis, but the follow-up time was only 2-3 years (Alexopoulos et al. 1989). Zhu and associates have recently found that the number of interstitial macrophages may predict a poor course in IgAN (Zhu et al. 2006). However, even if these last-mentioned results are similar to those in the present study, the low number (36) of patients must limit confidence (Zhu et al. 2006).

Also tubulointerstitial proinflammatory IL-1β were associated with progression of IgAN in the present study (**II**). No such findings were made in Japanese IgAN patients, where intrarenal IL-1β did not correlate with any studied clinical or histopathological factors (Taniguchi et al. 1996). Though the polymorphism of IL-10 may affect the prognosis of IgAN, the tubulointerstitial IL-10 level did not correlate with progression in the present study (Bantis et al. 2004) (**II**). However, only tubulointerstitial expression of CD3, in addition to hypertriglyceridemia, elevated serum creatinine and interstitial fibrosis, emerged as an independent risk factor for progression in IgAN (**II**). These findings emphasize the role of interstitial inflammation and other tubulointerstitial lesions when evaluating the prognosis of an individual patient with IgAN.

Only few studies, with incongruous results, have addressed factors predicting renal insufficiency in IgMN. In the present case with a larger number of patients, wide inclusion criteria and longer follow-up time, age and severity of chronic renal histopathological lesions were understandably related to a poorer course of IgMN (**IV**). Of critical clinical parameters hypertension and elevated baseline serum creatinine were the most significant risk factors for renal insufficiency or progression in IgMN, as is the case in almost all other kidney diseases. High BP with certain other factors may also anticipate the development of ESRD in IgMN.

Likewise a high serum C3 level seems to be associated with progression in IgMN regardless of the level of UPE (**V**). The role of complement in the prognosis of IgMN has not

previously been studied, but in IgAN more severe disease seems to be associated with low serum C3 level and/or high serum IgA/C3 ratio (Ishiguro et al. 2002, Komatsu et al. 2004). A Low serum C3 level has also been reported to predict severe course in the acute phase of glomerulonephritis (Friedman et al. 1985). This finding is taken to be attributable to depletion of C3 by a more intense inflammation process (Friedman et al. 1985). O'Donoghue and colleagues found very different risk factors in their IgMN material. They identified mesangial cell proliferation and sclerosis, and HU as independent risk factors for ESRD (O'Donoghue et al. 1991). In the present study these markers had no significant prognostic value; HU was rather a safety than risk factor **(IV)**. The causes and background of these discordant results remain obscure and call for further investigation with more homogeneous patient populations.

In summary, the present study confirms earlier findings in that the traditional high risk factors emerged. However, this study brought out new information regarding the significance of interstitial inflammation for the natural course of IgAN. Especially tubulointerstitial infiltration of CD3+ cells predicted progression in IgAN independently of other high-risk factors.

6.6.3. Development of FSGS in IgMN

Some investigators have reported a histologic transition from IgMN to FSGS (Gonzalo et al. 1985, Lin and Chu 1986, Saha et al. 1989, Ahmad and Tejani 2000). In the present study, typical FSGS was seen in five (45%) repeat biopsy specimens. No clinical or histopathological factors predicted the development of FSGS, possibly due to the small number of cases involved. However, all patients with FSGS in the repeat biopsy had NS or PU as their initial manifestation of kidney disease. This may partly account for the higher risk of progression in these patients than in patients with HU. Severe renal insufficiency was commonly seen in the context of FSGS in this material **(IV)**. Zeis and colleagues found transition to FSGS to be more common in patients with diffuse mesangial hypercellularity with mesangial IgM than in those without mesangial IgM (Zeis et al. 2001). This issue is important in view of the poor prognosis and poor response to medical therapy in FSGS (Korbet 1998). Accurate assessment of the frequency of IgMN developing to FSGS requires systematic repeated biopsies performed on a great number of IgMN patients. By reason of

potential serious complications associated with performing renal biopsy, serial biopsies are not ethically acceptable in these fairly asymptomatic subjects.

6.7. Vascular diseases in IgAN

6.7.1. Prevalence of VDs in IgAN patients compared to the general population

Strong evidence indicates that renal patients with severe renal insufficiency run a substantially elevated risk of cardiovascular diseases (Tonelli and Pfeffer 2007). The prevalence of CHD seems to be higher also among patients with mild renal insufficiency, but the risk rises linearly concomitant with decreasing GFR. This association seems to prevail independently of the degree of PU, which is also a clearly defined risk factor for CHD (Tonelli and Pfeffer 2007). The mechanisms underlying the increased CHD risk in chronic renal insufficiency may be a high prevalence of traditional or nontraditional risk factors and underusage of beneficial therapies (Vlagopoulos and Sarnak 2005, Tonelli and Pfeffer 2007). Chronic renal disease may also be a marker of the severity of pre-existing vascular disease (Tonelli and Pfeffer 2007). A similar association cannot be claimed in the case of patients with kidney diseases and normal renal function. In the present study, IgAN patients had VDs markedly more frequently than had the general population **(III)**. The prevalence of CeVD was especially high in IgAN. Since the results were similar among patients with stable renal disease, it would appear that there must be other factors, in addition to renal insufficiency, which explain the elevated risk of VDs in IgAN. One explanation may be the higher frequency of hypertension among IgAN patients. However, this difference in hypertension prevalence may be partly explained by the more strict blood pressure control by medication in IgAN than in the general population. It has been proposed that about half of the nephrons must be out of action before serum creatinine begins to rise. Even before that, other renal metabolic functions may have changed, which may partly explain the high rates of VDs in IgAN patients with normal serum creatinine.

However, serum cystatin C may be elevated in some of these patients. The serum cystatin C level may be a better indicator of lower grade renal dysfunction than estimated GFR or serum creatinine concentration (Stevens and Levey 2005, Schiffrin et al. 2007). High cystatin C levels were associated with a elevated risk of cardiovascular diseases in elderly

patients with estimated GFR ≥ 60 ml/min/1.73m^2 (Shlipak et al. 2006). This may reflect the influence of preclinical or subclinical kidney disease on the incidence of vascular diseases and their risk factors. Also atherosclerosis-accelerating inflammatory markers have been found to be elevated in early stages of IgAN (Nelson et al. 2005).

In summary, as in previous studies, VDs were here particularly common in patients with progressive renal disease. However, patients with stable disease also seem to carry an elevated risk of VDs compared to the general population. These patients may still have subclinical renal dysfunction without elevation of serum creatinine level.

6.7.2. Risk factors for VDs in IgAN

As in earlier studies, in the present IgAN material, progression of renal disease and elevated serum creatinine concentration predicted the development of VDs **(III)**. Male gender was also a risk factor for CHD in IgAN. Of traditional cardiovascular risk factors hypertension was also associated with VDs in IgAN. Almost all IgAN patients suffering from CeVD were hypertensive. Renal function tests may be used as sensors of the risk of VDs in hypertensive patients (Ruilope 2002). It is well known that high serum cholesterol, especially LDL, and triglycerides are associated with elevated CHD morbidity in the general population. A similar situation seems to prevail in IgAN based on the present findings.

Hyperuricemia is one of the factors associating with metabolic syndrome. Researchers' conceptions of the role of hyperuricemia as an independent risk factor for CHD in the general population are somewhat discordant. In the present study hyperuricemia correlated univariately, but not independently, with the occurrence of VD, CHD and CeVD **(III)**. This may be due to the strong correlation between serum uric acid and creatinine concentrations. The significant correlation between BMI and VDs may be attributable to other metabolic factors associating with metabolic syndrome, for example hypertension, dyslipidemia, insulin resistance and hyperuricemia. Even though the role of cigarette smoking in provoking the progression of renal disease remains obscure, it seems to affect the overall prognosis of IgAN by promoting the development of VDs.

Proteinuria >1g/24h was found to be risk factor for VDs in IgAN **(III)**. In fact there is in chronic kidney disease a graded direct relation between proteinuria and cardiovascular risk (Tonelli and Pfeffer 2007). Even microalbuminuria is associated with increased risk compared to patients with albumin-free urine (Tonelli and Pfeffer 2007). The influence of

proteinuria on VD risk may partly arise due to coexistent renal insufficiency and on the other hand microalbuminuria often precedes the development of renal insufficiency. However, since proteinuria and impaired renal function often exist independently, they may influence VD risk by different mechanisms (Tonelli and Pfeffer 2007).

The pathogenetic mechanisms of glomerulosclerosis and arteriosclerosis apparently have congruent features (Galle et al. 1999). As in the material of Ikee and colleagues (2006), the severity of glomerulosclerosis and hyaline arteriolosclerosis correlated significantly in the present IgAN material. Both of them, in addition to chronic tubulointerstitial lesions, were associated with VDs **(III)**. However, the association of hyaline arteriolosclerosis with the development of VDs was the strongest of these. Wu and associates reported that vascular changes in small arteries and arterioles may be more frequent in IgAN than in other glomerulonephritis forms (Wu et al. 2005). It would thus seem possible that VDs is more frequent in IgAN than in other glomerular diseases. It also seems clear that a manifestation of atherosclerosis in one organ reflects atherosclerosis in the overall vasculature.

In summary, the VD risk factor profile was similar in patients with IgAN and the general population. However, vascular changes seen in renal biopsies from IgAN patients predict the development of VDs.

7. SUMMARY AND CONCLUSIONS

Judging from previous findings factors associating with metabolic or insulin resistance syndrome, for example hypertension, diabetes, hyperlipidemia and also hyperuricemia, may promote the development of renal insufficiency in these diseases. This is especially observed in the context of IgAN. High-grade hyperuricemia evidently causes primarily damage in tubular region. Slightly elevated serum uric acid may also have an independent role in the development of tubulointerstitial histological changes. Of light microscopically evaluated parameters severe tubulointerstitial lesions seem to constitute the most significant histopathological risk factor for progression at least in IgAN. The same seems to apply to IgMN. In the present investigation the nature of interstitial inflammation and its relation to uric acid concentration and renal prognosis were further clarified.

In addition to terminal uremia, the overall prognosis of renal patients depends to a great extent on the development of cardiovascular diseases. This is especially noteworthy in Western countries like Finland, where these diseases are the most common causes of death. The present study presents the prevalence and risk factors for VDs in IgAN. These data were compared to those on the general population in the same district.

The summary and conclusions of the main findings in the present study are as follows:

1. All significant clinical risk factors for progression of IgAN correlated with morphological changes in renal tissue. While serum uric acid correlated most strongly with tubulointerstitial changes, blood pressure was the most significant factor predicting changes in vascular morphology. Also the level of serum triglycerides seemed to be associated with renal morphological changes in IgAN. Factors linked to metabolic or insulin resistance syndrome, especially uric acid, triglycerides and BMI, correlated with tubulointerstitial damage, which is widely accepted to be the most significant histopathological risk factor for progression in IgAN. Long-term prospective studies are needed in the future to establish whether uric acid-lowering therapy will have an influence on the prognosis of IgAN.

2. Inflammation of tubulointerstitial tissue clearly reflects a poor prognosis in IgAN. Especially investigation of tubulointerstitial CD3+ T-lymphocyte infiltrations and the

expression of intrarenal IL-1β by immunohistochemical methods, proved to be a useful tool in predicting outcome in IgAN. By evaluating the occurrence and level of active intrarenal inflammation, the medical therapy may be more accurately targeted to those patients who may derive the most significant benefit.

3. Vascular diseases, especially CeVD, are markedly more common in IgAN patients than in the general population. The risk factor profiles for VDs in IgAN and in the general population are similar. Male gender, hypertension, renal insufficiency, smoking and high serum triglyceride concentration were independently associated with some manifestation of VDs. Of factors earlier found to reflect risk of the progression of IgAN, hypertension and hypertriglyceridemia were also independently associated with VDs. A progressive renal disease is associated with the development of VDs in IgAN. Vascular changes seen upon renal biopsy in patients with IgAN signify an elevated risk of VDs.

4. It would seem that IgMN is a more severe disease than has previously been suggested. About one third of patients here developed at least mild stage of renal insufficiency. In over half of IgMN patients, hypertension developed during the follow-up of 15 years. Some IgMN patients, especially those with high-grade PU, seem to progress to FSGS, which is closely associated with ESRD. Judging from the present results IgMN may be divided into two different diseases or subgroups with similar renal morphology but different sex distribution and clinical outcome. Patients with only HU are mainly females and seem to have a lower progression rate.

5. Immunoglobulin and complement findings in the serum and intraglomerular area may be of prognostic significance in IgMN. Elevation of serum C3 correlated with clinical and histopathological factors indicating severe renal disease. Serum C3 was associated independently with progressive renal disease in the subgroup of IgMN patients without NS. Especially serum C3 measurement may bring out additional information in determining the long-term prognosis in IgMN.

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REFERENCES

Abe K, Miyazaki M, Koji T, Furusu A, Shioshita K, Tsukasaki S, Ozono Y, Harada T, Sakai H, Kohno S (2001) Intraglomerular synthesis of complement C3 and its activation products in IgA nephropathy. Nephron 87:231-239

Abe K, Miyazaki M, Shioshita K, Harada T, Koji T, Kohno S (2004). Clinical and immunohistochemical study of immunoglobulin A nephropathy (IgAN) before and after tonsillectomy. Acta Otolaryngol Suppl 555:20-24

Ahmad H, Tejani A (2000). Predictive value of repeat renal biopsies in children with nephrotic syndrome. Nephron 84:342-346

Akinsola A, Mbanefo CO, Iyun AO (1984). Serum immunoglobulins and complement in nephrotic syndrome. Afr J Med Sci 13:41-46

Alderman MH (2002). Uric acid and cardiovascular risk. Curr Opin Pharmacol 2:126-130

Al-Eisa A, Carter JE, Lirenman DS, Magil AB (1996). Childhood IgM nephropathy: comparison with minimal change disease. Nephron 72:37-43

Alexopoulos E, Seron D, Hartley RB, Nolasco F, Cameron JS (1989). The role of interstitial infiltrates in IgA nephropathy: a study with monoclonal antibodies. Nephrol Dial Transplant 4:187-195

Alexopoulos E, Papagianni A, Stangou M, Pantzaki A, Papadimitriou M (2000). Adult-onset idiopathic nephrotic syndrome associated with pure diffuse mesangial hypercellularity. Nephrol Dial Transplant 15:981-987

Altin JG, Sloan EK (1997). The role of CD45 and CD45-associated molecules in T cell activation. Immunol Cell Biol 75:430-445

Anonymous (1997). The sixth report of the Joint National Committee on prevention, detection, evaluation, and treatment of high blood pressure. Arch Intern Med 157:2413-2446

Anonymous (1996). Update on the 1987 Task Force Report on High Blood Pressure in Children and Adolescents: a working group report from the National High Blood Pressure Education Program. National High Blood Pressure Education Program Working Group on Hypertension Control in Children and Adolescents. Pediatrics 98:649-658

Aromaa A, Koskinen S, ed (2004). Health and functional capacity in Finland. Baseline results of the Health 2000 health examination survey. Publications of the National Public Health Institute B12/2004. Available at www.ktl.fi/terveys2000/index.uk.html

Arrizabalaga P, Sole M, Abellana R, Ascaso C, Cardesa A (2001). Renal expression of intercellular adhesion molecule-1 in immunoglobulin A nephropathy: tubulointerstitial injury and prognosis. Virchows Arch 439:27-34

Arrizabalaga P, Sole M, Abellana R, de las Cuevas, X., Soler J, Pascual J, Ascaso C (2003). Tubular and interstitial expression of ICAM-1 as a marker of renal injury in IgA nephropathy. Am J Nephrol 23:121-128

Aucouturier P, Monteiro RC, Noel LH, Preud'homme JL, Lesavre P (1989). Glomerular and serum immunoglobulin G subclasses in IgA nephropathy. Clin Immunol Immunopathol 51:338-347

Bakoush O, Grubb A, Rippe B, Tencer J (2001a). Urine excretion of protein HC in proteinuric glomerular diseases correlates to urine IgG but not to albuminuria. Kidney Int 60:1904-1909

Bakoush O, Torffvit O, Rippe B, Tencer J (2001b). High proteinuria selectivity index based upon IgM is a strong predictor of poor renal survival in glomerular diseases. Nephrol Dial Transplant 16:1357-1363

Bantis C, Heering PJ, Aker S, Klein-Vehne N, Grabensee B, Ivens K (2004). Association of interleukin-10 gene G-1082A polymorphism with the progression of primary glomerulonephritis. Kidney Int 66:288-294

Barratt J, Feehally J (2005). IgA nephropathy. J Am Soc Nephrol 16:2088-2097

Becker MA, Jolly M (2006) Hyperuricemia and associated diseases.. Rheum Dis Clin North Am 32:275-293

Bhasin HK, Abuelo JG, Nayak R, Esparza AR (1978). Mesangial proliferative glomerulonephritis. Laboratory Investigation 39:21-29

Biancone L, David S, Della Pietra V, Montrucchio G, Cambi V, Camussi G (1994). Alternative pathway activation of complement by cultured human proximal tubular epithelial cells. Kidney Int 45:451-460

Bitencourt-Dias C, Bahiense-Oliveira M, Saldanha LB, Barros RT, Woronik V (2004). Comparative study of IgA nephropathy with and without crescents. Brazilian Journal of Medical & Biological Research 37:1373-1377

Bonnet F, Deprele C, Sassolas A, Moulin P, Alamartine E, Berthezene F, Berthoux F (2001). Excessive body weight as a new independent risk factor for clinical and pathological progression in primary IgA nephritis. Am J Kidney Dis 37:720-727

Bonsib SM (2002). Differential diagnosis in nephropathology: an immunofluorescence-driven approach. Adv Anat Pathol 9:101-114

Border WA (1988). Distinguishing minimal-change disease from mesangial disorders. Kidney Int 34:419-434

Briganti EM, Dowling J, Finlay M, Hill PA, Jones CL, Kincaid-Smith PS, Sinclair R, McNeil JJ, Atkins RC (2001). The incidence of biopsy-proven glomerulonephritis in Australia. Nephrol Dial Transplant 16:1364-1367

Buemi M, Allegra A, Corica F, Aloisi C, Giacobbe M, Pettinato G, Corsonello A, Senatore M, Frisina N (2000). Effect of fluvastatin on proteinuria in patients with immunoglobulin A nephropathy. Clin Pharmacol Ther 67:427-431

Burnier M, Roch-Ramel F, Brunner HR (1996). Renal effects of angiotensin II receptor blockade in normotensive subjects. Kidney Int 49:1787-1790

Calls Ginesta J, Almirall J, Torras A, Darnell A, Revert L (1995). Long-term evolution of patients with isolated C3 mesangial glomerulonephritis. Clin Nephrol 43:221-225

Cameron JS (2006). Uric Acid and renal disease. Nucleosides Nucleotides Nucleic Acids 25:1055-1064

Cannon PJ, Stason WB, Demartini FE, Sommers SC, Laragh JH (1966). Hyperuricemia in primary and renal hypertension. N Engl J Med 275:457-464

Capasso G, Jaeger P, Robertson WG, Unwin RJ (2005). Uric acid and the kidney: urate transport, stone disease and progressive renal failure. Curr Pharm Des 11:4153-9

Cavallo T, Johnson MP (1981). Immunopathologic study of minimal change glomerular disease with mesangial IgM deposits. Nephron 27:281-284

Chan MK, Chan KW, Jones B (1987). Immunoglobulins (IgG, IgA, IgM, IgE) and complement components (C3, C4) in nephrotic syndrome due to minimal change and other forms of glomerulonephritis, a clue for steroid therapy? Nephron 47:125-130

Chan TM, Chan PC, Chan KW, Cheng IK (1991). IgM nephropathy in a patient with Kimura's disease. Nephron 58:489-490

Chen WP, Lin CY (1994). Augmented expression of interleukin-6 and interleukin-1 genes in the mesangium of IgM mesangial nephropathy. Nephron 68:10-19

Cho MH, Hong EH, Lee TH, Ko CW (2007). Pathophysiology of minimal change nephrotic syndrome and focal segmental glomerulosclerosis. Nephrol Suppl 12:S11-14

Cohen A, Border W (1982). Mesangial proliferative glomerulonephritis. Semin Nephrol 2:228-240

Cohen AH (1975). Massive obesity and the kidney. A morphologic and statistical study. Am J Pathol 81:117-130

Cohen AH, Border WA, Glassock RJ (1978). Nehprotic syndrome with glomerular mesangial IgM deposits. Lab Invest 38:610-619

Conger JD (1990). Acute uric acid nephropathy. Med Clin North Am 74:859-871

Corpa MV, Soares V (2002). Systemic hypertension in patients with glomerulonephritis. Ren Fail 24:347-352

Csiky B, Kovacs T, Wagner L, Vass T, Nagy J (1999). Ambulatory blood pressure monitoring and progression in patients with IgA nephropathy. Nephrol Dial Transplant 14:86-90

Culleton BF, Larson MG, Kannel WB, Levy D (1999). Serum uric acid and risk for cardiovascular disease and death: the Framingham Heart Study. Ann Intern Med 131:7-13

D'Amico G (2000). Natural history of idiopathic IgA nephropathy: role of clinical and histological prognostic factors. Am J Kidney Dis 36:227-237

D'Amico G, Gentile MG (1992). Effect of dietary manipulation on the lipid abnormalities and urinary protein loss in nephrotic patients. Miner Electrolyte Metab 18:203-206

Daniel L, Saingra Y, Giorgi R, Bouvier C, Pellissier JF, Berland Y (2000). Tubular lesions determine prognosis of IgA nephropathy. Am J Kidney Dis 35:13-20

Daskalopoulou SS, Mikhailidis DP, Elisaf M (2004). Prevention and treatment of the metabolic syndrome. Angiology 55:589-612

Davenport A, Maciver AG, Mackenzie JC (1992). C1q nephropathy: do C1q deposits have any prognostic significance in the nephrotic syndrome? Nephrol Dial Transplant 7:391-396

de Haij S, Woltman AM, Bakker AC, Daha MR, van Kooten C (2002). Production of inflammatory mediators by renal epithelial cells is insensitive to glucocorticoids. Br J Pharmacol 137:197-204

Delclaux C, Jacquot C, Callard P, Kleinknecht D (1993). Acute reversible renal failure with macroscopic haematuria in IgA nephropathy. Nephrol Dial Transplant 8:195-199

Dinarello CA (1996). Biologic basis for interleukin-1 in disease. Blood 87:2095-2147

Disciullo SO, Abuelo JG, Moalli K, Pezzullo JC (1988). Circulating heavy IgM in IgM nephropathy. Clin Exp Immunol 73:395-400

Donadio JV,Jr., Grande JP (1997). Immunoglobulin A nephropathy: a clinical perspective. J Am Soc Nephrol 8:1324-1332

Donadio JV, Grande JP (2002). IgA nephropathy. N Engl J Med 347:738-748

Donia AF, Sobh MA, Moustafa FE, Bakr MA, Foda MA (2000). Clinical significance and long-term evolution of minimal change histopathologic variants and of IGM nephropathy among Egyptians. J Nephrol 13:275-281

Eiro M, Katoh T, Sakuma Y, Sakurai K, Suzuki H, Asahi K, Watanabe K, Watanabe T (2003). Insulin resistance highly associates with hypertension in IgA nephropathy. Clin Nephrol 59:71-78

Emancipator SN (1994). IgA nephropathy: morphologic expression and pathogenesis. Am J Kidney Dis 23:451-462

Fakhouri F, Darre S, Droz D, Lemaire M, Nabarra B, Machet MC, Chauveau D, Lesavre P, Grunfeld JP, Noel LH, Knebelmann B (2002). Mesangial IgG glomerulonephritis: a distinct type of primary glomerulonephritis. J Am Soc Nephrol 13:379-387

Falk MC, Ng G, Zhang GY, Fanning GC, Roy LP, Bannister KM, Thomas AC, Clarkson AR, Woodroffe AJ, Knight JF (1995). Infiltration of the kidney by alpha beta and gamma delta T cells: effect on progression in IgA nephropathy. Kidney Int 47:177-185

Filler G, Bokenkamp A, Hofmann W, Le Bricon T, Martinez-Bru C, Grubb A (2005). Cystatin C as a marker of GFR--history, indications, and future research. Clin Biochem 38:1-8

Finnish Registry of Kidney Diseases: Annual reports. Helsinki. Available at http://www.musili.fi/fin/munuaistautirekisteri/finnish_registry_for_kidney_diseases/

Fliser D Pacini G, Engelleiter R, Kautzky-Willer A, Prager R, Franek E, Ritz E (1998). Insulin resistance and hyperinsulinemia are already present in patients with incipient renal disease. Kidney Int 53:1343-1347

Floege J, Feehally J (2000). IgA nephropathy: recent developments. J Am Soc Nephrol 11:2395-2403

Fogo AB (2001). Minimal change disease and focal segmental glomerulosclerosis. Nephrol Dial Transplant 6:74-76

Freese P, Norden G, Nyberg G (1998). Morphologic high-risk factors in IgA nephropathy. Nephron 79:420-425

Friedman R, Benderly A, Golan D, Levy J, Etzioni A (1985). Blood level of C3 complement component as an aid in the management of acute phase glomerulonephritis. Isr J Med Sci 21:118-122

Frimat L, Briancon S, Hestin D, Aymard B, Renoult E, Huu TC, Kessler M (1997). IgA nephropathy: prognostic classification of end-stage renal failure. L'Association des Nephrologues de l'Est. Nephrol Dial Transplant 12:2569-2575

Fu LW, Yang LY, Chen WP, Tsai SJ, Lin CY (1998). IgG subclass/IgM ratio and response to therapy in focal segmental glomerulonecrosis. Pediatr Nephrol 12:761-763

Galla JH (1995). IgA nephropathy. Kidney Int 47:377-387

Galle J, Heermeier K, Wanner C (1999). Atherogenic lipoproteins, oxidative stress, and cell death. Kidney Int Suppl 71:S62-65

Geddes CC, Rauta V, Gronhagen-Riska C, Bartosik LP, Jardine AG, Ibels LS, Pei Y, Cattran DC (2003). A tricontinental view of IgA nephropathy. Nephrol Dial Transplant 18:1541-1548

Giangiacomo J, Cleary TG, Cole BR, Hoffsten P, Robson AM (1975). Serum immunoglobulins in the nephrotic syndrome. A possible cause of minimal-change nephrotic syndrome. N Engl J Med 293:8-12

Gonzalo A, Mampaso F, Gallego N, Quereda C, Fierro C, Ortuno J (1985). Clinical significance of IgM mesangial deposits in the nephrotic syndrome. Nephron 41:246-249

Gupta R, Krieger DW (2005). Preventing ischemic stroke: choosing the best strategy. Cleve Clin J Med 72:433-444

Haas M (1997). Histologic subclassification of IgA nephropathy: a clinicopathologic study of 244 cases. Am J Kidney Dis 29:829-842

Haas M, Spargo BH, Wit EJ, Meehan SM (2000). Etiologies and outcome of acute renal insufficiency in older adults: a renal biopsy study of 259 cases. Am J Kidney Dis 35:433-447

Habib R, Girardin E, Gagnadoux MF, Hinglais N, Levy M, Broyer M (1988). Immunopathological findings in idiopathic nephrosis: clinical significance of glomerular "immune deposits". Pediatr Nephrol 2:402-408

Hall JE, Jones DW, Kuo JJ, da Silva A, Tallam LS, Liu J (2003a). Impact of the obesity epidemic on hypertension and renal disease. Curr Hypertens Rep 5:386-392

Hall JE, Kuo JJ, da Silva AA, de Paula RB, Liu J, Tallam L (2003b). Obesity-associated hypertension and kidney disease. Curr Opin Nephrol Hypertens 12:195-200

Hall YN, Fuentes EF, Chertow GM, Olson JL (2004). Race/ethnicity and disease severity in IgA nephropathy. BMC Nephrol 5:2

Hamuryudan V, Yurdakul S, Kural AR, Ince U, Yazici H (1991). Diffuse proliferative glomerulonephritis in Behcet's syndrome. Br J Rheumatol *30:63-64*

Harper SJ, Allen AC, Pringle JH, Feehally J (1996). Increased dimeric IgA producing B cells in the bone marrow in IgA nephropathy determined by in situ hybridisation for J chain mRNA. J Clin Pathol 49:38-42

Harper SJ, Pringle JH, Wicks AC, Hattersley J, Layward L, Allen A, Gillies A, Lauder I, Feehally J (1994). Expression of J chain mRNA in duodenal IgA plasma cells in IgA nephropathy. Kidney Int 45:836-844

Heaf J, Lokkegaard H, Larsen S (1889). The epidemiology and prognosis of glomerulonephritis in Denmark 1985-1997. Nephrol Dial Transplant 14:1889-1897

Heinig M, Johnson RJ (2006). Role of uric acid in hypertension, renal disease, and metabolic syndrome. Cleve Clin J Med 73:1059-1064

Helin H, Mustonen J, Pasternack A, Antonen J (1982). IgM-associated glomerulonephritis. Nephron 31:11-16

Hiki Y, Horie A, Yasuda Y, Iwase H, Sugiyama S (2004). IgA nephropathy and tonsils--an approach from the structure of IgA1 produced by tonsillar lymphocytes. Acta Otolaryngol Suppl 555:28-31

Hiki Y, Kokubo T, Iwase H, Masaki Y, Sano T, Tanaka A, Toma K, Hotta K, Kobayashi Y (1999). Underglycosylation of IgA1 hinge plays a certain role for its glomerular deposition in IgA nephropathy. J Am Soc Nephrology 10:760-769

Hirszel P, Yamase HT, Carney WR, Galen MA, Graeber CW, Johnson KJ, Kennedy TL, Lapkin RA, McLean RH, Rosenworcel E (1984). Mesangial proliferative glomerulonephritis with IgM deposits. Clinicopathologic analysis and evidence for morphologic transitions. Nephron 38:100-108

Honkanen T, Mustonen J, Kainulainen H, Myllymaki J, Collin P, Hurme M, Rantala I (2005). Small bowel cyclooxygenase 2 (COX-2) expression in patients with IgA nephropathy. Kidney Int 67:2187-2195

Hooke DH, Gee DC, Atkins RC (1987). Leukocyte analysis using monoclonal antibodies in human glomerulonephritis. Kidney Int 31:964-972

Horie A, Hiki Y, Odani H, Yasuda Y, Takahashi M, Kato M, Iwase H, Kobayashi Y, Nakashima I, Maeda K (2003). IgA1 molecules produced by tonsillar lymphocytes are under-O-glycosylated in IgA nephropathy. Am J Kidney Dis 42:486-496

Hotta O, Yusa N, Kitamura H, Taguma Y (2000). Urinary macrophages as activity markers of renal injury. Clin Chim Acta 297:123-133

Hsu SI, Ramirez SB, Winn MP, Bonventre JV, Owen WF (2000). Evidence for genetic factors in the development and progression of IgA nephropathy. Kidney Int 57:1818-1835

Hunley TE, Kon V (1999). IgA nephropathy. Curr Opin Pediatr 11:152-157

Ibels LS, Gyory AZ (1994). IgA nephropathy: analysis of the natural history, important factors in the progression of renal disease, and a review of the literature. Medicine 73:79-102

Ibels LS, Gyory AZ, Caterson RJ, Pollock CA, Mahony JF, Waugh DA, Coulshed S (1998). Recognition and management of IgA nephropathy. Drugs 55:73-83

Ikee R, Kobayashi S, Saigusa T, Namikoshi T, Yamada M, Hemmi N, Imakiire T, Kikuchi Y, Suzuki S, Miura S (2006.) Impact of hypertension and hypertension-related vascular lesions in IgA nephropathy. Hypertens Res 29:15-22

Ikezumi Y, Suzuki T, Imai N, Ueno M, Narita I, Kawachi H, Shimizu F, Nikolic-Paterson DJ, Uchiyama M (2006). Histological differences in new-onset IgA nephropathy between children and adults. Nephrol Dial Transplant 21:3466-3474

Ishiguro C, Yaguchi Y, Funabiki K, Horikoshi S, Shirato I, Tomino Y (2002). Serum IgA/C3 ratio may predict diagnosis and prognostic grading in patients with IgA nephropathy. Nephron 91:755-758

Janssen U, Bahlmann F, Kohl J, Zwirner J, Haubitz M, Floege J (2000). Activation of the acute phase response and complement C3 in patients with IgA nephropathy. Am J Kidney Dis 35:21-28

Jennette JC (1988). The immunohistology of IgA nephropathy. Am J Kidney Dis 12:348-352

Jennette JC, Hipp CG (1985). C1q nephropathy: a distinct pathologic entity usually causing nephrotic syndrome. Am J Kidney Dis 6:103-110

Johnson RJ, Kivlighn SD, Kim YG, Suga S, Fogo AB (1999) Reappraisal of the pathogenesis and consequences of hyperuricemia in hypertension, cardiovascular disease, and renal disease. Am J Kidney Dis 33:225-234

Johnston PA, Davison AM (1993). Hypertension in adults with idiopathic glomerulonephritis and normal serum creatinine. A report from the MRC Glomerulonephritis Registry. Nephrol Dial Transplant 8:20-24

Joles JA, Kunter U, Janssen U, Kriz W, Rabelink TJ, Koomans HA, Floege J (2000). Early mechanisms of renal injury in hypercholesterolemic or hypertriglyceridemic rats. J Am Soc Nephrol 11:669-683

Julian BA, Wyatt RJ, McMorrow RG, Galla JH (1983). Serum complement proteins in IgA nephropathy. Clin Nephrol 20:251-258

Julian BA, Novak J (2004). IgA nephropathy: an update. Curr Opin Nephrol Hypertens. 13:171-179

Kaartinen K, Syrjänen J, Porsti I, Harmoinen A, Pasternack A, Huhtala H, Niemela O, Mustonen J (2007). Insulin resistance and the progression of IgA glomerulonephritis. Nephrol Dial Transplant 22:778-783

Kang DH, Nakagawa T, Feng L, Watanabe S, Han L, Mazzali M, Truong L, Harris R, Johnson RJ (2002). A role for uric acid in the progression of renal disease. J Am Soc Nephrol 13:2888-2897

Kanno Y, Okada H, Saruta T, Suzuki H (2000). Blood pressure reduction associated with preservation of renal function in hypertensive patients with IgA nephropathy: a 3-year follow-up. Clin Nephrol 54:360-365

Kappel B, Olsen S (1980). Cortical interstitial tissue and sclerosed glomeruli in the normal human kidney, related to age and sex. A quantitative study. Virchows Arch A Pathol Anat Histol 387:271-277

Kasiske BL (1987) Relationship between vascular disease and age-associated changes in the human kidney. Kidney Int 31:1153-1159

Katafuchi R, Takebayashi S, Taguchi T (1988). Hypertension-related aggravation of Iga nephropathy: a statistical approach. Clin Nephrol 30:261-269

Kaysen GA, al Bander H (1990). Metabolism of albumin and immunoglobulins in the nephrotic syndrome. Am J Nephrol 10:36-42

Kemper MJ, Altrogge H, Ganschow R, Muller-Wiefel DE (2002). Serum levels of immunoglobulins and IgG subclasses in steroid sensitive nephrotic syndrome. Pediatr Nephrol17:413-417

Kishimoto H, Arakawa M (1999). Clinico-pathological characterization of mesangial proliferative glomerulonephritis with predominant deposition of IgM. Clin Exp Nephrol 3:110-115

Komatsu H, Fujimoto S, Hara S, Sato Y, Yamada K, Eto T (2004). Relationship between serum IgA/C3 ratio and progression of IgA nephropathy. Internal Medicine 43:1023-1028

Kopolovic J, Shvil Y, Pomeranz A, Ron N, Rubinger D, Oren R (1987). IgM nephropathy: morphological study related to clinical findings. Am J Nephrol 7:275-280

Korbet SM (1998). Primary focal segmental glomerulosclerosis. J Am Soc Nephrol 9:1333-1340

Korbet SM, Genchi RM, Borok RZ, Schwartz MM (1996). The racial prevalence of glomerular lesions in nephrotic adults. Am J Kidney Dis 27:647-651

Korpela M. Mustonen J. Teppo AM. Helin H. Pasternack A (1997). Mesangial glomerulonephritis as an extra-articular manifestation of rheumatoid arthritis. Br J Rheumatol 36:1189-95

Koyama A. Igarashi M. Kobayashi M (1997). Natural history and risk factors for immunoglobulin A nephropathy in Japan. Research Group on Progressive Renal Diseases. Am J Kidney Dis 29:526-32

Lai KN, Chan LY, Leung JC (2005). Mechanisms of tubulointerstitial injury in IgA nephropathy. Kidney Int Suppl 94:s110-115

Lau KK, Gaber LW, Delos Santos NM, Fisher KA, Grimes SJ, Wyatt RJ (2004). Pediatric IgA nephropathy: clinical features at presentation and outcome for African-Americans and Caucasians. Clin Nephrol 62:167-172

Lawler W, Williams G, Tarpey P, Mallick NP (1980). IgM associated primary diffuse mesangial proliferative glomerulonephritis. J Clin Pathol 33:1029-1038

Lee AH, Bass PS, Williams JH, Evans B, Jones DB, Theaker JM, Davies DR (1996). CD45RO and CD45RA positive cell populations in idiopathic membranous and IgA glomerulopathy. J Clin Pathol 49:43-47

Lee HS, Lee MS, Lee SM, Lee SY, Lee ES, Lee EY, Park SY, Han JS, Kim S, Lee JS (2005). Histological grading of IgA nephropathy predicting renal outcome: revisiting H. S. Lee's glomerular grading system. Nephrol Dial Transplant 20:342-348

Lee SM (1997). Prognostic indicators of progressive renal disease in IgA nephropathy: emergence of a new histologic grading system. Am J Kidney Dis 29:953-958

Lee SM, Rao VM, Franklin WA, Schiffer MS, Aronson AJ, Spargo BH, Katz AI (1982). IgA nephropathy: morphologic predictors of progressive renal disease. Hum Pathol 13:314-322

Li PK, Ho KK, Szeto CC, Yu L, Lai FM (2002). Prognostic indicators of IgA nephropathy in the Chinese--clinical and pathological perspectives. Nephrol Dial Transplant 17:64-69

Lim CS, Yoon HJ, Kim YS, Ahn C, Han JS, Kim S, Lee JS, Lee HS, Chae DW (2003). Clinicopathological correlation of intrarenal cytokines and chemokines in IgA nephropathy. Nephrology 8:21-27

Lin CY, Chen CH, Lee PP (1989). In vitro B-lymphocyte switch disturbance from IgM into IgG in IgM mesangial nephropathy. Pediatr Nephrol 3:254-258

Lin CY, Chu CM (1986). Studies of circulating immune complexes and lymphocyte subpopulations in childhood IgM mesangial nephropathy. Nephron 44:198-203

Little MA, Dorman A, Gill D, Walshe JJ (2000). Mesangioproliferative glomerulonephritis with IgM deposition: clinical characteristics and outcome. Ren Fail 22:445-457

Maeda A, Gohda T, Funabiki K, Horikoshi S, Shirato I, Tomino Y (2003). Significance of serum IgA levels and serum IgA/C3 ratio in diagnostic analysis of patients with IgA nephropathy. J Clin Lab Anal 17:73-76

Maesaka JK, Fishbane S (1998). Regulation of renal urate excretion: a critical review. Am J Kidney Dis 32:917-933

Maffei S, Buoncristiani U, Stefanelli M, Germini G, Morroni M, Cinti S (1990). Association between type II basement membrane nephropathy and mesangial IgM nephropathy. Contrib Nephrol 80:162-165

Malafronte P, Mastroianni-Kirsztajn G, Betonico GN, Romao JE,Jr., Alves MA, Carvalho MF, Viera Neto OM, Cadaval RA, Bergamo RR, Woronik V, Sens YA, Marrocos MS, Barros RT (2006). Paulista Registry of glomerulonephritis: 5-year data report. Nephrol Dial Transplant 21:3098-3105

Mampaso F, Gonzalo A, Teruel J, Losada M, Gallego N, Ortuno J, Bellas C (1981). Mesangial deposits of IgM in patients with the nephrotic syndrome. Clin Nephrol 16:230-234

Manjunath G, Tighiouart H, Ibrahim H, MacLeod B, Salem DN. Griffith JL, Coresh J, Levey AS, Sarnak MJ (2003). Level of kidney function as a risk factor for atherosclerotic cardiovascular outcomes in the community. J Am Coll Cardiol 41:47-55

Markovic-Lipkovski J, Muller CA, Risler T, Bohle A, Muller GA (1990). Association of glomerular and interstitial mononuclear leukocytes with different forms of glomerulonephritis. Nephrol Dial Transplant 5:10-17

Martinez-Maldonado M (2001). Role of hypertension in the progression of chronic renal disease. Nephrol Dial Transplant 1:63-66

Maruhashi Y, Nakajima M, Akazawa H, Shimoyama H, Nishiguchi M, Yamoto Y, Kamitsuji H, Yoshioka A (2004). Analysis of macrophages in urine sediments in children with IgA nephropathy. Clin Nephrol 62:336-343

Matsuda S, Arai T, Iwata K, Oka M, Nagase M (1999). A high-fat diet aggravates tubulointerstitial but not glomerular lesions in obese Zucker rats. Kidney Int Suppl 71:s150-152

Matsuo S, Morita Y, Mizuno M, Nishikawa K, Yuzawa Y (1998). Proteinuria and damage to tubular cells--is complement a culprit? Nephrol Dial Transplant 13:2723-2726

Mazzali M, Hughes J, Kim YG, Jefferson JA, Kang DH, Gordon KL, Lan HY, Kivlighn S, Johnson RJ (2001). Elevated uric acid increases blood pressure in the rat by a novel crystal-independent mechanism. Hypertension 38:1101-1106

Mazzali M, Kanellis J, Han L, Feng L, Xia YY, Chen Q, Kang DH, Gordon KL, Watanabe S, Nakagawa T, Lan HY, Johnson RJ (2002). Hyperuricemia induces a primary renal arteriolopathy in rats by a blood pressure-independent mechanism. Am J Physiol Ren Fluid Electrol Physiol 282:991-997

Mera J, Uchida S, Nagase M (2000). Clinicopathologic study on prognostic markers in IgA nephropathy. Nephron 84:148-157

Messerli FH, Frohlich ED, Dreslinski GR, Suarez DH, Aristimuno GG (1980). Serum uric acid in essential hypertension: an indicator of renal vascular involvement. Ann Intern Med 93:817-821

Miura M, Nomoto Y, Sakai H (1990). Evidence of enhanced uric acid clearance in macrohematuric patients with IgA nephropathy: prognostic significance of macrohematuria. Clin Nephrol 33:41-46

Moore KW, de Waal Malefyt, R., Coffman RL, O'Garra A (2001). Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol 19:683-765

Morita Y, Nomura A, Yuzawa Y, Nishikawa K, Hotta N, Shimizu F, Matsuo S (1997). The role of complement in the pathogenesis of tubulointerstitial lesions in rat mesangial proliferative glomerulonephritis. J Am Soc Nephrol 8:1363-1372

Muntner P, Coresh J, Smith JC, Eckfeldt J, Klag MJ (2000). Plasma lipids and risk of developing renal dysfunction: the atherosclerosis risk in communities study. Kidney Int 58:293-301

Muntner P, Hamm LL, Kusek JW, Chen J, Whelton PK, He J (2004). The prevalence of nontraditional risk factors for coronary heart disease in patients with chronic kidney disease. Ann Intern Med 140:9-17

Muso E, Mune M, Fujii Y, Imai E, Ueda N, Hatta K, Imada A, Takemura T, Miki S, Kuwahara T, Takamitsu Y, Tsubakihara Y, Kansai FGS LDL Apheresis Treatment (K-FLAT) Study Group (2001). Significantly rapid relief from steroid-resistant nephrotic syndrome by LDL apheresis compared with steroid monotherapy. Nephron 89:408-415

Mustonen J (1984). IgA glomerulonephritis and associated diseases. Ann Clin Res 16:161-166

Mustonen J, Pasternack A, Helin H, Nikkila M (1985). Clinicopathologic correlations in a series of 143 patients with IgA glomerulonephritis. Am J Nephrol 5:150-157

Mustonen J, Pasternack A, Helin H, Rilva A, Penttinen K, Wager O, Harmoinen A (1981). Circulating immune complexes, the concentration of serum IgA and the distribution of HLA antigens in IgA nephropathy. Nephron 29:170-175

Mustonen J, Syrjänen J, Rantala I, Pasternack A (2001). Clinical course and treatment of IgA nephropathy. J Nephrol 14:440-446

Mustonen JT, Rantala IS, Helin HJ, Pasternack AI (1991). IgA-IgM nephropathy. A subgroup of primary mesangial glomerulonephritis. Am J Clin Pathol 95:863-866

Nabokov A, Waldherr R, Ritz E (1997) Demonstration of the proliferation marker Ki-67 in renal biopsies: correlation to clinical findings. Am J Kidney Dis 30:87-97

Nakagawa T, Mazzali M, Kang DH, Kanellis J, Watanabe S, Sanchez-Lozada LG, Rodriguez-Iturbe B, Herrera-Acosta J, Johnson RJ (2003). Hyperuricemia causes glomerular hypertrophy in the rat. Am J Nephrol 23:2-7

Nakamura T, Ushiyama C, Hirokawa K, Osada S, Inoue T, Shimada N, Koide H (2002). Effect of cerivastatin on proteinuria and urinary podocytes in patients with chronic glomerulonephritis. Nephrol Dial Transplant 17:798-802

Nangaku M (2003). Complement regulatory proteins: are they important in disease? J Am Soc Nephrol 14:2411-2413

Nath KA, Hostetter MK, Hostetter TH (1985). Pathophysiology of chronic tubulo-interstitial disease in rats. Interactions of dietary acid load, ammonia, and complement component C3. J Clin Invest 76:667-675

Nelson CL, Karschimkus CS, Dragicevic G, Packham DK, Wilson AM, O'Neal D, Becker GJ, Best JD, Jenkins AJ (2005). Systemic and vascular inflammation is elevated in early IgA and type 1 diabetic nephropathies and relates to vascular disease risk factors and renal function. Nephrol Dial Transplant 20:2420-2426

Neugarten J, Acharya A, Silbiger SR (2000). Effect of gender on the progression of nondiabetic renal disease: a meta-analysis. J Am Soc Nephrol 11:319-329

Nickeleit V, Mihatsch MJ (1997). Uric acid nephropathy and end-stage renal disease--review of a non-disease. Nephrol Dial Transplant 12:1832-1838

Niemir ZI, Ondracek M, Dworacki G, Stein H, Waldherr R, Ritz E, Otto HF (1998). In situ upregulation of IL-10 reflects the activity of human glomerulonephritides. Am J Kidney Dis 32:80-92

Niemir ZI, Stein H, Dworacki G, Mundel P, Koehl N, Koch B, Autschbach F, Andrassy K, Ritz E, Waldherr R, Otto HF (1997). Podocytes are the major source of IL-1 alpha and IL-1 beta in human glomerulonephritides. Kidney Int 52:393-403

Nieuwhof C, Kruytzer M, Frederiks P, van Breda Vriesman PJ (1998). Chronicity index and mesangial IgG deposition are risk factors for hypertension and renal failure in early IgA nephropathy. Am J Kidney Dis 31:962-970

Nikolic-Paterson DJ, Atkins RC (2001) The role of macrophages in glomerulonephritis. Nephrol Dial Transplant 5:3-7

Nomura A, Morita Y, Maruyama S, Hotta N, Nadai M, Wang L, Hasegawa T, Matsuo S (1997). Role of complement in acute tubulointerstitial injury of rats with aminonucleoside nephrosis. Am J Pathol 151:539-547

Oda T, Hotta O, Taguma Y, Kitamura H, Sugai H, Onodera S, Horigome I, Suzuki K, Shouji Y, Furuta T, Chiba S, Yoshizawa N, Nagura H (1998). Clinicopathological significance of intratubular giant macrophages in progressive glomerulonephritis. Kidney Int 53:1190-1200

O'Donoghue DJ, Lawler W, Hunt LP, Acheson EJ, Mallick NP (1991). IgM-associated primary diffuse mesangial proliferative glomerulonephritis: natural history and prognostic indicators. QJM 79:333-350

Ohno I, Hosoya T, Gomi H, Ichida K, Okabe H, Hikita M (2001) .Serum uric acid and renal prognosis in patients with IgA nephropathy. Nephron 87:333-339

Packham DK, Hewitson TD, Yan HD, Elliott CE, Nicholls K, Becker GJ (1994). Acute renal failure in IgA nephropathy. Clin Nephrol 42:349-353

Pardo V, Berian MG, Levi DF, Strauss J (1979). Benign primary hematuria. Clinicopathologic study of 65 patients. Am J Med 67:817-822

Pardo V, Riesgo I, Zilleruelo G, Strauss J (1984). The clinical significance of mesangial IgM deposits and mesangial hypercellularity in minimal change nephrotic syndrome. American Journal of Kidney Diseases 3:264-269

Parfrey PS (2000). Cardiac disease in dialysis patients: diagnosis, burden of disease, prognosis, risk factors and management. Nephrol Dial Transplant Suppl 5:58-68

Park HC, Xu ZG, Choi S, Goo YS, Kang SW, Choi KH, Ha SK, Lee HY, Han DS (2003). Effect of losartan and amlodipine on proteinuria and transforming growth factor-beta1 in patients with IgA nephropathy. Nephrol Dial Transplant 18:1115-1121

Park YH, Choi JY, Chung HS, Koo JW, Kim SY, Namgoong MK, Park YS, Yoo KH, Lee KY, Lee DY, Lee SJ, Lee JE, Chung WY, Hah TS, Cheong HI, Choi Y, Lee KS (2005). Hematuria and proteinuria in a mass school urine screening test. Pediatric Nephrology 20:1126-1130

Payton CD, McLay A, Jones JM (1988). Progressive IgA nephropathy: the role of hypertension. Nephrol Dial Transplant 3:138-142

Perez-Fontan M, Miguel JL, Picazo ML, Martinez-Ara J, Selgas R, Sicilia LS (1986). Idiopathic IgA nephropathy presenting as malignant hypertension. Am J Nephrol 6:482-486

Perlstein TS, Gumieniak O, Hopkins PN, Murphey LJ, Brown NJ, Williams GH, Hollenberg NK, Fisher ND (2004). Uric acid and the state of the intrarenal renin-angiotensin system in humans. Kidney Int 66:1465-1470

Pettersson E (1997). IgA nephropathy: 30 years on J Intern Med 242:349-353

Polenakovic MH, Grcevska L, Dzikova S (2003). The incidence of biopsy-proven primary glomerulonephritis in the Republic of Macedonia-long-term follow-up. Nephrol Dial Transplant 26

Praga M, Hernandez E, Morales E, Campos AP, Valero MA, Martinez MA, Leon M (2001). Clinical features and long-term outcome of obesity-associated focal segmental glomerulosclerosis. Nephrol Dial Transplant 16:1790-1798

Purkerson J, Isakson P (1992). A two-signal model for regulation of immunoglobulin isotype switching. FASEB J 6:3245-3252

Racusen LC, Solez K, Colvin RB, Bonsib SM, Castro MC, Cavallo T, Croker BP, Demetris AJ, Drachenberg CB, Fogo AB, Furness P, Gaber LW, Gibson IW, Glotz D, Goldberg JC, Grande J, Halloran PF, Hansen HE, Hartley B, Hayry PJ, Hill CM, Hoffman EO, Hunsicker LG, Lindblad AS, Yamaguchi Y, et al (1999). The Banff 97 working classification of renal allograft pathology. Kidney Int 55:713-23

Radford MG,Jr., Donadio JV,Jr., Bergstralh EJ, Grande JP (1997). Predicting renal outcome in IgA nephropathy. Journal of the Am Soc Nephrol 8:199-207

Rafey MA, Lipkowitz MS, Leal-Pinto E, Abramson RG (2003). Uric acid transport. CurrOpin Nephrol Hypertens 12:511-516

Rai A, Nast C, Adler S (1999). Henoch-Schonlein purpura nephritis. J Am Soc Nephrol 10:2637-2644

Rantala I, Collin P, Holm K, Kainulainen H, Mustonen J, Maki M (1999). Small bowel T cells, HLA class II antigen DR, and GroEL stress protein in IgA nephropathy. Kidney Int 55:2274-2280

Rantala I, Mustonen J, Hurme M, Syrjänen J, Helin H (2001). Pathogenetic aspects of IgA nephropathy. Nephron 88:193-198

Rauta V, Teppo AM, Törnroth T, Honkanen E, Grönhagen-Riska C (2002). Factors associated with progression of IgA nephropathy are related to renal function--a model for estimating risk of progression in mild disease. Clin Nephrol 58:85-94

Rehan A, Johnson K (1986). IgM nephropathy associated with penicillamine. Am J Nephrol 6:71-74

Rich MW (2000). Uric acid: is it a risk factor for cardiovascular disease? Am J Cardiol 85:1018-1021

Ridao N, Luno J, Garcia de Vinuesa, S., Gomez F, Tejedor A, Valderrabano F (2001). Prevalence of hypertension in renal disease. Nephrol Dial Transplant 1:70-73

Ritz E (2003). Minor renal dysfunction: an emerging independent cardiovascular risk factor. Heart 89:963-964

Ritz E, McClellan WM (2004). Overview: increased cardiovascular risk in patients with minor renal dysfunction: an emerging issue with far-reaching consequences. J Am Soc Nephrol 15:513-516

Rivera F, Lopez-Gomez JM, Perez-Garcia R, Spsnish Registry of, G (2002). Frequency of renal pathology in Spain 1994-1999. Nephrol Dial Transplant 17:1594-1602

Roccatello D, Picciotto G, Torchio M, Ropolo R, Ferro M, Franceschini R, Quattrocchio G, Cacace G, Coppo R, Sena LM (1993). Removal systems of immunoglobulin A and immunoglobulin A containing complexes in IgA nephropathy and cirrhosis patients. The role of asialoglycoprotein receptors. Lab Invest 69:714-723

Rodriguez-Iturbe B, Johnson RJ (2006). Role of inflammatory cells in the kidney in the induction and maintenance of hypertension. Nephrol Dial Transplant 21:260-263

Ronkainen J, Ala-Houhala M, Autio-Harmainen H, Jahnukainen T, Koskimies O, Merenmies J, Mustonen J, Örmälä T, Turtinen J, Nuutinen M (2006). Long-term outcome 19 years after childhood IgA nephritis: a retrospective cohort study. Pediatr Nephrol 21:1266-1273

Ruilope LM (2002). The kidney as a sensor of cardiovascular risk in essential hypertension. J Am Soc Nephrology Suppl 13:s165-168

Saha H, Mustonen J, Pasternack A, Helin H (1989). Clinical follow-up of 54 patients with IgM-nephropathy. Am J Nephrol 9:124-128

Said R, Hamzeh Y (1990). IgM nephropathy associated with familial Mediterranean fever. Clin Nephrol 33:227-231

Said R, Hamzeh Y, Said S, Tarawneh M, al-Khateeb M (1992). Spectrum of renal involvement in familial Mediterranean fever. Kidney Int 41:414-419

Sakai H, Abe K, Kobayashi Y, Koyama A, Shigematsu H, Harada T, Yoshikawa N, Arakawa M, Itoh H, Osawa G, et al. (1995). Clinical guidelines of IgA nephropathy. Nippon Jinzo Gakkai Shi 37:417-421

Sano T, Hiki Y, Kokubo T, Iwase H, Shigematsu H, Kobayashi Y (2002). Enzymatically deglycosylated human IgA1 molecules accumulate and induce inflammatory cell reaction in rat glomeruli. Nephrol Dial Transplant 17:50-56

Sarnak MJ, Levey AS (2000). Cardiovascular disease and chronic renal disease: a new paradigm. Am J Kidney Dis Suppl 35:s117-131

Schiffrin EL, Lipman ML, Mann JF (2007). Chronic kidney disease: effects on the cardiovascular system. Circulation 116:85-97

Scolari F, Scaini P, Savoldi S, Prati E, Sacchi G, Amoroso A, Mazzola G, Canale L, Borelli I, Cristinelli L (1990). Familial IgM mesangial nephropathy: a morphologic and immunogenetic study of three pedigrees. Am J Nephrol 10:261-268

Segerer S, Mac KM, Regele H, Kerjaschki D, Schlondorff D (1999). Expression of the C-C chemokine receptor 5 in human kidney diseases. Kidney Int 56:52-64

Shlipak MG, Katz R, Sarnak MJ, Fried LF, Newman AB, Stehman-Breen C, Seliger SL, Kestenbaum B, Psaty B, Tracy RP, Siscovick DS (2006). Cystatin C and prognosis for cardiovascular and kidney outcomes in elderly persons without chronic kidney disease. Ann Intern Med 145:237-246

Shu KH, Lian JD, Yang YF, Lu YS, Wang JY (1988). Serum IgE in primary glomerular diseases and its clinical significance. Nephron 49:24-28

Shu KH, Ho WL, Lu YS, Cheng CH, Wu MJ, Lian JD (1999). Long-term outcome of adult patients with minimal urinary abnormalities and normal renal function. Clin Nephrol 52:5-9

Simon P, Ramee MP, Boulahrouz R, Stanescu C, Charasse C, Ang KS, Leonetti F, Cam G, Laruelle E, Autuly V, Rioux N (2004). Epidemiologic data of primary glomerular diseases in western France. Kidney Int 66:905-908

Sinniah R (1983). Occurrence of mesangial IgA and IgM deposits in a control necropsy population. J Clin Pathol 36:276-279

Siu YP, Leung KT, Tong MK, Kwan TH (2006). Use of allopurinol in slowing the progression of renal disease through its ability to lower serum uric acid level. Am J Kidney Dis 47:51-59

Snapper CM, Mond JJ (1993). Towards a comprehensive view of immunoglobulin class switching. Immunol Today 14:15-17

Statistical database of Finnish National Cardiovascular Diseases Register. Nationa Public Health Institute. Available at http://www3.ktl.fi/stat/

Statistics Finland. Causes of Death 2005. Available at http://tilastokeskus.fi/til/ksyyt/2005/ksyyt_2005_2006-10-31_tie_001_en.html

Steinsson K. Hirszel P. Weinstein A (1983). Mesangial IgM nephropathy in a patient with HLA-B27 spondylarthropathy. Arthritis Rheum 26:1056

Stenvinkel P, Alvestrand A, Angelin B, Eriksson M (2000). LDL-apheresis in patients with nephrotic syndrome: effects on serum albumin and urinary albumin excretion. Eur J Clin Invest 30:866-870

Stevens LA, Levey AS (2005). Measurement of kidney function. Med Clin North Am 89:457-473

Stratta P, Segoloni GP, Canavese C, Sandri L, Mazzucco G, Roccatello D, Manganaro M, Vercellone A (1996). Incidence of biopsy-proven primary glomerulonephritis in an Italian province. Am J Kidney Dis 27:631-639

Strife CF, Jackson EC, Forristal J, West CD (1986). Effect of the nephrotic syndrome on the concentration of serum complement components. Am J Kidney Dis 8:37-42

Subias R, Botey A, Darnell A, Montoliu J, Revert L (1987). Malignant or accelerated hypertension in IgA nephropathy. Clin Nephrol 27:1-7

Suzuki S, Joh K (2004). Applicability of steroid therapy in 275 adult patients with IgA nephropathy determined using a histological scoring system and degree of proteinuria. Clin Exp Nephrol 8:109-116

Syrjänen J, Hurme M, Lehtimaki T, Mustonen J, Pasternack A (2002). Polymorphism of the cytokine genes and IgA nephropathy. Kidney Int 61:1079-1085

Syrjänen J, Mustonen J, Pasternack A (2000). Hypertriglyceridaemia and hyperuricaemia are risk factors for progression of IgA nephropathy. Nephrol Dial Transplant 15:34-42

Tang Z, Wu Y, Wang QW, Yu YS, Hu WX, Yao XD, Chen HP, Liu ZH, Li LS (2002). Idiopathic IgA nephropathy with diffuse crescent formation. Am J Nephrol 22:480-486

Taniguchi Y, Yorioka N, Oda H, Yamakido M (1996). Platelet-derived growth factor, interleukin (IL)-1 beta, IL-6R and tumor necrosis factor-alpha in IgA nephropathy. An immunohistochemical study. Nephron 74:652-660

Tchilian EZ, Beverley PC (2006). Altered CD45 expression and disease. Trends Immunol 27:146-153

Tejani A, Nicastri AD (1983). Mesangial IgM nephropathy. Nephron 35:1-5

Tejani A (1985). Morphological transition in minimal change nephrotic syndrome. Nephron 39:157-159

Tencer J, Bakoush O, Torffvit O (2000). Diagnostic and prognostic significance of proteinuria selectivity index in glomerular diseases. Clin Chim Acta 297:73-83

Tencer J, Torffvit O, Thysell H, Rippe B, Grubb A (1998). 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 54:2098-2105

Tesch GH, Yang N, Yu H, Lan HY, Foti R, Chadban SJ, Atkins RC, Nikolic-Paterson DJ (a1997). Intrinsic renal cells are the major source of interleukin-1 beta synthesis in normal and diseased rat kidney. Nephrol Dial Transplant 12:1109-1115

Tesch GH, Lan HY, Atkins RC, Nikolic-Paterson DJ (b1997). Role of interleukin-1 in mesangial cell proliferation and matrix deposition in experimental mesangioproliferative nephritis. Am J Pathol 151:141-150

Tishkov I, Razboynikov S, Tchoukanov C, Shipkova P (1978): Biochemical methods for the assessment of the selectivity of proteinuria. Int Urol Nephrol 10:245-249

Tomana M, Matousovic K, Julian BA, Radl J, Konecny K, Mestecky J (1997) Galactose-deficient IgA1 in sera of IgA nephropathy patients is present in complexes with IgG. Kidney Int 52:509-516

Tomino Y, Suzuki S, Imai H, Saito T, Kawamura T, Yorioka N, Harada T, Yasumoto Y, Kida H, Kobayashi Y, Endoh M, Sato H, Saito K (2000). Measurement of serum IgA and C3 may predict the diagnosis of patients with IgA nephropathy prior to renal biopsy. J Clin Lab Anal 14:220-223

Tonelli M, Pfeffer MA (2007). Kidney disease and cardiovascular risk. Annu Rev Med 58:123-139

Topham PS, Jethwa A, Watkins M, Rees Y, Feehally J (2004). The value of urine screening in a young adult population. Fam Pract 21:18-21

van de Putte LB, de la Riviere GB, van Breda Vriesman PJ (1974). Recurrent or persistent hematuria. Sign of mesangial immune-complex deposition. N Engl J Med 290:1165-1170

van den Wall Bake AW, Daha MR, Haaijman JJ, Radl J, van der Ark A, van Es LA (1989). Elevated production of polymeric and monomeric IgA1 by the bone marrow in IgA nephropathy. Kidney Int 35:1400-1404

Vangelista A, Frasca G, Biagini G, Bonomini V (1981). Long term study of mesangial proliferative glomerulonephritis with IgM deposits. Proc Eur Dial Transplant Assoc18:503-507

Varis J, Rantala I, Pasternack A, Oksa H, Jantti M, Paunu ES, Pirhonen R (1993). Immunoglobulin and complement deposition in glomeruli of 756 subjects who had committed suicide or met with a violent death. J Clin Pathol 46:607-610

Vaziri ND, Liang K (2004). Effects of HMG-CoA reductase inhibition on hepatic expression of key cholesterol-regulatory enzymes and receptors in nephrotic syndrome. Am J Nephrol 24:606-613

Vilches AR, Turner DR, Cameron JS, Ogg CS, Chantler C, Williams DG (1982). Significance of mesangial IgM deposition in "minimal change" nephrotic syndrome. Lab Invest 46:10-15

Vlagopoulos PT, Sarnak MJ (2005). Traditional and nontraditional cardiovascular risk factors in chronic kidney disease. Med Clin North Am 89:587-611

Vuorinen-Markkola H, Yki-Jarvinen H (1994). Hyperuricemia and insulin resistance. J Clin Endocrinol Metab 78:25-29

Wagrowska-Danilewicz M, Danilewicz M (2002). A correlation between immunoexpression of CD44, alpha-SMA and CD68+ cells in IgA-nephropathy and in mesangial proliferative IgA-negative glomerulonephritis. Pol J Pathol 53:155-162

Waldherr R, Noronha IL, Niemir Z, Kruger C, Stein H, Stumm G (1993). Expression of cytokines and growth factors in human glomerulonephritides. Pediatr Nephrol 7:471-478

Wang HH, Fu LW, Yang LY, Chen WP, Tsai SJ, Lin CY (1997). A study of the relationship between IgG subclass/IgM and idiopathic nephrotic syndrome. Chung-Hua Min Kuo Hsiao Erh Ko i Hsueh Hui Tsa Chih 38:21-27

Warshaw BL, Check IJ (1989). IgG subclasses in children with nephrotic syndrome. Am J Clin Pathol 92:68-72

Welch TR, McAdams J (1998). Immunoglobulin M and C1q mesangial labeling in IgA nephropathy. Am J Kidney Dis 32:589-592

Wheeler DC (2001). Lipid abnormalities in the nephrotic syndrome: the therapeutic role of statins. J Nephrol Suppl 14:s70-75

Wilson PW (2004). Assessing coronary heart disease risk with traditional and novel risk factors. Clin Cardiol Suppl 27:s7-11

Wirta O, Mustonen J, Helin H, Pasternack A (2008). Incidence of biopsy-proven glomerulonephritis. Nephrol Dial Transplant 23:193-200

Wolf-Maier K, Cooper RS, Banegas JR, Giampaoli S, Hense HW, Joffres M, Kastarinen M, Poulter N, Primatesta P, Rodriguez-Artalejo F, Stegmayr B, Thamm M, Tuomilehto J, Vanuzzo D, Vescio F (2003). Hypertension prevalence and blood pressure levels in 6 European countries, Canada, and the United States. JAMA 289:2363-2369

Woo KT, Wong KS, Lau YK, Chiang GS, Lim CH (1988). Hypertension in IgA nephropathy. Ann Acad Med Singap 17:583-588

Wu J, Chen X, Xie Y, Yamanaka N, Shi S, Wu D, Liu S, Cai G (2005). Characteristics and risk factors of intrarenal arterial lesions in patients with IgA nephropathy. Nephrol Dial Transplant 20:719-727

Wyatt RJ, Julian BA (1988). Activation of complement in IgA nephropathy. Am J Kidney Dis 12:437-442

Wyatt RJ, Julian BA, Baehler RW, Stafford CC, McMorrow RG, Ferguson T, Jackson E, Woodford SY, Miller PM, Kritchevsky S (1998). Epidemiology of IgA nephropathy in central and eastern Kentucky for the period 1975 through 1994. Central Kentucky Region of the Southeastern United States IgA Nephropathy DATABANK Project. J Am Soc Nephrol 9:853-858

Yang N, Isbel NM, Nikolic-Paterson DJ, Li Y, Ye R, Atkins RC, Lan HY (1998). Local macrophage proliferation in human glomerulonephritis. Kidney Int 54:143-151

Yoshikawa N, Iijima K, Ito H (1999). IgA nephropathy in children. Nephron 83:1-12

Yoshioka K, Takemura T, Murakami K, Okada M, Yagi K, Miyazato H, Matsushima K, Maki S (1993). In situ expression of cytokines in IgA nephritis. Kidney Int 44:825-833

Yun EJ, Meng MV, Carroll PR (2004). Evaluation of the patient with hematuria. Med Clin North Am 88:329-343

Zeis PM, Kavazarakis E, Nakopoulou L, Moustaki M, Messaritaki A, Zeis MP, Nicolaidou P (2001). Glomerulopathy with mesangial IgM deposits: long-term follow up of 64 children. Pediatr Int 43:287-292

Zhang L, Zuo L, Wang F, Wang M, Wang S, Lv J, Liu L, Wang H (2006). Cardiovascular disease in early stages of chronic kidney disease in a Chinese population. J Am Soc Nephrol 17:2617-2621

Zhu G, Wang Y, Wang J, Tay YC, Yung T, Rangan GK, Harris DC (2006). Significance of CD25 positive cells and macrophages in noncrescentic IgA nephropathy. Ren Fail 28:229-235

Zwirner J, Burg M, Schulze M, Brunkhorst R, Gotze O, Koch KM, Floege J (1997). Activated complement C3: a potentially novel predictor of progressive IgA nephropathy. Kidney Int 51:1257-1264

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