From DEPARTMENT OF CLINICAL SCIENCE,
INTERVENTION AND TECHNOLOGY (CLINTEC)
DIVISION OF PEDIATRICS
Karolinska Institutet, Stockholm, Sweden

Renal function and renal histopathology in assessment of course and prognosis in Henoch Schönlein Nephritis and IgA nephropathy

Stella Edström Halling

Karolinska Institutet

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ABSTRACT

Background: Henoch Schönlein Nephritis (HSN) is a common childhood vasculitis that generally has a self-limiting course but the long-term outcome varies with the clinical picture at onset. Morbidity is high among the most severe cases, and therefore there is a need for intervention. Immunoglobulin A nephropathy (IgAN) is the most frequent glomerulonephritis in the world, and the risk of disease progression to chronic renal disease (CKD) is as high in paediatric population as among adults. There is no consensus regarding treatment strategies in the two diseases. **Aim:** To identify patients at risk and to identify predictors of a poor outcome in HSN and IgAN patients and to study the results of treated patients with severe forms of HSN and IgAN. **Results:** In s**tudy I** 73 patients with HSN, investigated within 5 years from onset, we observed that GFR at the first investigation was lowest among patients with nephrotic syndrome or with a nephritic-nephrotic picture at onset. The clinical picture at onset was related to the histology findings. Advanced biopsy findings were found in 60% of patients with nephrotic syndrome and in 70% of patients with a nephritic-nephrotic picture at onset. Among patients with non- nephrotic proteinuria, generally considered to be a benign group, 69% showed advanced biopsy findings, despite the fact that their GFR showed only a moderate reduction at onset. In **study II** the results of treatment of the most severe cases of HSN (n=24) and IgAN (n=19) were presented. All patients were treated with ACEi/ARB. In group A (n=18) Methylprednisolone/oral prednisolone was combined with Cyclophosphamide given as 3-6 monthly pulses. In group B (n=25) 15 patients received corticosteroids and 10 only ACEi/ARB. In group A proteinuria was reduced after Methylprednisolone and further declined after Cyclophophamide treatment. GFR improved during follow-up in group A. In group B the proteinuria decreased during follow-up and the GFR remained unchanged. There was a greater fall in the protein reduction in the group treated with corticosteroids and ACEi/ARB than in the group treated without corticosteroids. The results presented in **study III** identified the predictors of a poor outcome in 78 HSN patients followed mean 5 years. 26% progressed to a poor outcome (active renal disease or CKD stage 4-5/ESRD). Both severe clinical features at onset and advanced biopsy findings were related to a poor outcome. Proteinuria at one year follow-up was assessed as a strong individual predictor. The combination of proteinuria at one year and the histology grading showed highest discriminative ability. The results in **study IV** validated the new Oxford classification and assessed the predictability of the of the histology findings identified in the Oxford MEST score: mesangial (M) and endocapillary (E) hypercellularity, segmental glomerulosclerosis (S) and tubular atrophy/interstitial fibrosis (T). Ninety-nine children were followed > 5 years. Eighteen per cent progressed to a poor outcome. Ninety biopsies were reviewed according to the MEST score: M, E and T were each associated with a poor outcome but S did not reach significance. Instead, presence of crescents and of global sclerosis was predictive of poor outcome in our cohort. **Conclusion:** Morbidity is high among severe cases of HSN and IgAN. Identification of predictors of a poor prognosis will improve medical intervention and reduce the risk of deterioration of the diseases.

LIST OF PUBLICATIONS

I. **Henoch Schönlein Nephritis**

Clinical findings related to renal function and morphology

Edström Halling S. F, Söderberg M.P, Berg U.B

Pediatric Nephology 2005 Jan; 20 (1):46-51

II. **Treatment of severe Henoch–Schönlein and Immunoglobulin A nephritis**

A single center experience

Edström Halling S, Söderberg M.P, Berg U.B

Pediatric Nephrology 2009 Jan; 24 (1):91-7

III. **Predictors of outcome in Henoch Schönlein nephritis**

Edström Halling S, Söderberg M.P, Berg U.B

Pedatric Nephrology 2010, June; 25 (6): 1101-1108

IV. **Predictors of outcome in paediatric IgA nephropathy with regard to clinical and histopathologic variables (Oxford Classification)**

Edström Halling S, Söderberg M.P, Berg U.B

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LIST OF ABBREVIATIONS

ACEi	angiotensin converting enzyme inhibitor
ARB	angiotensin receptor blocker
BSA	body surface area
CKD	chronic kidney disease
ESRD	end stage renal disease
GFR	glomerular filtration rate
HSN	Henoch Schönlein Nephritis
IgAN	Immunoglobulin A nephropathy
ISKDC	International Study of Kidney Diseases in Children
RAAS	Renin Angiotensin Aldosterone System
Ualb/c	urine albumin/urine creatinine ratio

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INTRODUCTION

1.1 Brief historical remarks

Henoch Schönlein Purpura (HSP) is a leukocytoclastic vasculitis with a multi-organ engagement, primarily affecting the skin but also the joints, the intestine and the kidneys. The first report of the disease was published 1802 by Heberden [1, 2] and in 1837 Schönlein found the association between rash (purpura) and joint pain. Henoch added the gastrointestinal symptoms in 1874 and 25 years later renal engagement was explored. From the 1920s it was known that the morbidity of the HSP syndrome, at the time called Anaphylactoid purpura, was due to the severity of glomerulonephritis. The immunofluorescence technique developed during the late 1960s and in 1968 Berger and Hinglais reported a form of glomerulonephritis, in which the renal damage was due to identified mesangial accumulation of IgA deposits. The disease was named Berger´s disease [3]. In the same decade IgA deposits were also identified in renal biopsies from patients with Henoch Schönlein nephritis (HSN) [4].

1.2 Epidemiology

Henoch Schönlein purpura (HSP) is the most common form of vasculitis in childhood with an annual incidence of 10-20/100 000 children [5-7]. The estimated annual incidence varies among different ethnic groups in paediatric populations and is higher among Asian children than among Caucasian children [7]. The age distribution at onset range from 2 to 18 years and the peak incidence occurs at 4-6 years. It is considered to be a self- limiting disease in a majority of the cases. The long-term prognosis is related to the renal engagement, Henoch Schönlein Nephritis, which occurs in 35% (40-60%) of the HSP patients [2, 3, 8-10] and accounts for 10-15% of the paediatric glomerulonephritis [11]. In biopsy data from our center during the years 1994-2000 HSN accounted for 8% of all biopsies. Children who are older at onset tend to have a higher risk of renal involvement [2, 8] and of advanced renal disease [12].

Immunoglobulin A nephropathy (IgAN) is the most common type of glomerulonephritis in the world with frequency that varies geographically. As in HSN, there is a higher frequency in Asian than in Caucasian populations. The annual incidence in adult population in France is 25-30/million, in Japan 45/million and in USA 12/million [13]. The incidence figures can be explained by the different regional sampling procedures, such as indications for a renal biopsy and there can also be genetic explanations. The male/female ratio indicates that males are more likely to be affected by the disease, generally in the second and third decade

of life [13, 14], but the onset of the disease can occur in children as young as 2-3 years of age.

1.3 Pathogenesis

1.3.1 Trigger factors

HSN and IgAN are considered to be related diseases with a common pathogenetic pathway. The diseases are usually proceeded by a bacterial or viral infection [2, 3, 15]. Allergic manifestations caused by drugs and insects bites have also been reported as stimulus for HSP induction, but no causal relationship has been determined [16, 17]. Regardless of which antigen triggers, an immunologic process starts and forms immune complexes which circulate in the blood and then form depositions primarily in the mesangial matrix (Figure 1). With increasing mesangial proliferation and mesangial matrix expansion, the architecture of the glomerular capillary wall is gradually destroyed. If the renal damage proceeds, the glomerular filtration is reduced temporarily or permanently.

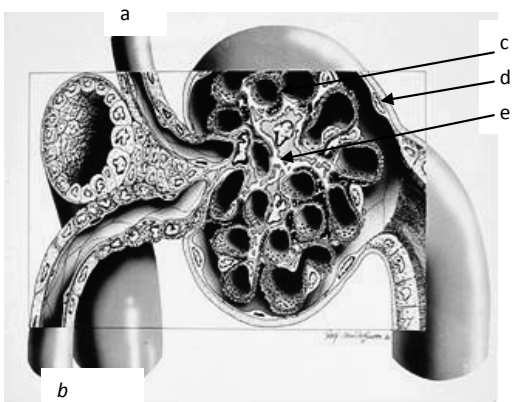

Figure 1. Normal glomerulus

a. afferent arterioli, b. efferent arterioli,

c. capillary loops, d. Bowman´s capsule,

e. mesangium

1.3.2 Impaired IgA synthesis

Most of the systemic IgA is produced in the bone marrow, lymph nodes and spleen and appears in monomeric form. The polymeric IgA is produced mainly by lymphocytes and plasma cells in the gastrointestinal and respiratory tracts [18]. Increased synthesis and release of the polymeric IgA1 (pIgA1) into the circulation is one of the factors involved in the pathogenesis of the diseases [19].Various mechanisms have been described and several aspects of the dysregulated IgA immune system have been discussed (Figure 2); Increased production, defective synthesis, decreased clearance of IgA1 and enhanced mesangial binding of IgA1 complex [20]. At present time the attention has focused on the defective galactosylation of the molecule [21] and poorly galactosylated IgA1 has been identified in the mesangium and sera from most IgAN patients [22].

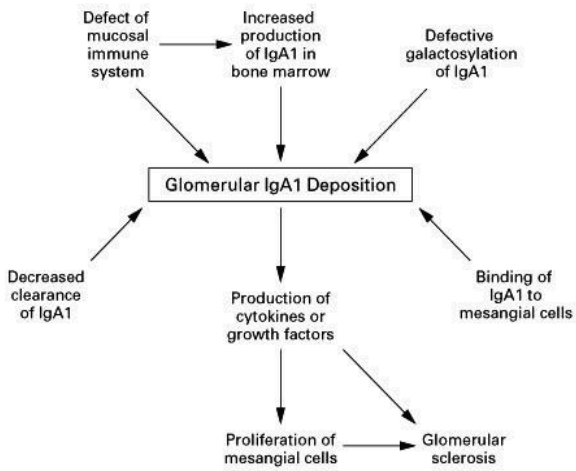

Figure 2.

Potential mechanisms underlying glomerular deposition of IgA and progression of renal disease in IgA nephropathy.

Adapted from Donadio et al [84].

The heavy chains of IgA1 with its hinge region contains up to six oligosaccharide chains composed by N-acetylgalactosamine (GalNAc) and attached residues [21, 23] (Figure 3).

In patients with IgAN a galactose deficient form of IgA1 (Gal-deficient IgA1) is identified and appears to have a key role in the pathogenetic process [20, 21, 24]. Recent investigation suggest that HSN share this pathway [25, 26]. Increased levels of Gal-deficient IgA1 in plasma has also been observed in patients with HSP who develop glomerulonephritis, but not in those without glomerulonephritis [21, 23, 25, 27].

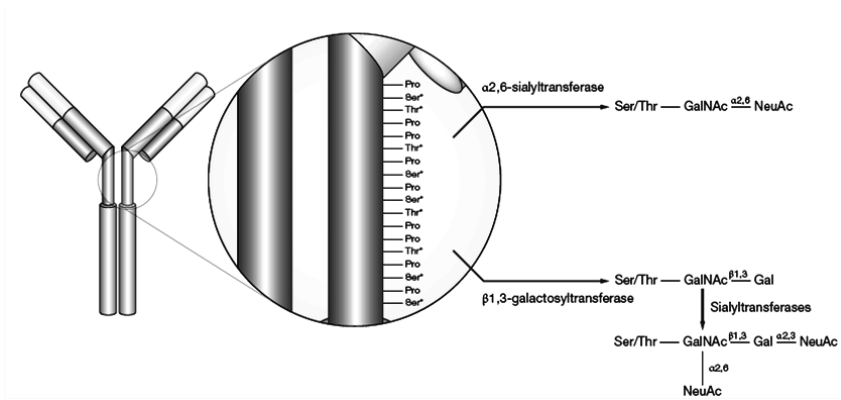

Figure 3. IgA1 and its hinge region with amino acid sequences.

The lower structure shows normal galactosylation in healthy individuals and the upper structure the galactose deficient form found at elevated levels in patients with IgAN. Adapted from Beerman [28].

1.3.3 Mesangial deposition of IgA1

The serum IgA molecule has a half-life of 4-5 days, before it binds to the receptor in the hepatocyte where it is catabolized and excreted. In IgAN the increased production and the decreased clearance leads to a longer lifespan of the Gal-deficient IgA1 [21]. The molecules are recognized by specific antibodies and form immunocomplexes which, due to their size, are less effectively taken up by receptors in the liver. Instead they pass from the blood to the mesangium through the endothelial fenestrae in the glomerular capillary wall, which permits the entry of large molecules [21]. The Gal-deficient IgA1 immunocomplexes have a high affinity to mesangial cells and the binding is facilitated by factors promoting mesangial trapping and mesangial proliferation [20, 21, 26, 29]. Of the complement components primarily C3 (60-100%) are co-deposited together with IgA1 and in some cases also with IgM (9-66%) and IgG (15-87%). The normal serum complement levels are consistent with an activity of the alternative or lectin pathway in the diseases, whereas complement components of the classical pathway such as C1q are not verified [18, 19, 30-33]. Mesangial deposition of the immunocomplexes can induce complement activation which can influence the extent of the glomerular injury [3]. The complement deposits can be observed many years after the primary onset of disease [34, 35].

1.4 Grading of the glomerular damage

1.4.1 Grading of glomerular damage in HSN

The defining pathology in HSN and IgAN is the deposition of IgA1 in the mesangium and histological findings of mesangial proliferative glomerulonephritis.

International Study of Kidney Disease in Children´s classification of Henoch Schönlein Nephritis	
grade	
I	**minimal glomerular alteration**
II	**mesangial proliferation**
IIIa	**focal proliferation with < 50% crescents/ segmental lesions**
IIIb	**diffuse proliferation with < 50% crescents/ segmental lesions**
IVa	**focal proliferation with 50-75% crescents/ segmental lesions**
IVb	**diffuse proliferation with 50-75% crescents/segmental lesions**
Va	**focal proliferation with > 75% crescents/ segmental lesions**
Vb	**diffuse proliferation with > 75% crescents/segmental lesions**
VI	**membranoproliferative picture**

Table 1. The International Study of Kidney Disease in Children´s classification of Henoch Schönlein Nephritis [17, 36]

The histology pattern in HSN is graded according to the classification by the International Society of Kidney Disease in Children (ISKDC) in which the severity of renal damage is assessed and divided into six grades (Table 1). Grade I shows minimal glomerular changes. Grade II shows mesangial proliferation (Figure 4a) with or without endocapillary hypercellularity or mesangial matrix expansion. The amount of mesangial proliferation varies and can either be located diffusely or focally/segmentally. Severe mesangial proliferation is often accompanied with leucocyte infiltration and fibrin deposition in the

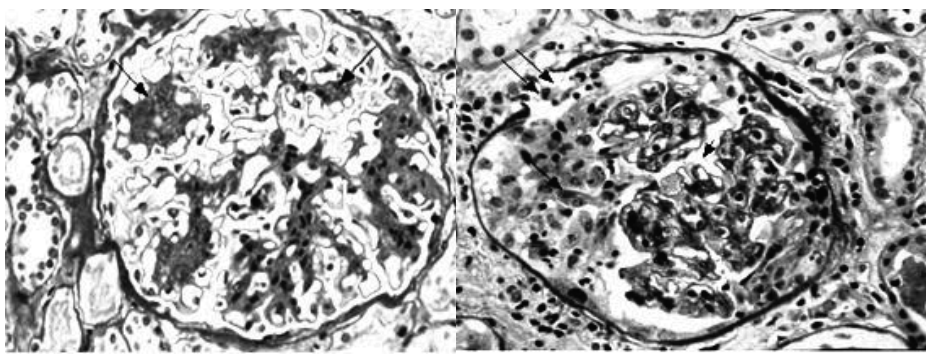

Fig 4a. Mesangial proliferation. ISKDC II **Fig 4b. Cellular crescent. ISKDC ≥ III**

Figure 4a. Arrows show hypercellularity and mesangial matrix expansion. Figure 4b. Arrows show rupture of the basement membrane of Bowman´s capsule and infiltration of leukocytes and fibrin deposits

glomeruli can also be found [37]. Grades I-II are defined as mild histology findings whereas presence of cellular/ fibrocellular crescents (Figure 4b) or fibrous crescents and/or segmental or global glomerulosclerosis as seen in grade III-V, are defined as advanced renal injury. In grade VI the glomeruli show a membranoproliferative picture, which is uncommon, and the high numerical grade gives a misleading impression of its severity, which is mainly determined by the frequency crescents. The formation of crescents is caused by proliferation of the epithelial cells of Bowman´s capsule, and may vary in size from small segmental/cellular to large global circumferential with fibrin deposits. Cellular/fibrocellular crescents are considered as markers of disease activity whereas fibrous crescents are signs of permanent injury, as well as segmental and global glomerulosclerosis [17, 38]. Repeated biopsies can show a spontaneous reduction of the number of glomeruli with fibrocellular or cellular crescent formations and also a complete reduction of mesangial proliferation [17, 33]. Apart from the glomerular findings the amount of tubular atrophy, interstitial inflammation and interstitial fibrosis also reflect the severity of renal damage [39]. Therefore, to obtain a differentiated pattern of the renal injury the tubulointerstitial findings can be evaluated separately. Andreoli and Bergstein [40] developed a scoring system for IgAN to differentiate acute from chronic histology

findings. Foster used introduced a scale for HSN biopsies that reflected the disease activity/chronicity highlighting the importance of tubulointerstitial findings in indicating the severity of the disease. [41].

1.4.2 Grading of glomerular damage in IgAN

The histologic pattern in IgAN varies widely, from signs of mild injury to advanced renal damage. The classification systems for IgAN have been less straight forward than for HSN. Based on numerous reports on which lesions that best predict a poor outcome, consensus was not reached until recently, when the Oxford classification was published in 2009 [42, 43], a result of international collaboration in the International IgA Nephropathy Network and the Renal Pathology Society. The classifications previously used (the Lee classification [44, 45] and the Haas classification [46]) are now gradually replaced by the Oxford classification, in which four lesions with the most prognostic importance are identified: Mesangial hypercellularity, endocapillary hypercellularity, segmental glomerulosclerosis and tubular atrophy/interstitial fibrosis. The results have been analysed using a systematic approach, in order to develop a reproducible classification with a scoring system (MEST score) which can improve individual patient prognostication (Table 2). The Oxford classification MEST score has also been applied in paediatric biopsies [47]. The validity of the scoring system to assess the severity of the renal lesion and its usefulness as a prospective tool has not been established previously in paediatric IgAN.

Histology finding	Definition	Score
Mesangial hypercellularity	< 4 mesangial cells/mesangial area=0 4-5 mesangial cells/mesangial area=1 6-7 mesangial cells/mesangial area=2 >8 mesangial cells/mesangial area=3	M0≤ 0.5 M1> 0.5[1]
Endocapillary hypercellularity	Hypercellularity due to increased number of cells within the glomerular capillary causing narrowing of the capillary lumina	E0= absent E1= present
Segmental glomerulosclerosis	Any amount of the tuft involved in the sclerosis but not involving the whole tuft, or presence of adhesions	S0= absent S1= present
Tubular atrophy/interstitial fibrosis	% of cortical area affected by tubular atrophy or interstitial fibrosis, whichever is greater	T0=0-25% T1=25-50% T2 >50%

Table 2. Definitions of histology findings used in the Oxford Classification of IgA Nephropathy [42].

[1] The mean score for all glomeruli is calculated but if >50% of the glomeruli have > 3 cells in the mesangial area it is categorized as M1

1.4.3. Differences in renal pathology findings in HSN compared to IgAN

Renal morphology findings in HSN are identical to those of IgAN although the frequency and distribution may differ. Cellular or fibrocellular crescents, deposits of fibrin in the glomeruli and endocapillary proliferative findings are often more found in severe in HSN than in severe IgAN [30]. Deposits of IgA along the capillary wall have been associated with more severe clinical manifestations and a poor outcome both in HSN and in IgAN. This finding is also more frequent in HSN than in IgAN [18, 48].

1.5 Clinical onset and course of the disease

1.5.1 Clinical features at onset and relation to outcome in HSN

HSN is in most cases a self-limited disorder, but there is a risk of permanent renal damage and deteriorating renal function [12, 49]. The long-term prognosis of HSN is related to the clinical features at onset and to the renal biopsy findings. Patients with mild symptoms i.e hematuria and/or mild proteinuria at onset are generally considered to have a good prognosis, with a risk of long-term impairment of less than 2% [2, 6, 50-52]. The risk of a poor ourcome increases for patients with severe symptoms at onset (Table 3). Patients who presents a nephritic, nephrotic or a mixed nephritic-nephrotic picture, have an estimation of long-term impairment of 20% [1, 12, 33, 36 , 52-54]. However, the clinical course in children is more unpredictable than among adults [55] and the renal function can deteriorate many years after apparent remission [54].

Study	n HSP/HSN	Poor outcome [3] n (%)	Follow-up years	Mild[1] symptoms n (%)	Severe[2] symptoms n (%)
Steward et al [6]	270/55	1 (2)	8.3	0	1/18 (5)
Saulsbury et al [2]	100/40	1 (2)	-	0	1/9 (11)
Goldstein et al [54]	78	22 (28)	23	5/39 (13)	17/39 (44)
Schärer et al [56]	64	21 (33)	6.1	3/26 (8)	18/38 (47)
Ronkainen et al [12]	47/38	9 (23)	24	2/18 (11)	7/20 (35)

Table 3.

HSN patients with poor outcome related to severity of renal symptoms at onset in the five largest studies.

1 = Hematuria ± proteinuria with Ualb/c < 200 mg/mmol, 2 = Nephritic or nephrotic or mixed clinical picture

3 = Active renal disease (hypertension ± proteinuria ± decreased GFR) or renal failure/ERSD

1.5.2 Clinical features at onset and 10 year survival in IgAN patients

The initial features at presentation in paediatric IgAN varies geographically. In Japanese series, in which patients at risk are identified by urinary screening, the clinical presentation at onset is most often microscopic hematuria (60%), whereas in studies from the United States and Europe, macroscopic hematuria is the most frequent initial finding (50-80%) [39, 57-59]. The onset is rarely acute nephritic or nephrotic syndrome (10%) [60]. In paediatric patients with isolated microscopic hematuria, the proteinuria often occurs after several years (mean 5 years) [39]. In several centers the indication for a renal biopsy is limited to patients with macroscopic hematuria or microscopic hematuria in combination with proteinuria. Therefore the disease can proceed undiagnosed for many years.

Compared to adults, children are less likely to have impaired GFR at time of biopsy, tend to have lower blood pressure at time of biopsy and are more likely to have had recurrences of macroscopic hematuria [39]. The most common clinical course is a slow progression to renal insufficiency, but the rate of progression is variable and the outcome is difficult to predict [18]. Few cases of full clinical recovery exists [39, 59, 61, 62], in some cases with a disappearance or diminution of mesangial IgA deposits [48]. Previously IgAN was considered to be less progressive in young ages. However, according to several reports the 20-year renal survival analysis showed that children have as progressive disease as adults [14, 59, 62, 63]. The actuarial 10-year renal survival in adult patients with childhood onset, shows apparent geographic variations according to the most accurate studies during the years 1984-2000; Europe 83-94%, Asia 74-91% and North America 57-78% [14]. More recent studies show an improved expected 10-year survival in the majority of the cases (Table 4). Studies have shown that IgAN patients diagnosed in childhood have better long term survival than those diagnosed as adults [59, 64, 65]. The differences might be due to improved follow-up routines and also to the fact that adult biopsies reveal more advanced chronic histology lesions, reducing the opportunities for medical intervention [64].

Study	Patients n	at 10 years (%)	at 20 years (%)	Ethnicity
Wyatt 1995[63]	103	87	73	Caucasian/AfroAm
Nozawa 2005 [66]	181	92	89	Japanese
Ronkainen 2006 [59]	55	93	87	Finnish
Hastings 2007 [67]	67	91	80	Caucasian
Haas 2008 [64]	99	87	-	Caucasian

Table 4.
Predicted renal survival of IgAN with childhood onset adapted after Coppo [39]

AIM

The aim of the study was to identify risk factors of a poor prognosis in HSN and IgAN

- To assess how the clinical picture at onset was related to renal function and to morphology in HSN (study I)
- To study clinical and functional results of treated patients severe HSN and IgAN (study II)
- To assess predictors of a poor prognosis in HSN (study III) and IgAN (study IV)
- To validate the prognostic significance of the new Oxford classification of IgAN in pediatric patients (study IV)

METHODS AND MATERIAL

3.1 Renal function

3.1.1. Clearance investigations and definition of CKD

The clearance of one substance from a compartment is defined as the ratio between the elimination rate of the substance from that compartment, and the concentration of the substance in the same compartment. The calculated value of clearance is expressed as ml/min and is corrected for a standard body surface area (BSA) of 1.73 m^2.

The measurement of glomerular filtration rate (GFR) requires a biologically inert substance, which passes freely through the glomerulus and is excreted without being reabsorbed, metabolized or secreted in the tubuli. Inulin, a polymer of fructose, has been considered the ideal substance for GFR measurement. This marker is given intravenously either as a constant infusion or as a bolus injection (single injection). Renal clearance (C) is calculated using the formula C = U x V/P in which U= urinary concentration of the substance (mg/ml), V= diuresis (ml/min) and P= plasma concentration of the substance (mg/ml). However, this method has its disadvantages. It is time consuming, and the bladder has to be emptied completely to avoid measure defaults. Additionally, the increased costs for Inulin (Inutest), has created a demand for an alternative filtration marker. Therefore the single injection technique with Iohexol, a low-osmolar contrast medium, has gradually replaced Inulin over the last five years. The method is cheaper and does not require urinary sampling. Comparisons have shown a good agreement between the renal clearance with Inulin and the plasma clearance with Iohexol [68].

The effective renal plasma flow (ERPF) is determined with a marker which is totally cleared from the plasma by glomerular filtration and tubular secretion. Para-aminohippuric acid (PAH) is a suitable substance for these estimations. The filtration fraction (FF) is calculated as a ratio between GFR and ERPF.

All clearance values in studies I-IV are expressed as ml/min/1.73m^2 adjusted to BSA which is calculated from the Haycock formula [69].

In the year 2002 the National Kidney Foundation´s Kidney Disease Outcomes Quality Initiative (NFK/K/DOQI) set guidelines to classify levels (stages 1-5) of chronic kidney disease (CKD) in adults according to the level of GFR [70]. The stages and the associated recommendations have also been assessed for children > 2 years of age and for adolescents [71]. In this classification CKD is defined as 1) presence of kidney damage defined by structural or functional abnormalities with or without reduction of GFR or 2) GFR $\leq$ 60 ml/min/1.73m^2 for three months or more irrespectively of the diagnosis. Kidney failure is

defined as GFR ≤ 15 ml/min/1.73m^2 or end stage renal disease (ESRD) requiring dialysis or transplantation.

3.1.2 Clearance of Inulin and of PAH during water diuresis

A prime dose (64 mg/kg) of Inulin (Inutest, 25% Fresenius Kabi Austria GmbH, Graz, Austria) is injected intravenously and a prime dose (9 mg/kg) of PAH (PAH, 20%, MSD, West Point, USA) followed by a continuous infusion of Inulin 1-2 mg/kg/min and PAH 0.15-0.3 mg/kg/min. After an equilibration period of 60 minutes, the infusion is continued for another two hours. A total of four urinary collections are obtained with an interval of 30 minutes. In the middle of each collection period a blood sample is drawn. Water diuresis is obtained by oral water intake of 20 ml/kg during the first hour, and then 5 ml/kg every 30 minute up to a maximum of 1200 ml and 300 ml respectively and no catheter is used. A mean value of the four collected periods is calculated [72]. Inulin concentrations in blood and urine is determined by an enzymatic technique [73] and PAH by photometric analysis.

3.1.2 Clearance of Iohexol

Iohexol (Omnipaque 300 mg/ml, GE Healthcare, Stockholm, Sweden) 5 ml is injected intravenously and blood samples are drawn from the contra-lateral arm after 180, 200, 220 and 240 minutes if the GFR is predicted to be > 50 ml/min/1.73m^2. The investigation continued and a blood sample is drawn after 420 minutes if predicted GFR is 20-50 and after 1440 minutes if predicted GFR is < 20 ml/min/1.73m^2. Clearance is calculated from the slope of the plasma concentrations using a pharmacokinetic one-compartment model with the modification of Bröckner Mortensson [74, 75]. Plasma concentration of Iohexol is determined by high-performance liquid chromatography [76].

3.2 Proteinuria

3.2.1 Filtration barrier

The blood is filtered through the glomerulus to the urinary space through several layers; the endothelial cell surface layer (ESL), the endothelial cell coat layer of fenestrated cells, the basement membrane (GBM) and a layer of glomerular epithelial cells (podocytes) which are separated by narrow gaps (slit membranes) [77]. The basement membrane consists mainly of collagen type IV with high density, an effective filtration barrier which works primarily selectively as it forms a network restraining larger molecules

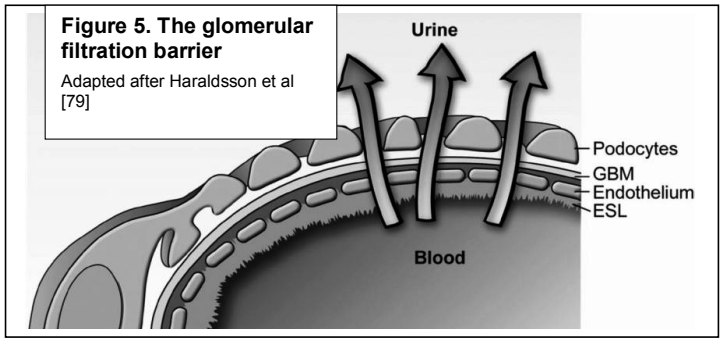

Figure 5. The glomerular filtration barrier
Adapted after Haraldsson et al [79]

(> 45 Å in diameter) while the smaller ones (< 20 Å) pass [78]. The barrier also constitutes a charge selectivity that repels the negative albumin molecules. The podocyte and the slit pores with their specialized differentiated functions, play a pivotal role in the intact barrier. The size and charge selectivity of the glomerular barrier has been questioned during the last decade, but the classical view still holds [79, 80].

3.2.2 Proteinuria detection levels

Quantification of proteinuria has traditionally demanded a timed urinary collection, usually performed over 24-hours. The timing and volume problems in the young children, who have not achieved continence, and the need to correct the protein excretion rate for body surface area, make this method cumbersome. The Clinical Practice Guidelines for Chronic Renal Disease in Children and Adolescents published in the NKF-K/DOQI recommends untimed (spot) urinary samples, in which the ratio of the urine albumin/urine creatinine (Ualb/c, mg/mmol) can be calculated to detect and monitor proteinuria in children [71]. Studies have shown a high correlation between Ualb/c ratio to 24-hour collected protein excretion [81, 82], and the method requires no correlation to BSA. The method provides the most accurate measurement of albumin clearance and provides a good marker of glomerular permeability [70].

Levels of albuminuria	Protein excretion	Dipstick	Ualb/c	24-hour collection
Normo	insignificant	0		< 4mg/m^2/h
Mirco	<0.2 g/L	trace	2-25	30-300 mg/d
	<0.3 g/L	+1		
Mild	<1 g/L	+2	>25-200	
Moderate			>200-400	
Nephrotic	3 g/L	+3	>400	>40mg/m^2/h (>1g/1.73m^2/d)
	>20 g/L	+4		

Table 5. Levels of proteinuria and their quantification measurement methods

3.3 Renal biopsy

3.3.1 Indication of investigation with a renal biopsy

In HSN the diagnosis is based on the clinical picture and a renal biopsy is not mandatory for diagnostic purposes, in the majority of the cases. However, as the long-term prognosis is related to the severity of renal disease at onset, it is a common strategy to perform an early renal biopsy if the clinical presentation shows an acute nephritic or nephritic syndrome or a mixed clinical picture [15, 51, 53, 83]. If the duration of a non-nephrotic proteinuria persists more than a few months from onset, or if the GFR declines, we recommend a biopsy, which to our knowledge is less likely to be a common strategy.

The investigations recommended for patients with suspected IgAN differs geographically. The indication for a renal biopsy in our center is persistent asymptomatic isolated hematuria for minimum two years. In other centers there is a more restricted policy with a biopsy reserved for patients with macroscopic hematuria or persistent microscopic hematuria with additional proteinuria > 1g/d or impaired renal function [24, 63, 84]. A re-biopsy of IgAN patients is undertaken in our center, if there is a deterioration of renal function and/or an increase in albuminuria despite RAAS blockade.

3.3.2. Ultrasound guidance

The renal biopsies are performed percutaneously with ultrasound guidance, using an automatic device with a 16-gauge needle (Bard Magnum Biopsy Instrument and Core Tissue Biopsy Needle, Urological, Covington, CA, USA). The method has been validated in a previous study by our group [85].

3.3.3 Preparation and examination of the biopsy

The biopsies are fixed in 4% paraformaldehyde in phosphate buffer and analysed based on three micron PAS-stained sections. The preparations of tissue for immunofluorescence are frozen in liquid nitrogen, cut 5 μm thick and incubated with standard anti-antibodies. All biopsies are examined with light microscopy and immunofluorescence investigation and if the diagnostic criteria were not fulfilled, also with electron microscopy.

The biopsies in all four studies were examined by the same pathologist (MPS), who was blinded to patient outcome at the time of his investigation. The ISKDC classification was used in HSN biopsies in study I-III and the Oxford classification for the cases in study IV. In study II the ISKDC classification was used for both HSN and IgAN cases. In the articles

I-III we used the ISKDC classification without the subgroups a) and b) and thus we did not differentiate focal proliferation from diffuse (see Table 1). In study I-III amount of the mesangial proliferation, mesangial matrix expansion, interstitial inflammation and tubulointerstital findings were graded in a four -graded scale (0-3). In study II the biopsies are additionally classified according to signs of activity/chronicity [40, 41]. Our modified scoring system was based on mesangial matrix expansion/mesangial proliferation (0-3) and per cent of glomeruli with cellular or fibrocellular crescents (0-3) as indicators of activity. Indicators of chronicity were per cent of glomeruli with global or segmental glomerulosclerosis (0-2) and the amount of interstitial fibrosis (0-3). The scoring of interstitial fibrosis was doubled due to its impact on progressive histological damage [46, 48, 86-88].

3.4 Blood pressure

Office blood pressure was measured with an oscillometric device (Dynamap Newport, USA). The blood pressure was measured from the right arm, in a sitting position after 5 minutes of rest. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured and mean arterial blood pressure was calculated as DBP + (SBP-DBP/3). Hypertension was defined according to recommendations from the Working Group of National High Blood Pressure Educational Program and adjusted for age, sex and height [89].

3.5 Patients and controls

The cohorts in studies I-IV mainly consisted of referral patients with a high proportion of cases with severe disease. This fact is expressed in Table 6 in which shows a high age at onset, a high proportion of biopsied patients and a high proportion of treated patients. The age matched control group consisted of healthy patients who had undergone a renal function investigation due to a suspicion of a renal disease, which could not be verified. The number of patient included in each study and their overlapping is shown in Figure 6.

Article	I	II	III	IV
			all/ study group	
N	73	43	103/78	99
Age at onset years	8	12	9/9	12
Male (%)	44	58	49/47	59
Follow up time years	<5	3.1	4.6/5.2	13
HSN (%)	100	56	100	0
IgA (%)	0	44	0	100
Biopsied patients (%)	54	100	68/75	90
Treated patients (%)	0	100	/38	35
ESRD patients (%)	0	14	/4	13
Controls (n)	49	50	50	59

Table 6.
Descriptive data of patients and number of controls included in studies I-IV

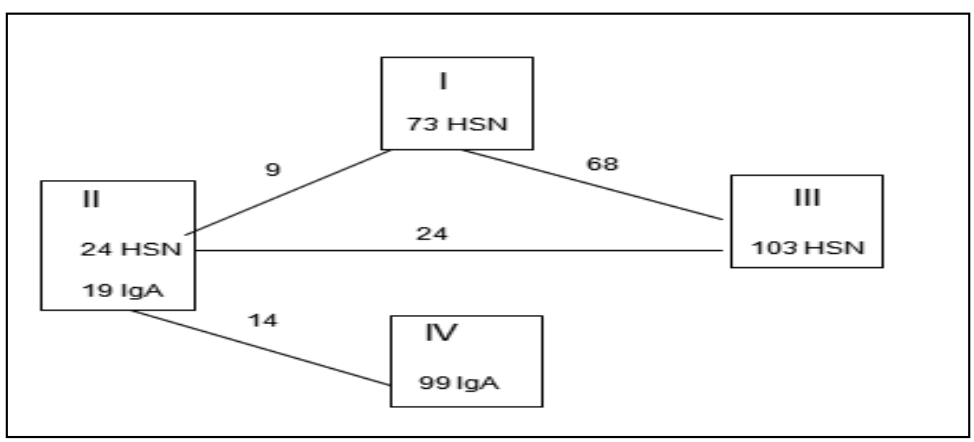

Figure 6.
Number of patients in studies I-IV and their overlapping
The figures above the lines refer to the number of patients included in both studies.

3.6 Statistical methods

3.6.1 Descriptive statistics

The data was presented using mean and standard deviation, when normally distributed. When data was not normally distributed it is presented with median and range (minimum-maximum).

3.6.2 Overview of statistical methods

All calculations were done using Statistica v 7.0, Tulsa USA. Student´s t test was used for normally distributed data and the Mann-Whitney U-test for data with a skewed distribution. The chi-square test was used to test for differences between proportions, i.e. categorical variables. For comparisons of repeated measurements in skewed data Wilcoxon´s matched pairs test was used. To compare mean in variables with more than two groups, one-way analysis of variance (ANOVA) was used, followed by the post-hoc test of Tukey-Kramer for pairwise comparison. Linear regression with the Pearson´s correlation was used when comparing continuous variables and the Spearman correlation coefficient when ordered categorical data were compared. To estimate the discriminative efficacy of predictors of poor outcome logistic regression was used with univariate and multivariate analyses.

Renal survival data were explored using the Kaplan-Meier curve with the corresponding log rank calculations between groups of clinical and pathologic variables, with respect to time-to-poor outcome. Cox proportional hazard regression (HR) was used to estimate the hazard ratio between groups within each factor for the time-to-poor outcome.

Estimates were presented with their corresponding 95% confidence intervals (CIs).

Multiple hazard regression was used to estimate a model including a combination of the independent factors of interest, and was also used to explore which factors were the overall most important discriminators for time-to-poor outcome. All tests were two-sided and a *P*-value < 0.05 was considered as statistically significant.

Study	I	II	III	IV
Method				
ANOVA	x			
Student´s paired t-test		x	x	x
Mann-Whitney U-test		x	x	x
Chi square test		x	x	x
Wilcoxon´s matched pairs test		x		
Linear regression			x	x
Spearman rank correlation coefficient	x		x	x
Logistic regression (Odds ratio)			x	
Cox proportional hazard regression				x
Kaplan- Meier survival analysis				x

Table 7.
Statistical methods used in studies I-IV

ETHICAL APPROVALS

The studies in article I-III were approved by the Ethics committee at the Karolinska University Hospital, Huddinge (no 262/97) and the study in article IV was approved by the Regional Ethical Review Board (no 2010/290-31/3).

RESULTS AND DISCUSSION

5.1 Clinical features and histology findings in HSN patients

Results of study I

The main factors in defining risk patients in HSN are highlighted in the study of 73 HSN patients biopsied within five years from onset. The relation between clinical and functional results at onset was compared to histology findings. The clinical features at onset/ biopsy in studies I-III are shown in Table 8.

Study		I n=73		II n=43		III n=78	
HEM	micro/ macro hematuria	12		0		12	
PROT	persistent mild proteinuria[1]	27		14		34	
AN	acute nephritic syndrome[2]	17	34 (47%)	8	29 (67%)	10	32 (41%)
NS	acute nephrotic syndrome[3]	6		12		8	
AN+NS	mixed clinical picture	11		9		14	

Table 8.
Number of patients in relation to symptoms at onset/at biopsy in study I-III

[1] Urine albumin < 1g/L or Ualb/c< 200 mg/mmol ± hematuria

[2] Ualb/c ≥200-400 mg/mmol, hematuria, decreased GFR ± hypertension [89]

[3] Ualb >40 mg/h/ m^2 BSA or Ualb/c >400 mg/mmol and serum albumin < 25g/L ± oedema

In study I the GFR at the first investigation (GFR:first) was lowest among patients with nephrotic syndrome or with a nephritic-nephrotic picture at onset. It was significantly lower compared to patients with only hematuria at onset and to controls. Among patients with non- nephrotic proteinuria, GFR:first was only slightly reduced compared to controls. There was an inverse correlation between GFR:first and the amount of proteinuria. GFR:first was significantly lower in patients with proteinuria in the nephrotic range compared with normo/micro-albuminuric patients. Grade III was the most frequent ISKDC

grade and 68 % of the biopsies showed ISKDC ≥ III. The frequency of the biopsy findings according to their classification in studies I-IV is shown in Table 9.

		ISKDC histology grading of HSN		Oxford classification MEST score of IgAN	
Study	n	Mild I-II n (%)	Advanced III-V n (%)	No MEST C0/GGS0 n (%)	Any MEST C1/GGS1 n (%)
I	40	13 (32)	27 (68)		
II	43	8(19)	35 (81)		
III	59	21 (36)	38 (64)		
IV	90			50 (55)	40 (45)

Table 9.

Frequency of biopsy findings according to the ISKDC or to the Oxford classification in studies I-IV

ISKDC I-V: Definitions as in Table 1 and in the text section 1.4.1.

Oxford classification: Definitions as in Table 2 and in the text section 1.4.2

C0=No cellular/fibrocellular crescents, C1=presence of cellular/fibrocellular crescents

GGS0= No global glomerulosclerosis, GGS1= presence of global glomerulosclerosis

In study I the clinical picture at onset was related to the histology findings. Advanced biopsy findings were frequent among patients with nephritic syndrome or a nephritic-nephritic onset (60% and 70% respectively). Unexpectedly, in patients with mild to moderate proteinuria, a group that is generally considered to be benign, the frequency advanced biopsy findings was equally high (69%) despite the fact that their GFR showed only a moderate reduction at onset. Patients with cellular or fibrocellular crescents had lower GFR and more proteinuria than patients without this lesion. GFR deteriorated and proteinuria increased with more advanced histology findings.

The clinic-pathological correlations have been observed in a number of studies [1, 33, 36, 55, 90-92] in which severe symptoms are well correlated with the severity of the histology findings, thereby presenting the prognostic dependence thereof. More recent studies specifically highlight the risk of a poor outcome in patients with nephritic syndrome or with nephritic-nephrotic onset [12, 51, 56, 93]. Our results show that not only nephrotic proteinuria, but also persistent non-nephrotic proteinuria correlate with advanced biopsy findings and impaired GFR at time of biopsy. These patients therefore need close monitoring and follow-up.

5.2 Effect of treatment in patients with severe HSN and IgAN

Results of study II

The results of two treatment strategies were retrospectively studied in 43 patients (24 HSN, 19 IgAN) with severe forms of disease. All patients received ACEi and/or ARB. Eighteen patients received treatment A: Methylprednisolone pulses followed by oral prednisolone (n=12), starting dose 1 mg/kg daily and then tapered during 3-6 months and additionally Cyclophosphamide pulses given 3-6 times monthly. Twenty-five patients received treatment B: ACEi and/or ARB with (n=15) or without (n=10) corticosteroids given as in treatment A. In group A all except one patient had severe clinical features at biopsy and advanced biopsy findings. The corresponding figures for patients in group B were 48% and 72% and respectively.

Proteinuria was reduced in both groups after treatment with Methylprednisolone. In group A proteinuria was further reduced after treatment with Cyclophosphamide. The effect was sustained up to10 years with a median of 3 years. In group B, the 15 patients treated with corticosteroids in combination with ACEi/ARB had a greater fall in proteinuria, than in the group with no corticosteroid treatment. GFR in group A increased after treatment and remained unchanged in group B. In the nine re-biopsied patients, the activity index, based on the percentage of cellular/fibrocellular crescents and the amount of mesangial proliferation, decreased. The chronicity index, based on percentage of segmental and global glomerulosclerosis and the amount of interstitial fibrosis, increased. The frequencies of patients in the different outcome groups in studies II-IV are shown in Table 10.

Outcome	**n**	**A**	**B**	**C**	**D**
Study		n (%)	n (%)	n (%)	n (%)
II					
all	43	18 (42)	8 (18)	11 (26)	6 (14)
HSN	24	12 (50)	3 (12)	7 (30)	2 (8)
IgAN	19	6 (32)	5 (26)	4 (21)	4 (21)
III	78	48 (61)	10 (13)	17 (22)	3 (4)
		good outcome		**poor outcome**	
IV	99	81 (82)		18 (18)	

Table 10. Number of patients in the different outcome groups in studies II-IV
A: no or minor urinary abnormalities, B: persistent mild proteinuria and a normal GFR, C: active renal disease Ualb/c ≥ 200 mg/mmol and/or hypertension and/or GFR 41-93 ml/min/1.73m^2 (study II) GFR 31-93 ml/min/1.73m^2 (study III), D: GFR ≤ 40 ml/min/1.73 m^2 or ESRD (study II); GFR ≤ 30 ml/min/1.73m^2 or ESRD (study III). Definition of outcome in study IV: Poor outcome if GFR:last is reduced >50% from GFR at biopsy or if deterioration to ESRD (see section 5.4)

In study II 40% of the patients the disease deteriorated to a poor outcome, found in 9/18 (50%) of the patients in group A and 8/25 (33%) in group B. Since patients in group A in a higher proportion had a more advanced disease than patients in group B no conclusions on treatment efficacy can be made. There were no significant differences in the results of any clinical variable between HSN and IgAN patients at any point in time during follow-up, and no significant differences in outcome between HSN and IgAN.

The recommendation regarding how to treat severe cases has changed over time. Immunosuppression was not commonly used before the years 1998-2000, and the use of RAAS blockade has also increased over the years in both diseases. The improved regimen is shown to ameliorate the outcome in study II. Patients with onset of the disease before 1998 more frequently progressed to a poor outcome, than patients with a later onset ($P<0.01$). On the other hand several factors make the nephrologist reluctant to use aggressive immunosuppression. Aware of the fact that spontaneous resolution in HSN has been described [6, 36] and the concern of the significant side effects of Methylprednisolone and of Cyclophosphamide [94], the question of treatment requires careful consideration. Therefore an overview of the most important studies in the field is presented below.

5.3 Overview of treatment strategies

Treatment in HSN patients

Treatment aims at preventing long-term renal morbidity in high-risk patients, but there is no consensus on treatment recommendations. In studies of patients with severe HSN there have been several observational studies of various regimens, but there are few randomized controlled trials offering firm evidence of the best therapeutic practice. Due to the scarcity of the severe cases, the majority of the observational studies published include few patients (in 13/18 studies ≤ 20 patients are included) [95]. The studies show effectiveness of several immuosuppressive treatment strategies: Methylprednisolone followed by oral corticosteroids [93], corticosteroids in combination with Azathioprine [41, 96] , Cyclophophamide [51, 97-102], with or without anticoagulants [103], with or without plasmapheresis [56, 104, 105], Cyclosporine A [106-108], Mycophenolate mofetil [35, 109] or Immunoglobulin therapy [110]. In contrast, in one of the few randomized controlled study in HSN, the authors found no evidence favouring Cyclophoshamide versus supportive therapy [98]. There is still insufficient data to present evidence-based recommendations for the use of pulse or oral corticosteroids, either alone or in combination with Azatioprine or Cyclophosphamide. The use of ACEi and ARB to HSN patients with

mild to moderate proteinuria is frequently used based on the satisfactory results obtained with this regimen in IgAN (see below).

Treatment in IgAN patients

Several treatment approaches have been explored in IgAN [111], but no specific curative treatment has been established. In paediatric studies the long time period from apparent clinical onset to progression of ESRD creates a need for surrogate markers of a poor prognosis to enable evaluation of interventions [112].

In adult IgAN patients treatment with ACEi [113], with or without the combination of ARB [114, 115] or ARB alone [116] in both normotensive and hypertensive patients has been shown to be effective in preserving GFR and reducing proteinuria. A superior effect of a combination therapy of ACEi plus ARB has been clearly demonstrated in the COOPERATE trial [117] of adult non-diabetic patients, of which 50% had IgAN. The study establishes that ACEi and ARBs preserve renal function equally and that the combination of the two preserves the renal function more effectively than either therapy alone.

The most convincing study of the effect of ACEi in IgA children and adults (9-35 years old) with moderate proteinuria is an Italian randomized controlled study from 2007 [118] (Table 11). Sixty-six patients (32 children) with proteinuria > 1g < 3g/day/BSA and normal or moderately reduced renal function (GFR > 50 ml/min/1.73m^2 were included and randomized to Benazepril (ACEi) or placebo. The end point was a 30% decrease in creatinine clearance or worsening of proteinuria exceeding nephrotic range. The results demonstrated a beneficial effect of ACEi. Patients in the treated group had a significant reduction of proteinuria and less deterioration of GFR compared to the placebo group.

The beneficial effect of ACEi in 500 Japanese children with IgAN was reported in a retrospective study where cohorts from the years 1976-89 and 1990-2004 were compared. The renal survival of these patients has improved since 1990, with the introduction of ACEi [119].

The use of corticosteroids alone or in combination with cytotoxic agents (Cyclophosphamide/Azatioprine or Cyclosporin A) and treatment with cytotoxic agents alone has been evaluated in a systematic review including 13 randomized controlled studies with over 600 adult patients [120]. All three subsets were each compared to controls/placebo/no treatment. The result shows that the use of steroids to high risk patients lower the risk of ESRD compared to controls/placebo/no treatment. The use of steroids

combined with cytotoxic agents or cytotoxic agents alone did not. However, the reports concluded that the examined populations were too small and did not meet methodological quality for certainty of the results.

One of the most referred adult randomized controlled study (Pozzi et al), with a follow-up time of more than 15 years, compared the effectiveness of steroid treatment (iv and oral during 6 months) with supportive therapy including ACEi or ARB [121, 122]. The 10-year renal survival was significantly better and the protein excretion was significantly reduced in the steroid treated group compared to the group with supportive treatment.

There is an on-going debate among paediatric nephrologists on which patient group that will benefit from steroid treatment, and if there is sufficient evidence to recommend additional immunosuppression. With the expanding use in the 1990s of corticosteroids and immunosuppressive drugs in diffuse proliferative IgAN, many observation studies but few trials, have been reported in childhood IgAN. The overall level of evidence is low [112]. The results of the three largest trials are summarized in Table 11.

Study	N	treatment	Proteinuria	GFR decline	Histology deterioration
Yoshikawa [124]	80	1.Pred+AZA+ anticoagulation 2. pred alone	More reduced in 1	No difference	No increase in 1 but in 2
Hogg [125]	96	1.Pred 2. OmegaIII 3. placebo	No benefit of treatment 1 or 2 versus 3	No benefit of treatment 1 or 2 versus 3	Not evaluated
Coppo [118]	66 (32 children)	1.ACEi 2.placebo	Significant reduction in 1	Trend effect	Not evaluated

Table 11. Result of trials in paediatric IgAN , adapted after Coppo et al [39].
AZA= azatioprine, pred=prednisolone, ACEi=angiotensin converting enzyme inhibitor

In a Japanese study [124] the effect of steroids in combination with Azatioprine and anticoagulants was compared with the effect of steroids alone. The regimen was given for 2 years to patients with diffuse mesangial proliferation. The result favoured the combined therapy, which was more efficient in reducing proteinuria and preventing

glomerulosclerosis. RAAS blockade was not used in the referred study. The side effects of the treatment in the referred study, which were also used in and earlier large Japanese randomized controlled study, were few [123,124].

A recent multicentre-study has evaluated the effect of prednisolone with placebo and omega III [125]. Ninety-six children with biopsy proven IgAN, GFR > 50 ml/min/1.73m^2 and moderate to severe proteinuria, were randomly assigned to one of the treatments for 2 years. In group 1, prednisone with the dose tapered from 60 mg/m^2 BSA. Group 2 received Omega III and group 3 was given placebo. Hypertensive patients were given ACEi. The results showed no benefit neither from steroid therapy or omega III compared to placebo, when the outcome was defined as GFR < 60 % of baseline and no difference in proteinuria. Other studies with Omega III have shown conflicting results and at present time there is not sufficient evidence for treatment recommendations [118, 126].

5.4 Predictors of outcome in patients with HSN

Results of study III

We summarize the predictors of a poor outcome in a study group of 78 patients with mean 5.2 up to 17 years of follow-up. The cohort includes patients with mild as well as severe (41%) features at first visit, and with biopsies showing mild and advanced (64%) histology findings (Table 8 and 9). Twenty patients (26%) progressed to poor outcome defined as active renal disease or CKD stage 4-5/ESRD (Table 10). Both severe clinical features at onset and advanced biopsy findings were related to a poor outcome. The frequency of patients with poor outcome in relation to symptoms at onset is in the same range as in studies of the same size showed in Table 3.

The clinical independent predictors of a poor outcome were assessed in the univariate analysis. They were high age at onset, high levels of proteinuria at one year follow-up, severe clinical features at onset and high systolic blood pressure. Of the histology predictors the amount of mesangial proliferation, mesangial matrix expansion, interstitial inflammation and interstitial fibrosis were found to discriminate between good and poor prognosis. Multivariate analysis showed that the combination of proteinuria at one year and the ISKDC grading gave the highest predictive power. The cut-off level of proteinuria at one year follow-up predicting a poor outcome was as low as 144 mg/mmol.

The relationships between clinical and histological findings and the outcome has been discussed by several authors [1, 6, 12, 33, 50-56, 83, 91, 93]. Several authors agree that patients with nephrotic syndrome or a nephritic-nephrotic picture at onset seem to be of highest risk of a poor outcome. In accordance with others [51, 56, 83, 93, 101-104] we advocate an early biopsy in patients with nephrotic range proteinuria before the decision of the treatment strategy is made (see section 3.3.1). In certain cases of prolonged severe symptoms a repeated biopsy can be of value [83]. In our study III patients with nephrotic syndrome or a nephritic-nephrotic picture showed a high recovery-rate (59%). Half of the patients in this group were treated.

Our contribution to the previous knowledge in the field is the special attention to patients with persistent mild to moderate proteinuria in which 18% of the patients had a poor outcome. These observations have also been verified in a recent study of 433 patients [127] and in another of 65 patients [128]. The prognostic value of follow-up proteinuria has been recognized by others [53, 100] but it has not previously been specified to time or amount.

Goldstein et al [55] conducted a long-term follow-up of mean 23 years showing that active renal disease and renal insufficiency were potential outcomes many years after apparent

remission for all forms of clinical presentation. Therefore the prediction by clinical features at onset was not reliable in the assessment of the individual patient.

Many authors have discussed the predictive value of an early biopsy in severe HSN [54-56, 83, 93, 96]. Several authors state that cellular or fibrocellular crescents carry a prognostic significance [1, 6, 33, 36, 56, 91, 98, 100]. The predictive role of crescentic lesions in glomerular diseases has been considered unquestionable. However, the definition of crescents varies between the studies compared, and in some cases the cellular versus fibrous nature of the crescents is not stated. Another source of error is that the distribution of crescents is unevenly spread in the biopsy specimen and may give a false impression of the glomeruli involved. The risk increases in levels less than 20% crescents [129] .

	Good outcome	Poor outcome	Summary
CRE absent	26	4	30
CRE present	19	10	29
	45	14	59

Table 12.
Presence and absence of crescents in relation to outcome in study III

We found no significant difference between presence and absence of crescents in relation to outcome (Table 12) which is in concordance with others [53, 87, 88, 127, 130]. Our conclusions can be due to the retrospect nature of our study with an inevitable pre-selection bias of treated cases. Thirty-eight per cent of all patients in the study group were treated which is 47% of the subgroup who were biopsied and 21/29 (72%) of patients with crescents. We assume that treatment has improved the course of the disease. However, our present outcome data does not show that treatment has a positive effect on outcome. The explanation might be that treatment was given to the most severe cases. Figure 7 shows the distribution of cellular or fibrocellular crescents in groups of patients with increasing amount (absent, < 20%, 20-50% and > 50%), and the proportion of patients with a poor outcome in each group (13, 31, 33 and 50% respectively).

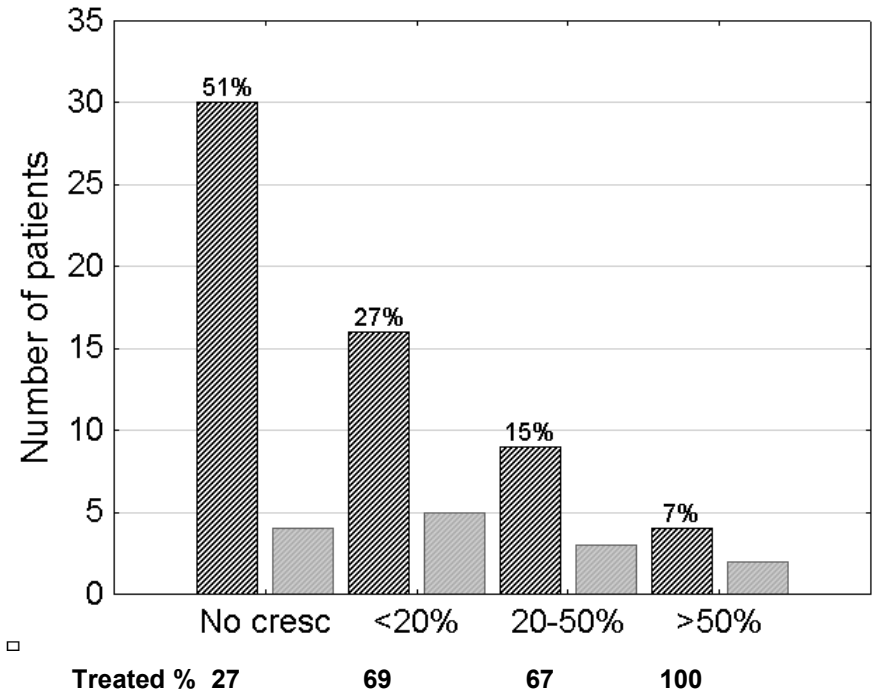

Figure 7.

Distribution of cellular or fibrocellular crescents and their relation to poor outcome at the last visit in the 59 biopsied patients with HSN in study III.

Total number of patients in each histology group (dark)

Subgroups of patients with poor outcome in each histology group (light)

The percentage treated patients in each group is shown in the lower part of the figure

Apart from crescents we found active lesions such as mesangial proliferation and mesangial matrix expansion as predictors in the univariate analysis. These findings were strongly interrelated with crescents (r= 0.35, P= 0.006, r=0.49, P< 0.001 respectively) crescents also correlated to interstitial inflammation (r= 0.46, P < 0.001) but not to interstitial fibrosis or global glomerulosclerosis. As in adults a poor outcome was associated with tubulointerstitial findings [88, 131]. However, in our study the frequency of patients with interstitial fibrosis was low (n=10) and the amount of was mild to moderate (grade 1-2) with no cases of advanced (grade 3) findings.

5.5 Predictors of outcome in paediatric IgAN

Results of study IV

The aim of this study was to identify the clinical and histological predictors of a poor outcome in paediatric IgAN. Furthermore, we validated the histological predictors of a poor outcome, identified in the Oxford classification of 265 patients (59 children) [42, 43], applied to our cohort of 99 children. Ninety biopsies were available for re-evaluation according to the Oxford MEST score (section 1.4.2). Our inclusion criteria and the clinical findings at biopsy and at follow-up differed in several aspects compared to the international study. The comparisons are shown in Table 13. The end points were identically defined in our and the referred study. Poor outcome was defined as a reduction of GFR at the last investigation of more than 50% GFR at the time of biopsy or ESRD.

	Oxford study		Present study
	adults	children	
	n=206	n=59	n=99
At time of biopsy			
Median age (years)	30	13	13
Female (%)	28	25	41
Follow up time (years)	6.4	5.1	13
Follow up time < 1 year (%)	0	0	4
Normal GFR at biopsy (%)	77	-	72
GFR stage 4-5 at biopsy (%)	0	0	5
Nephrotic (%)	30	27	12
Microalbuminuric (%)	-	-	43
Hypertensive (%)	31	15[1]	30[2]
Median time to biopsy (mths)	9	2	19
At follow-up			
RAAS blockade (%)	80	56	24
Immunosuppression (%)	23	47	11
Rate of decline of GFR (ml/min/1.73m^2/year)	-3.7 ± 6.6	-2.7 ± 1.1	-2.0 ± 18[3]
>50% decline of GFR (%)	22 (all)		3
ESRD (%)	13 (all)		15

Table 13.
Clinical findings comparing patients in study IV to the international study [42-43, 47].

[1] Blood pressure adjusted to adult value > 130/90 (MAP s.d. score >1 using age and gender specific constants) or number of patients taking antihypertensive medication [42] [2] Definition of hypertension, see section 3.4
[3] Excluding outliers; four patients with GFR deterioration > 50 ml/min/1.73m^2/year

In study IV the individual clinical predictors of a poor outcome were an impaired GFR at biopsy and during follow-up at 1, 3 and 5 years, a high blood pressure at biopsy, and a high amount of proteinuria at biopsy and during follow-up. Proteinuria at biopsy was identified as the strongest predictor (HR 2.31, 95% CI 1.66-3.20). We found that the higher level of proteinuria at biopsy, the higher the risk of a poor prognosis. The identification of these clinical prognostic markers is in concordance with other authors [14, 18, 57, 62, 132-135], with focus on proteinuria as being the strongest. The increased risk of a poor prognosis also in patients with moderate proteinuria at biopsy has been discussed [113, 136]. The search for predictors in several studies has focused both on levels of proteinuria at biopsy, but also on the predictive value of follow-up proteinuria [14, 134].

Thirty per cent of our patients were hypertensive at time of the biopsy. High blood pressure was identified as a predictor only at time of biopsy and not during follow-up. Compared to adult series hypertension is less frequently a predictor among children with IgAN. The frequency of high blood pressure is often lower in children [65, 137] and is often reversible in children compare to adults with IgAN [48].

In the identification of the histological predictors of a poor outcome we support the conclusions in the Oxford classification [42]. We found three out of the four histology lesions in the Oxford MEST score to be predictive of a poor prognosis: A high mesangial proliferation score (M), presence of endocapillary hypercellularity (E) and a high amount of tubular atrophy/interstitial fibrosis (TA/IF), but presence of segmental sclerosis (S) did not reach significance in our study. We additionally found presence of cellular or fibrocellular crescents and presence of global glomerulosclerosis to be predictive in the univariate analysis. Although a small sample size, we identified endocapillary hypercellularity as a strong predictor (HR 7.15, 95% CI 2.21-23.13), which was not the case in the referred study. Tubular atrophy/interstitial fibrosis was a strong independent predictor in our study, which is in concordance with the international study and other authors [14, 47, 138]. This lesion correlated strongly to global glomerulosclerosis (r= 0.83) in our and in the international study. The discriminative ability of all histology lesions was reduced when they were compared with proteinuira at biopsy (Model A), indicating that the influence of proteinuria was strong. When histology was combined with proteinuria at one year follow-up (Model B), the discriminative power of the histology lesion was more pronounced, due to a smaller sample size and a selection of the more severe cases. A low GFR at biopsy and during follow -up indicated an unfavourable prognosis in the univariate analysis, but not in the multivariate analysis. Thus, according to our results, biopsy findings were not independent of the impact of clinical data in the prognostic assessment which was found in the international study. However, due to the low number of events (n=18) we could not expand the multivariate models further.

CONCLUDING REMARKS AND FUTURE PERSPECTIVE

In summary the clinical course of HSN is unpredictable, which requires close monitoring and a long-term follow-up. Risk patients are those with severe symptoms at onset and those with persistent mild-moderate proteinuria at onset, as well as those with proteinuria at follow-up. The clinical picture at onset and the grading of the histological findings as well as the amount of proteinuria at follow-up are powerful predictors of a poor prognosis, highly informative value for the decision regarding treatment.

In paediatric IgAN the clinical course is as serious as in adults, with a high risk of disease progression. Impaired GFR and the amount of proteinuria at biopsy and during follow-up were identified as individual clinical predictors. We support the prognostic significance of the histological findings defined in the Oxford classification MEST score. We found three out of four lesions to be of prognostic importance; mesangial and endocapillary hypercellularity and tubular atrophy/interstitial fibrosis. Additionally we found cellular/fibrocellular crescents and glomerulosclerosis to be predictive.

Identification of reliable clinical and histopathologic prognostic factors in HSN and IgAN may contribute to permit an appropriate design for future therapeutic prospective studies. There is a need for an evaluation of the long-term effect of ACEi/ARB treatment in mild and moderately severe HSN, and further studies are needed to evaluate the efficacy of different immunosuppressive drugs in treating severe HSN and IgAN. Large prospective longitudinal multicentre studies with strict and narrow inclusion criterias, strict protocols for medical interventions and a close follow-up are needed, to evaluate the outcome among patients with a similar degree of severity of disease. The objective should be to replace the present variety of treatments with more formalized recommendations.

In the future treatment strategies will most likely focus on the defects in the IgA1 glycosylation, and the prevention of the mesangial depositions of the IgA complex. Moreover, with expanding knowledge about the components of the glomerular barrier and its conditions and mechanisms of permeability, a new therapeutic treatment may emerge.

SVENSK SAMMANFATTNING

Henoch Schönlein Nefrit (HSN) och IgA nefropati (IgAN) diagnosticeras i njurvävnadsprov med fynd av IgA depositioner i njurvävnaden i kombination specifika avvikelser talande för sjukdomsutveckling sekundär till denna deposition. Sjukdomarna har olika långtidsförlopp: HSN anses vara en akut sjukdom med multi-organ engagemang (hud, leder och magtarmkanal) och vanligtvis ett spontant läkningsförlopp och generellt en god prognos. IgAN beskrivs däremot som en kronisk sjukdom med en långsam progress och hög risk för utveckling av kronisk njursjukdom. Förloppet och prognosen för de båda sjukdomarna är emellertid svårt att förutsäga. Syftet med avhandlingsarbetet är att öka kunskaper och identifiera riskfaktorer för sjukdomsprogress och dålig prognos bland HSN och IgA patienter samt att studera effekten av behandling till de svåra fallen. I **studie I** sammanställdes resultat från 73 HSN patienter. Sambanden mellan klinik och histologi studerades. Klinisk bild vid symtomdebut (grad av proteinutsöndring (proteinuri) och resultat av njurfunktion (GFR) undersökning) jämfördes med resultat av njurvävnadsprov (njurbiopsi). Patienter med uttalad proteinuri (nefrotisk) hade ofta förekommande avancerade patologiska förändringar och nedsatt GFR. Avancerad njurpatologi förekom även i hög frekvens bland patienter med lägre nivå av proteinuri (icke-nefrotisk). Proteinnivå ökade och GFR nivån minskade med ökad grad av patologi i njurbiopsin. I **studie II** studerades behandlingseffekt vid svår sjukdom (n=43, 24 HSN, 19 IgAN). Grupp A, n=18, fick behandling med kortison och ytterligare immunhämmande läkemedel (Cyklofosfamid) samt proteinreducerande läkemedel (RAAS blockad). Av de 25 patienterna i grupp B fick 15 kortison och RAAS blockad och övriga 10 endast RAAS blockad. Graden av proteinuri minskade efter behandling med kortison och ytterligare efter behandling med Cyklofosfamid i grupp A. GFR förbättras vid uppföljning under median 3 år, upp till 10 år. Proteinurin minskade även efter behandling i grupp B och GFR nivån var stabil under uppföljning. Markörer för utveckling av dålig prognos bland 78 HSN patienter identifierades i **studie III**. Dålig prognos definierades som kvarvarande aktiv njursjukdom alternativ kronisk njursjukdom med GFR $\leq$ 30 ml/min/1.73m^2 alternativ terminal njursvikt som kräver dialys eller transplantation. Riskfaktorer för utveckling av dålig prognos var hög ålder vid debut, svår klinisk bild vid debut, hög grad av proteinuri vid ett års uppföljning samt hög grad avancerade förändringar i njurbiopsi. Nivån av proteinuri vid ett års uppföljning i kombination med graden njurvävnadsskada utgjorde tillsammans den bästa möjligheten att förutsäga dålig prognos. Det prognostiska värdet av en ny klassifikation för IgAN (Oxfordklassifikationen) samt betydelsen av och sambandet med kliniska parametrar studerades i **studie IV**. 99 IgA patienter följdes under medel 13 år och 90/99 biopsier bedömdes enligt Oxfordklassifikationens s.k MEST score baserat på fyra njurvävnadsfynd som ansågs vara prognostiskt ogynnsamma. Nedsatt GFR, högt blodtryck vid biopsi och hög nivå av proteinuri både vid biopsi och under uppföljning identifierades som kliniska markörer för dålig prognos. Tre av de fyra njurvävnadsfynden i MEST score visade sig ha prognostisk betydelse.

Slutsatser av detta arbete kan bidra till kunskap för omhändertagandet av pediatriska HSN och IgA patienter, så att medicinska insatser till de svåra fallen kan förhindra eller förskjuta utvecklingen av kronisk njursjukdom.

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ORIGINAL ARTICLE

Stella F. Edström Halling · Magnus P. Söderberg · Ulla B. Berg

Henoch Schönlein nephritis: clinical findings related to renal function and morphology

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Abstract We evaluated the renal hemodynamics and the urine protein excretion rates of 73 children with Henoch-Schönlein nephritis (HSN). In 40 children we also performed a renal biopsy. The glomerular filtration rate (GFR) and effective renal plasma flow were determined by the clearances of inulin and para-aminohippurate during water diuresis. Urine albumin and IgG excretion were assessed in short-term timed samples. The mean GFR at the first examination was reduced in the HSN patients and most reduced in those with nephrotic proteinuria. There was an inverse correlation between the GFR at the first examination and the amount of albuminuria and urinary IgG excretion. Among the 40 patients with some degree of proteinuria who underwent a renal biopsy, 9 of 13 patients with mild to moderate proteinuria had severe morphological changes. GFR correlated inversely and fractional albumin and IgG excretion directly with the severity of the pathological findings on the biopsy, and with segmental and global sclerosis, the grade of mesangial proliferation, and interstitial inflammation. In conclusion, GFR is moderately reduced early in HSN and more reduced in patients with more proteinuria and in those with more advanced morphological changes. Moreover, even mild to moderate proteinuria may indicate severe morphological changes, which increase the indications for early renal biopsy in these patients.

Keywords Henoch-Schönlein glomerulonephritis · Glomerular filtration rate · Renal hemodynamics · Proteinuria · Albuminuria · Urinary IgG · Renal morphology

S. F. E. Halling (✉) · U. B. Berg
Department of Pediatrics,
Karolinska University Hospital,
Huddinge, 141 86 Stockholm, Sweden
e-mail: stella.edstrom.halling@klinvet.ki.se
Tel.: +46-8-58580000
Fax: +46-8-58581410

M. P. Söderberg
Department of Pathology,
Karolinska University Hospital,
Huddinge, 141 86 Stockholm, Sweden

Introduction

Henoch-Schönlein purpura (HSP) is the most common systemic vasculitis in childhood. In the United Kingdom, the incidence is 14–20 per 100,000 children per year and the estimated annual incidence is highest between 4 and 6 years of age [1, 2]. Skin biopsy shows a leukocytoclastic vasculitis that involves the small vessels. The vasculitis may affect several organ systems, but mainly affects the skin, joints, gastrointestinal tract, and kidneys. Glomerulonephritis (HSN), the principal manifestation of HSP, occurs in 40%–50% of cases [3]. Many authors have shown an association between the renal symptoms at onset and the long-term outcome [4]. We therefore wished to study further how the clinical findings at onset of HSN are related to renal function and to the histological changes in a renal biopsy performed within 5 years of onset of the disease. We also investigated how the proteinuria was related to renal hemodynamics and to the biopsy findings.

Materials and methods

Between 1975 and 2002, we studied 73 children with HSN, 32 boys, with a median age of 8.1 (2.0–16.6) years at the onset of the disease. The clinic has a regional population of 100,000 children between 0 and 18 years of age and a catchment area that includes greater Stockholm and patients from other parts of Sweden. Our hospital is one of two referral hospitals in Sweden for children with kidney diseases. Nearly all those from our referral area (i.e., about two-thirds of Swedish children), who need a kidney biopsy, are referred to us. The patients were referred at various stages of the disease and they were divided into five groups, according to their clinical findings at onset. The first group of patients had only hematuria (HEM) at onset. The second group had proteinuria (PROT) with macro- or microscopic hematuria. The third group had the acute nephritic syndrome (AN) with hypertension and/or an increase in the serum creatinine level and hematuria with or without proteinuria. The fourth group had the nephrotic syndrome (NS)

Table 1 Relationship between clinical findings at onset and renal hemodynamics (mean±SD) (*GFR* glomerular filtration rate, *ERPF* effective renal plasma flow, *FF* filtration fraction)

	n	GFR	ERPF	FF
		(ml/min per 1.73 m^2)		(%)
1. Hematuria (HEM)	12	118±20	572±69	20.8±3.2*[1], *[2]
2. Proteinuria±hematuria (PROT)	27	108±18	573±126	19.9±2.9*[3],
3. Acute nephritic syndrome (AN)	17	106±26	588±132	18.0±3.5
4. Nephrotic syndrome (NS)	6	88±27	603±140	14.7±3.3
5. Nephritic/nephrotic (AN+NS)	11	79±26	585±99	14.6±2.5*[4]
Total number of patients	73	103±25*[5]	580±115	18.0±3.5*[6]
Controls	49	116±11	611±90	19.3±2.5

*[1] P=0.003 vs. NS, *[2] P=0.0001 vs. AN+NS, *[3] P=0.005 vs. AN+NS, *[4] P=0.003 vs. controls, *[5] P=0.002 vs. controls, *[6] P =0.048 vs. controls

with proteinuria >40 mg/m^2 per hour and a serum albumin level below 25 g/l with or without edema. The fifth group of patients had a mixed nephritic-nephrotic (AN+NS) picture at onset.

On admission, the urinary protein excretion rate, blood pressure, and serum creatinine concentration were determined in the children and hemodynamic studies were performed. The median duration of the disease at the first examination was 0.48 (0.01–3.0) years. Renal hemodynamics were assessed by the glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) determined by the clearances of inulin and para-aminohippuric acid during water diuresis, using a standard clearance technique with continuous infusion and repeated urine sampling [5]. The filtration fraction (FF) was calculated as the ratio between GFR and ERPF. The control group for renal function comprised 49 healthy children aged 9.9±4.5 years. The GFR of the control group was 116±11 ml/min per 1.73 m^2, the ERPF was 611±90 ml/min per 1.73 m^2, and the FF 19.3±2.5%.

At the time when the renal function study was performed, we collected a short-term timed urine sample and a serum sample for analysis of albumin, IgG, β_2-microglobulin, and creatinine in both blood and urine. The detection level of albuminuria was 4 mg/l. The ratios of urinary albumin/creatinine (Ualb/UCr) (mg/mmol) and urinary IgG/creatinine (UIgG/UCr) (mg/mmol) were calculated. Since the range of Ualb/UCr was large and not normally distributed, the ratio was log-transformed. Microalbuminuria was defined as Ualb/UCr of 2.5–25 mg/mmol [6], which corresponds to log Ualb/UCr of 0.4–1.4. Nephrotic proteinuria was defined as Ualb/UCr $\geq$400, which corresponds to log Ualb/UCr $\geq$2.6.

Hypertension was defined in accordance with the Task Force Report on High Blood Pressure in Children and Adolescents [7]. Two patients were treated with enalapril at a low dose at the time of biopsy**.** Three patients had been treated with low doses of oral corticosteroids (1 mg/kg) for 1 or 2 weeks, respectively, prior to the biopsy due to bowel pain.

Renal biopsy was performed in 40 children within 5 years of the onset of their disease. The biopsies were performed 0.04–4.9 (median 0.7) years from the onset. Some AN and/or NS patients entered a spontaneous remission without an early biopsy, which explains the wide time range. In the PROT group the median time for the biopsy was 2.1 years, while in the two nephrotic groups (NS and AN+NS) the median time was 0.08 and 0.12 years, respectively. The indication for renal biopsy was based on the clinical findings. The patients were sedated with midazolam and morphine and were given lidocaine local anesthesia. The biopsy was performed percutaneously, under ultrasound guidance, using an automatic device with a 16-gauge needle (Bard Magnum Biopsy Instrument and Core Tissue Biopsy Needle, Urological, Covington, Calif., USA). Specimens were taken for light microscopy (LM) and immunofluorescence (IF). The biopsies were fixed in 4% paraformaldehyde in phosphate buffer. All patients showed predominantly IgA with IF. In this paper, we discuss only the LM results.

The degree of glomerular involvement was determined by calculating the total number of glomeruli and then the percentages of glomeruli with global or segmental sclerosis and of glomeruli with crescents. Signs of mesangial matrix expansion, mesangial proliferation, interstitial inflammation, and interstitial fibrosis with tubular atrophy were noted and semi-quantitatively graded on a four-point scale (0–3). All biopsy findings were then classified on a five-point scale (I–V) as recommended by the International Study of Kidney Disease in Children (ISKDC) [8]. The study protocol was approved by the Ethics Committee of Karolinska Institutet at Huddinge University Hospital.

Statistical analysis

The data are expressed as mean values±1 standard deviation (SD) if they were normally distributed. Statistical comparison of patient groups was performed using ANOVA with the post hoc Tukey test. Spearman rank correlations were used to assess the relationship between renal hemodynamics and proteinuria with various biopsy parameters. The statistical program of Statistica 6.0 was used. P<0.05 was chosen as the level of significance.

Results

On the first investigation, the mean GFR of all 73 HSN patients was 103±25 ml/min per 1.73 m^2 and the FF 18.0±3.5%, which is significantly lower than the results of the controls (Table 1). The ERPF was similar to the controls.

Table 1 shows the clinical findings at onset and the renal hemodynamics at the first investigation. The GFR differed in the five groups at the first investigation (Fig. 1 and Table 1). It was normal in the HEM group and low in the other groups, being lowest in the NS and AN+NS groups, in whom 50% and 70%, respectively, had GFR below –2 SD of that of the controls. In the PROT group, the GFR was only moderately reduced and differed from the AN+NS group. The ERPF was similar in all the groups. The FF was significantly lower in the AN+NS group than in controls and in the HEM and PROT groups. The FF in the NS group differed significantly from that in the HEM group. We found no gender differences in GFR in the various clinical groups.

Figure 2 shows the GFR in relation to the degree of albuminuria at the time of the first renal functional examination. The GFR was significantly higher in patients with normo- and microalbuminuria than in those with albuminuria in the nephrotic range. There were 8% of patients with microalbuminuria that had GFR below –2 SD, compared with 66 % in the group of patients with nephrotic proteinuria. Moreover, there was an inverse correlation between log Ualb/UCr and GFR (r=–0.55,

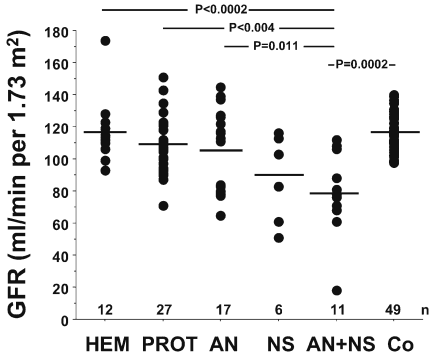

Fig. 1 Glomerular filtration rate (*GFR*) (ml/min per 1.73 m^2) on the first investigation of the patient groups according to their clinical findings at onset. The number of patients is given in the lower part of the figure (*HEM*) hematuria, *PROT* proteinuria±hematuria, *AN* acute nephritic syndrome, *NS* nephrotic syndrome, *Co* controls)

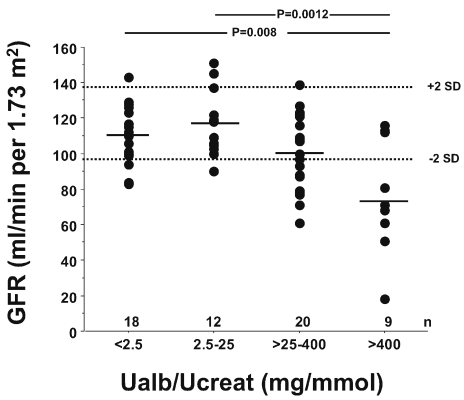

Fig. 2 GFR (ml/min per 1.73 m^2) on the first investigation in relation to normoalbuminuria (Ualb/UCr <2.5), microalbuminuria (Ualb/UCr 2.5–25), albuminuria (Ualb/UCr >25–400), and albuminuria in the nephrotic range (Ualb/UCr >400 mg/mmol). The *dotted lines* indicate ±2 SD of those of the controls. The number of patients is given in the lower part of the figure

n=44, P=0.0001) and between UIgG/UCr and GFR (r=–0.53, n=33, P=0.0014) at the time of the first investigation.

Table 2 shows the clinical findings at the onset in relation to the renal biopsy changes, which were graded according to the ISKDC. The biopsies were taken from 40 patients in groups 2–5 with no patients from group 1 (hematuria only). The most common histological changes were grade III (mesangial proliferation with crescents or glomerular sclerosis in <50% of the glomeruli), seen in 19 of 40 (47%) of the biopsies; 6 of 40 (15%) patients had grade IV (mesangial proliferation with crescents and/or glomerulosclerosis 50%–75%) and 2 of 40 (5%) had

Table 2 ISKDC grading of the renal biopsies in the patients who were grouped according to their clinical symptoms at onset

	n	Grade				
		I	II	III	IV	V
1. HEM	0					
2. PROT	13	1	3	7	1	1
3. AN	12	3	2	6	1	0
4. NS	5	0	2	2	1	0
5. AN+NS	10	1	1	4	3	1
Total	40	5	8	19	6	2

grade V (mesangial proliferation with crescents or glomerulosclerosis >75%). Thus 68% of all biopsies showed grade III changes or more. In the PROT group, 13 of 27 (48%) were biopsied and 9 of 13 (69%) of the biopsies had grade III or more changes, compared with 58% in the AN group, 60% in the NS group, and 70% in the AN+NS group. There was no statistically significant difference in proteinuria between the biopsied and the non-biopsied patients in the PROT group. The STAT exact test showed no statistically significant difference between the clinical findings at onset and the biopsy findings.

The GFR was significantly lower in patients with crescents (80±26 ml/min per 1.73 m^2) than in those without (111±20 ml/min per 1.73 m^2, P=0.0002). Those with crescents had more proteinuria, measured as log Ualb/UCr and UIgG/UCr, than those without (P=0.00014 and P=0.011, respectively). In patients with global and segmental sclerosis, we found inverse correlations between the percentages of glomeruli with global and segmental sclerosis and GFR (r=–0.637, n=13, P=0.019 and r=–0.489, n=22, P=0.021, respectively). Figure 3a shows the GFR in 36 patients from the various ISKDC groups at the time of the biopsies. Renal hemodynamics were not studied in 4 patients at the time of the biopsy. The GFR deteriorates with more advanced pathological findings, i.e., it was significantly lower in those with grade IV changes than in the controls and those with grade I changes. Figure 3b and c shows log Ualb/UCr and UIgG/UCr in the ISKDC groups. Albuminuria and urinary IgG excretion increased with more advanced pathology on the biopsies (Table 3). Figure 3b also shows that all patients, except one with ISKDC grades IV and V changes, had proteinuria in the nephrotic range.

Table 3 shows the significant correlation coefficients between the biopsy findings and the GFR, log Ualb/UCr, UIgG/UCr, systolic and mean arterial blood pressure (MAP). The correlations with proteinuria were calculated only in patients with measurable urinary albumin and urinary IgG levels, respectively, at the time of the biopsy. We found decreases in the GFR and increases in the fractional albumin and IgG excretion with increasing grades of interstitial inflammation, mesangial proliferation, and ISKDC groups on the biopsy.

An inverse correlation was noted between the GFR at the time of the biopsy and log Ualb/UCr and UIgG/UCr, respectively (r=–0.570, n=31, P=0.0008 and r=–0.518, n=23, P=0.011), but no significant correlation between

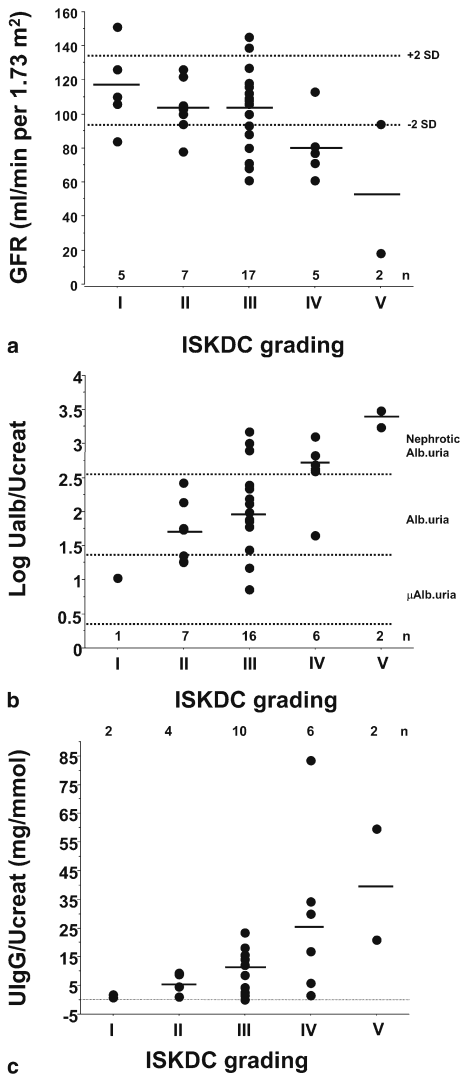

Fig. 3 **a** GFR (ml/min per 1.73 m^2) at the time of the biopsy in the various groups. The *dotted lines* indicate ±2 SD of those of the controls. The number of patients is given in the lower part of the figure. **b** Proteinuria (log Ualb/UCr) at the time of the biopsy in the various groups. The *dotted lines* indicate the ranges of microalbuminuria and albuminuria. The number of patients with measurable Ualb is given in the lower part of the figure. **c** Proteinuria (UIgG/UCr) at the time of the biopsy in the various groups. The number of patients with measurable UIgG is given in the upper part of the figure

Uβ_2/UCr and the biopsy findings. Direct correlations were detected between the systolic blood pressure or MAP and the ISKDC grading and mesangial proliferation (Table 3). None of the blood pressure data were matched

Table 3 Spearman correlations between pathological changes and GFR at the time of the biopsy, log Ualb/UCr and UIgG/UCr in patients with measurable Ualb and UIgG, respectively, and systolic blood pressure (syst BP) and mean arterial blood pressure (MAP) (*MM* mesangial matrix, *MP* mesangial proliferation, *II* interstitial inflammation)

	GFR			Log Ualb/UCr			UIgG/UCr			Syst BP			MAP		
	n	*r*	*P*	*n*	*r*	*P*	*n*	*r*	*P*	*n*	*r*	*P*	*n*	*r*	*P*
MM	36	−0.297	0.079	32	0.480	0.0055	24	0.232	0.275	37	0.252	0.131	37	0.229	0.174
MP	36	−0.422	0.010	32	0.552	0.001	24	0.686	0.0002	37	0.387	0.018	37	0.358	0.0298
II	36	−0.447	0.006	32	0.636	0.00009	24	0.590	0.0024	37	0.285	0.088	37	0.227	0.176
ISKDC	36	−0.387	0.0196	32	0.606	0.00024	24	0.622	0.0012	37	0.482	0.0025	37	0.335	0.042

for age or length, but the mean ages of the patients in the various biopsy groups were similar.

Discussion

The aim of this study was to evaluate the relationship between the clinical findings at onset and (1) the renal function and (2) histological findings in HSN patients biopsied within 5 years of disease onset. The two main findings were that HSN patients as a group had a lower GFR at the first examination than controls and that severe morphological changes occurred not only in patients with proteinuria in the nephrotic range, but also in those with mild proteinuria (Ualb/Cr 25–400 mg/mmol). Thus the clinical findings at the onset of the disease cannot predict the morphological changes. Moreover, the more severe the morphological changes, the greater the proteinuria, the lower the GFR, and the higher the blood pressure at the time of the biopsy. Among the pathological findings in the biopsy, the percentage of global and segmental sclerosis**,** mesangial proliferation, and interstitial inflammation seemed to correlate best with poor renal function, albuminuria, and IgG excretion.

Our patients are both selected and unselected, since those from our area had all stages of the disease, and most had mild renal involvement. Many patients with more advanced stages of the disease were referred to us from other parts of the country for a renal biopsy. In our study, 34 of 73 (47%) of the patients had AN, NS, or AN+NS at the onset. The distribution of our study population therefore differs from that of Koskimies et al. [9], who reported 27% with AN, NS, or AN+NS among 29 unselected HSN patients.

The lower GFR found in the entire HSN group resembles that in previous studies on children with IgA nephropathy from our unit [10, 11]. The normal GFR in the HEM group may indicate a good prognosis. This is in accordance with the findings of others who reported that fewer than 5% developed renal failure in this group [4, 9, 12]. Patients in the PROT group had a slightly reduced GFR, despite the high proportion of severe morphological changes. The inverse correlation between GFR and albuminuria in the present study resembles our findings in children with IgA nephropathy [11]. Coppo et al. [13] compared the prognosis of HSN in adults with that of children and found no definite level of proteinuria in children that was associated with a poor prognosis. However, the clinical outcome in children with absent or mild proteinuria was usually better than in those with higher levels of proteinuria, but the difference was not statistically significant. Therefore the clinical course was more unpredictable in children than in adults [13]. We found the lowest GFR in the NS and AN+NS groups, which accords with the findings of Schärer et al. [14], who reported that the presence of renal insufficiency or NS at the onset predicts the development of chronic renal disease. The lower GFR in our patients with crescents than in those without also agrees with the results of Schärer et al. [14], who found that patients with crescentic glomerulonephritis had a poor prognosis.

Of 40 biopsies, 68% had histological findings of grade III changes or greater. However, one should remember that none of the patients from the HEM group underwent a biopsy. Our study shows that proteinuria at the onset is frequently associated with advanced renal pathology. The log Ualb/UCr and UIgG/UCr correlated with the ISKDC grade in the biopsy. In groups ISKDC III–V, 10 of 24 (41%) and in groups IV–V, 7 of 8 (87%) of the patients had proteinuria in the nephrotic range at the time of the biopsy. Patients with nephrotic or nephritic-nephrotic findings at the onset, and those with a high proportion of crescents in the renal biopsy, may have a poor prognosis [13, 15, 16, 17, 18]. Our study shows that a renal biopsy is indicated not only in patients with nephrotic symptoms, but also in those with mild proteinuria. The amount of proteinuria should be closely monitored and if severe or the nephrotic syndrome develops, the biopsy must be performed immediately to decide whether treatment is necessary. Several protocols have been used but there is no golden standard [19, 20, 21]. Renal function can deteriorate many years after signs of recovery and the patients therefore need a long-term follow-up [4].

In our study the proteinuria correlated with the ISKDC grade. The log Ualb/UCr and UIgG/UCr correlated with both glomerular mesangial proliferation and interstitial inflammation. The main risk factor for progression of glomerular disease is the severity of the proteinuria [22]. This is shown by the selectivity index (SI) based on the ratio of IgG to transferrin clearances. The SI correlated with the severity of the histological lesions in patients with glomerular diseases [23, 24]. Patients with high or moderately selective proteinuria showed less severe tubulointerstitial damage than those with non-selective proteinuria. According to D'Amico and Bazzi [24], the fractional urinary excretion of IgG correlated with the tubulointerstitial damage, but not with the glomerulosclerosis. In the present study, we found a correlation between UIgG/UCr and interstitial inflammation, but no correlation with interstitial fibrosis, probably because of the short time elapsed between the onset and the biopsy.

We also found higher blood pressure in patients with more severe morphological changes. Yoshikawa et al. [18] reported that patients presenting with hypertension and/or acute renal insufficiency were more likely to develop chronic renal failure, while Schärer et al. [14] in a multivariate regression analysis could not show that initial hypertension was a significant predictor of a poor outcome. Coppo et al. [13] found that, in contrast to adults, no definite level of proteinuria or hypertension was associated with a poor prognosis in children.

To summarize, we noted that more advanced morphological lesions in the renal biopsy were reflected by a lower GFR and more marked proteinuria. The GFR was lower in HSN patients as a group than in the controls, and especially in those with nephrotic syndrome at the onset or advanced biopsy findings. Our study clearly shows that proteinuria is a marker of renal damage in HSN. In pa-

tients with non-nephrotic proteinuria at the onset, we found severe histological changes in 9 of 13 biopsies, despite only a moderate reduction in the GFR. Thus patients with HSN should be followed both by determining GFR and fractional protein excretion rates, and those with persistent proteinuria need an early kidney biopsy. These findings increase the understanding of risk factors predicting a poor prognosis in HSN, although a longer follow-up is needed to obtain more conclusive results.

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ORIGINAL ARTICLE

Treatment of severe Henoch–Schönlein and immunoglobulin A nephritis. A single center experience

Stella Edström Halling · Magnus P. Söderberg · Ulla B. Berg

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Abstract Our aim was to report the effect of two treatment regimens in 43 cases of severe Henoch–Schönlein nephritis (HSN) and immunoglobulin A nephritis (IgAN) (24 HSN, 19 IgAN). Group A, 11 HSN and 7 IgAN, 88% with an International Study of Kidney Disease in Children (ISKDC) biopsy grade ≥ III and severe clinical features, were treated with corticosteroids, cyclophosphamide (CYC-P) and angiotensin-converting enzyme inhibitor/angiotensin receptor blocker (ACEi/ARB). Group B, 12 HSN and 13 IgAN, 72% with biopsy findings as above and 52% with severe clinical features, were treated with ACEi/ARB ± corticosteroids. The outcome classification was: (a) healthy; (b) mild proteinuria, normal glomerular filtration rate (GFR); (c) active renal disease; (d) chronic renal failure. Twenty-six patients had a good outcome (a+b). The 17 children with poor outcome (c+d) had lower GFR at onset and at follow-up, higher albumin excretion at follow-up, and higher percentage of segmental glomerulosclerosis in the renal biopsy, than those with good outcome. Treatment with corticosteroids, CYC-P and ACEi/ARB was effective in increasing GFR, reducing proteinuria and decreasing the disease activity index. The proteinuria had decreased at follow-up in both groups. In group A, GFR increased and histopathological activity index declined after treatment. The outcome did not differ between groups A and B. The effects of treatment did not differ between HSN and IgAN.

Keywords Methylprednisolone · Cyclophosphamide · Renal biopsy findings · Proteinuria · Glomerular filtration rate · Segmental glomerulosclerosis · IgA nephritis · Henoch–Schönlein nephritis

S. Edström Halling (✉) · U. B. Berg
Department of Clinical Science, Intervention and Technology,
Division of Pediatrics, Karolinska Institutet,
Karolinska University Hospital, Huddinge,
14186 Stockholm, Sweden
e-mail: stella.edstrom.halling@ki.se

M. P. Söderberg
Department of Laboratory Medicine, Division of Pathology,
Karolinska University Hospital, Huddinge,
Stockholm, Sweden

Introduction

The management of severe Henoch–Schönlein nephritis (HSN) and immunoglobulin A nephritis (IgAN) is still controversial. Severe cases are rare, but the morbidity rate among these patients is high. Severe disease is defined according to the clinical features and duration of proteinuria [1] and renal biopsy findings showing a high proportion of glomeruli with crescents or sclerosis as well as interstitial inflammation or fibrosis [2–5].

Various regimens of treatment in severe HSN and IgAN have been described, such as intravenous administration of methylprednisolone (MP) followed by oral treatment with corticosteroids, or corticosteroids in combination with azathioprine (AZA) or cyclophosphamide (CYC-P), with or without anticoagulants, with or without plasmapheresis, cyclosporine A (CyA) or mycophenolate mofetil [6–21]. Treatment with angiotensin-converting enzyme inhibitors (ACEis) [22] with or without the combination of angiotensin II receptor blockers (ARBs) [23] in both normotensive and hypertensive patients has been shown to be effective in preserving glomerular filtration rate (GFR) and reducing proteinuria in IgAN patients.

The aim of this study was to describe the effects of treatment of two groups of patients. Group A had severe disease and were treated with corticosteroids in combination

with CYC-P and ACEi /ARB, whereas group B consisted of patients both with milder and severe disease. All group B patients were treated with ACEi/ARB with or without corticosteroids.

Materials and methods

The study was longitudinal and retrospective. We examined 43 children (25 boys), of whom 24 had HSN (14 boys) and 19 IgAN (11 boys), admitted to the department of Pediatric Nephrology at the Karolinska University Hospital, Hudddinge, between May 1990 and December 2005. Our clinic is the larger of two tertiary centers for children with kidney diseases in Sweden. Thus, the patients were mainly referral patients with a severe clinical presentation at onset. Their median age at onset was 12.0 (4.1–17.7) years, and they were followed for a median of 3.1 (0.25–10.5) years. The age at onset did not differ significantly between the groups.

In Group A there were 18 patients (11 HSN, 7 IgAN), of whom 17 had renal biopsy findings according to the International Study of Kidney Disease in Children (ISKDC) of ≥ grade III, in combination with nephrotic proteinuria at onset, and all except one had severe clinical features. They were initially treated intravenously with MP 30 mg/kg for three alternate days. Twelve patients were also treated orally with prednisolone at a starting dose of 1 mg/kg daily, which, 1 month later, was changed to alternate-day dosing and slowly tapered thereafter. The duration of the oral treatment with corticosteroids was a median 0.6 (0.4–3.1) years. After MP, the regimen also included intravenous treatment with CYC-P 500–750 mg/m^2 body surface area, 1–3 pulses (14 patients), or six pulses monthly (2 patients), or oral administration of CYC-P 2 mg/kg daily for 8 weeks (2 patients). All patients received ACEis/ARBs.

Group B consisted of 25 patients (13 HSN, 12 IgAN) of whom12 had severe clinical features. Eighteen patients had undergone a biopsy showing ISKDC grade ≥ III, but only five of these 18 had nephrotic proteinuria. Five patients had biopsy findings showing ISKDC grade II, and two patients had ISKDC grade I. All 25 patients were treated with ACEis/ARBs with (15 patients) or without (10 patients) corticosteroids. The duration of the latter medication was a median 0.3 (0.02–0.7) years.

The recommendations of treatment have changed over time. In group A only 3/18 patients had been treated before 1998, compared with 6/25 in group B. At the time, aggressive treatment with immunosuppression not commonly used.

The patients were classified into five categories according to their renal manifestations at onset and at biopsy [24]: (1) micro- or macroscopic hematuria; (2) persistent mild proteinuria (<1 g/l or urine albumin/creatinine ratio (Ua/c) < 200 mg/mmol) ± hematuria; (3) nephritic syndrome (moderate proteinuria (Ua/c≥ 200–400 mg/mmol), decreased GFR, hematuria and/or hypertension [25]); (4) nephrotic syndrome (urinary albumin excretion >40 mg/h per square meter body surface area or Ua/c ≥ 400 mg/mmol, serum albumin <25 g/l); (5) mixed nephritic/nephrotic syndrome at onset. Classes 1 and 2 were considered as mild clinical features, and classes 3–5 as severe. Severe features at onset were present in 66% of group A patients and 48% of group B patients, while, at the time of the first renal biopsy, the corresponding values were 94% and 48%, respectively.

On admission, all patients had undergone a renal function test and a renal biopsy at a median 0.2 years after onset. Nine patients (eight from group A, one from group B; 7/9 HSN, 2/9 IgAN) underwent a second renal biopsy at a median 1.5 years from onset, so that the effect of treatment could be assessed or because of progression of the disease. The GFR and effective renal plasma flow (ERPF) were determined by urinary clearances of inulin and para-amino-hippurate (PAH) during water diuresis [26]. Fifty children with no renal disease served as controls (Table 1).

The outcomes were categorized as: (a) normal and minor urinary abnormalities; this category included both healthy patients without urinary abnormalities and those with microalbuminuria (Ua/c 2.5–25 mg/mmol) with or without hematuria (b) Persistent mild proteinuria and GFR ≥ 94 ml/min per 1.73 m^2 (two standard deviations below the mean value of the controls); (c) active renal disease (i.e. hypertension with mean arterial pressure (MAP) > 95th percentile and/or Ua/c ≥ 200 mg/mmol and/or GFR 40–94 ml/min per 1.73 m^2; (d) chronic renal failure (CRF) (GFR ≤ 40 ml/min per 1.73 m^2) or end-stage renal disease (ESRD, requiring dialysis and/or renal transplantation). Categories a and b were considered as good outcomes and categories c and d as poor outcomes.

All renal biopsies were examined by the same pathologist (M.P.S.) and classified in accordance with the recommendations of the ISKDC [27]. The specimens were also scored for signs of activity and chronicity [6, 28]. Our modified scoring system was based on mesangial matrix expansion/mesangial proliferation (0–3) and percentage of glomeruli with crescent formation (0–3) as indicators of activity, and on percentage of global glomerular sclerosis (GGS) and segmental glomerulosclerosis (SGS) (0–2) and extent of interstitial fibrosis (0–3) as indicators of chronicity. The scoring of interstitial fibrosis was doubled, due to its impact on progressive histological damage [4, 29, 30].

Statistical analysis

Statistica 7.0 (Statsoft Inc., Trulsa USA) was used for all calculations. All data were expressed as mean (standard

Table 1 Urine albumin/creatinine ratio (Ua/c), serum albumin and GFR at first visit, at 1 year after start of treatment, and at the last visit, in groups A and B. For Ua/c, serum albumin (*S-albumin*) and GFR, the *P* values are calculated for comparison with the first visit in each group, using the Wilcoxon pairs test (*n.s.* not significant)

Parameter	First visit		One year after treatment			Last visit		
Ua/c	Number	mg/mmol	Number	mg/mmol	*P* vs first visit	Number	mg/mmol	*P* vs first visit
Group A	18	739 (149–3335)	17	40 (2–587)	0.0004	17	23 (2–406)	0.0003
Group B	25	237 (4–1263)	23	18 (1–397)	0.0005	21	18 (1–1245)	0.0001
S-albumin	Number	g/l	Number	g/l	*P* vs first visit	N	g/l	*P* vs first visit
Group A	18	24 (10–35)	17	35 (27–42)	0.0003	17	39 (31–44)	0.0003
Group B	25	30 (9–45)	21	40 (13–45)	0.001	19	40 (23–47)	0.0003
GFR	Number	ml/min per 1.73 m^2	Number	ml/min per 1.73 m^2	*P* vs first visit	Number	ml/min per 1.73 m^2	*P* vs first visit
Group A	18	77*** (4–120)	17	108 (23–143)	0.023	17	104** (7–133)	n.s.
Group B	25	100* (2–147)	22	112 (10–164)	n.s.	21	102*** (10–139)	n.s.

***$P<0.001$, **$P<0.01$, *$P<0.05$ vs control subject value of 116 (98–140) ml/min per 1.73 m^2 body surface area (Mann–Whitney U test)

deviation) or median (minimum–maximum) if not normally distributed. Comparisons between groups were made with Student's *t*-test or Mann–Whitney U test, and repeated measurements with Wilcoxon's matched pairs test. A chi-square test was used for comparisons of proportions. $P<0.05$ was considered statistically significant.

Results

There were no differences in any variable [GFR, ERPF, serum albumin, MAP, Ua/c, urine immunoglobulin G/creatinine ratio (UIgG/c)] at any time point between IgAN and HSN patients. Figure 1 illustrates the Ua/c during follow-up. Thus, we have pooled the results from both disease populations.

Group A ($n=18$) treatment with MP and CYC-P in combination with ACEi/ARB

After three pulses of MP intravenously, the patients' Ua/c ratios decreased from a median of 739 (149–3335) mg/mmol to 293 (77–1647) mg/mmol, $P=0.0012$ (Table 1). After the patients had received additional CYC-P, their Ua/c ratios decreased further to 99 (2–1094) mg/mmol, $P=0.00002$ compared with the first visit. This effect was maintained at 1 year after start of treatment and at the last visit. Figure 1a shows that the effects of treatment were similar in IgAN and HSN patients. UIgG/c decreased from a median of 29 (0.1–69) mg/mmol to 7 (1–48) mg/mmol, $P=0.022$, after treatment with MP and CYC-P, and this response was maintained at 1 year and at the last visit. Serum albumin increased significantly through the follow-up (Table 1). GFR increased from a median 77 (4–120) ml/min per 1.73 m^2 to 105 (8–133) ml/min per 1.73 m^2 after treatment, $P=0.02$, and remained normal during follow-up (Table 1). ERPF (data not shown) was normal at onset and did not change significantly during follow-up. MAP was normal at onset and did not change during the follow-up.

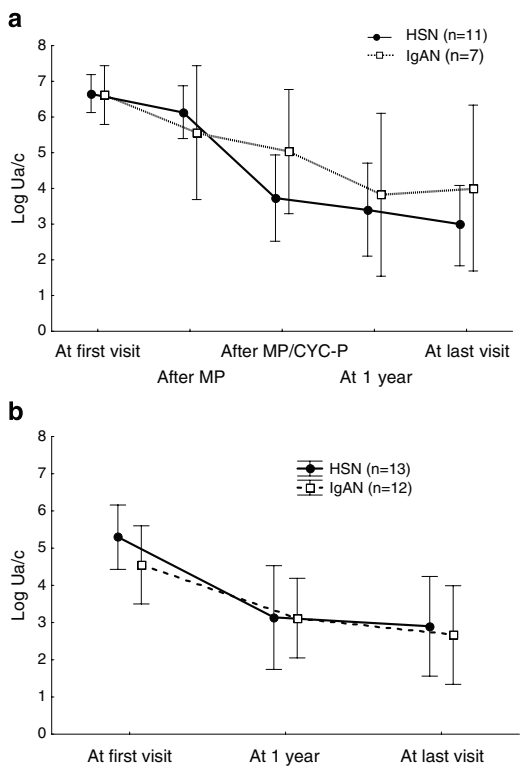

Fig. 1 **a** Urine albumin/creatinine ratio in 11 HSN and 7 IgAN patients in group A during follow-up. *MP* methylprednisolone, *CYC-P* cyclophosphamide. **b** Urine albumin/creatinine ratio in 13 HSN and 12 IgAN patients in group B during follow-up. Data are presented as means with 95% confidence intervals

Group B ($n=25$) treatment with ACEi/ARB with or without corticosteroids

Ua/c decreased and serum albumin increased significantly during follow-up (Table 1). Figure 1b shows that the effects of treatment on Ua/c were similar in HSN and IgAN patients. However, patients treated with ACEi/ARB and corticosteroids had a higher Ua/c at first visit, a median 391 (44–1,263) mg/mmol, than patients treated with ACEi/ARB alone, a median 44 (4–778) mg/mmol, $P=0.012$. The ACEi/ARB- and corticosteroid-treated group showed a greater fall in Ua/c (a median decrease of 286 mg/mmol, 73% of the first value) than the group not treated with corticosteroid (a median decrease in Ua/c of 27 mg/mmol, 61% of the first value). GFR was unchanged during follow-up.

Renal biopsy

ISKDC ≥ grade III was found in 94% of patients in group A and in 72% in group B. Group A patients had significantly more crescents, a median 22 (0–67)%, than group B, a median 0 (0–65)%, $P=0.0007$, and the mean activity index was 2.72±0.75 in group A, significantly higher than the 1.64±1.29 in group B, $P=0.0027$.

In the nine patients that had undergone repeat biopsy, comparisons between the first and second renal biopsy showed that the activity index had declined from 3.11±0.8 to 1.55±1.5, $P=0.0008$. On the other hand, the chronicity index had increased from 2.11±1.8 to 4.33±2.2, $P=0.008$, with the SGS having increased from a median of 11% to 31%, $P=0.049$, and the GGS from a median 0% to 20%, $P=0.036$.

Patients who developed end stage renal disease

The rate of development of ESRD was 14% (four IgAN and two HSN). Table 2 shows the clinical parameters in the six patients (four boys) who developed ESRD in comparison with the 37 who did not. In summary, the ESRD patients had lower GFR and higher Ua/c and U IgG/c at the first visit and at the follow-up visits than did the group that did not have ESRD. All ESRD patients had been over 9 years old at onset, and all except one patient had severe features at onset. The renal biopsy results showed ISKDC grade III in two patients, ISKDC IV in three patients and ISKDC V in one patient. The six patients who had progressed to ESRD had higher a percentage of SGS (median 33) in the first biopsy than did the patients without ESRD (median 8), $P=0.014$.

Outcome

Of the 43 patients, 26 (60%) had a good outcome (9/18 in group A, 17/25 in group B) and 17 (40%) had a poor outcome (9/18 in group A, 8/25 in group B). A total of 18 patients (42%) had a complete recovery (7/18 in group A, 11/25 in group B) and were healthy at the last visit. Age at onset did not predict outcome.

The clinical picture at onset progressed in 14 patients to the time of the first renal biopsy. All five patients with a primary presentation of hematuria had developed proteinuria at the time of renal biopsy, and seven patients with mild proteinuria at onset had progressed to nephritic, nephrotic or nephritic–nephrotic syndrome at renal biopsy, and an additional two patients from nephritic or nephrotic syndrome to a mixed form. In view of this progressive worsening of clinical features between onset and the time of renal biopsy, we have chosen to present the outcome in relation to clinical features at the time of renal biopsy (Table 3). Sixteen of 29 patients (55%) with severe clinical features had a good outcome compared with 10/14 (71%) of the patients with mild clinical features [P not significant (n.s.)].

Table 3 also gives the outcome in relation to biopsy findings. The duration of the disease before the biopsy did not differ between the group with good (1.4±2.4 years) and with poor (0.4±0.75 years, $P=0.058$) outcome. Of all the renal biopsies, 81% showed advanced findings

Table 2 Patients who had progressed to ESRD vs those who had not. GFR and proteinuria at the first visit, at 1 year after start of treatment, and at the last visit.

Parameter	With ESRD ($n=6$) median (range)	Without ESRD ($n=37$) median (range)	P
GFR, ml/min per 1.73 m^2	42 (2–73)	98 (4–147)	0.004
Ua/c, mg/mmol	1,255 (568–1,754)	388 (4–3,335)	0.006
UIgG/c, mg/mmol	47 (13–83)	6 (0–69)	0.02
At 1 year after treatment	$n=5$	$n=35$	
GFR, ml/min per 1.73 m^2	32 (10–68)	114 (54–164)	0.0007
Ua/c, mg/mmol	316 (4–587)	18 (1–397)	0.02
UIgG/c, mg/mmol	11 (0–32)	2 (0–19)	0.03
At the last visit	$n=5$	$n=33$	
GFR, ml/min per 1.73 m^2	11 (7–20)	106 (60–139)	0.0004
Ua/c, mg/mmol	388 (62–1,245)	14 (0–247)	0.002

Table 3 Outcome in relation to clinical features at the time of renal biopsy and to ISKDC grading at the first renal biopsy

Clinical features at biopsy	Outcome groups				
	Number	a	b	c	d
Group A	18	7	2	5	4
Mild	1	0	0	1	0
Severe	17	7	2	4	4
Group B	25	11	6	6	2
Mild	13	6	4	3	0
Severe	12	5	2	3	2
		Good outcome (a+b) 26		Poor outcome c+d) 17	
Mild features at biopsy		10		4	
Severe features at biopsy		16		13	
Biopsy grade (ISKDC) Outcome groups					
Group A	18	7	2	5	4
ISKDC < III	1	1	0	0	0
ISKDC ≥ III	17	6	2	5	4
Group B	25	11	6	6	2
ISKDC < III	7	5	1	1	0
ISKDC ≥ III	18	6	5	5	2
		Good outcome (a+b) 26		Poor outcome (c+d) 17	
ISKDC < III		7		1	
ISKDC ≥ III		19		16	

χ^2 P=n.s.

(ISKDC ≥ III). Nineteen of 35 (54%) of these had a good outcome in comparison with 7/8 (87%) with milder renal biopsy findings (ISKDC < III), P=n.s.. Thus, neither clinical features at renal biopsy nor renal biopsy grade significantly predicted outcome. However, patients with poor outcome had significantly lower GFR at the first visit and lower GFR, higher MAP and Ua/c, and lower serum albumin (S-albumin) 1 year after treatment than did those with a good outcome. Comparing renal biopsy findings between the groups we found a higher percentage of SGS and a higher amount of interstitial fibrosis and interstitial inflammation in the group of patients with poor outcome (Table 4).

There was no significant difference in outcome between the HSN group and the IgAN group. Fifteen of 24 (63%) patients in the HSN group and 11/19 (58%) in the IgAN group had a good outcome. Neither the clinical features nor the ISKDC grading could predict outcome in these two groups.

Discussion

Severe HSN and IgAN in childhood are not common, and there is no consensus on treatment strategies. The aim of this study was to describe our experience at a single center of the

Table 4 Clinical features at first visit, at 1 year after start of treatment and first renal biopsy findings in relation to outcome

Clinical features	Good outcome (n=26) Median (range)	Poor outcome (n=17) Median (range)	P
At first visit			
GFR, ml/min per 1.73 m^2	115 (4–147)	62 (2–100)	0.00026
One year after treatment			
GFR, ml/min per 1.73 m^2	119 (80–164)	68 (10–127)	0.00002
MAP, mmHg	78 (58–96)	86 (68–105)	0.0085
Ua/c, mg/mmol	13 (1–397)	116 (2–587)	0.0048
Serum albumin, g/l	40 (25–45)	33 (13–41)	0.004
Renal biopsy findings			
SGS, %	0 (0–33)	22 (0–75)	0.0002
Interstitial fibrosis	0 (0–1)	1 (0–2)	0.002
Interstitial inflammation	0 (0–2)	1 (0–2)	0.0125

effects of treatment in 43 cases of severe HSN and IgAN in a 10-year follow-up study. The study was retrospective and, therefore, has its limitations. The patients' data were retrospectively analyzed and grouped according to the treatment given. The study groups both differed and overlapped in the severity of clinical features and renal biopsy morphology, and therefore no comparisons to prove efficacy of the different treatments can be made. The treatment tradition has also been changed over time. However, some important observations can be made. Intravenous treatment with MP was associated with a decline in Ua/c, and, after additional oral treatment with corticosteroids and CYC-P in combination with ACEis and ARBs, GFR increased and both Ua/c and UIgG/c decreased further. In the group not treated with CYC-P, the Ua/c was also reduced, while the GFR remained unchanged through the follow-up.

Although several questions are left unsolved regarding the indication and timing of the treatment, many investigators emphasize the importance of early treatment in severe IgAN and HSN, as the morbidity rate is high among patients with ISKDC grades ≥ III and nephrotic range proteinuria [11, 17, 28, 31]. We found a decrease in proteinuria and an increase in GFR following the corticosteroid treatment, and, in group B, 68% of the patients had a good outcome at the last visit. The decline in proteinuria and preservation of GFR after treatment with corticosteroids in severe IgAN and HSN have been shown by others [11–13]. The further decline in Ua/c after additional CYC-P therapy found in our study has also been shown in small series of high-risk patients [8–10, 15, 16]. On the other hand, CYC-P as single therapy was not shown to be more effective than supportive therapy [32]. In our study 50% of the patients treated with corticosteroids and CYC-P had a good outcome after a median 3.1 years, and no severe side effects were seen.

In our study neither the clinical features (mild or severe) nor the ISKDC grade of the biopsy (< III or ≥ III) could predict outcome, which has also been reported by other authors [2, 16, 27, 31, 33]. An early biopsy is, however, informative and of great importance for the further decision to treat. A high proportion of crescents in the first renal biopsy indicated treatment but did not predict poor outcome in our study. Instead, we found that a high percentage of SGS was more frequent among patients with poor outcome. Several investigators have reported that the number of crescents in the biopsy have a strong relation to outcome [15, 16, 34], while others have indicated that the predictive role of crescents is more controversial [1, 3, 35]. In our study, one patient who was anuric and had 67% of crescents in the first biopsy was treated as in group A and underwent peritoneal dialysis for 5 days. The patient recovered and 3 months later, he had normal GFR and blood pressure and only microalbuminuria. Four years after onset he is still healthy and takes no medication. In contrast, another patient who had nephrotic proteinuria, reduced GFR and a high percentage of SGS in the first biopsy, which were all resistant to intervention, went to ESRD within 6 months, despite aggressive treatment.

Fourteen percent of the patients progressed to ESRD, which is in agreement with other reports [15, 32, 33, 36]. Those patients had a lower GFR, greater proteinuria, and more severe morphological findings in their first renal biopsy, as has been reported in other studies [1, 4, 35, 37, 38].

In conclusion, we emphasize the value of an early biopsy in patients with severe clinical features. Treatment with MP and CYC-P in combination with ACEi/ARB, and treatment with corticosteroids in combination with ACEi/ARB, was efficient in reducing proteinuria and improving GFR in both HSN and IgAN patients. Although further studies are needed to determine the role of immunosuppression, our study shows that 50% of the patients undergoing the combined treatment had a good outcome at follow-up, despite severe clinical features and advanced renal biopsy findings.

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ORIGINAL ARTICLE

Predictors of outcome in Henoch–Schönlein nephritis

Stella Edström Halling · Magnus P. Söderberg · Ulla B. Berg

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Abstract Factors predictive of renal outcome were studied in 78 children with Henoch–Schönlein nephritis followed up for as long as 17 (mean 5.2) years. Patients with a good outcome (74%) were healthy or had microalbuminuria or mild proteinuria at the final follow-up (FU), and those with poor outcome (26%) had active renal disease or chronic kidney disease at stages IV–V. Patients with mild symptoms at onset (hematuria ± mild proteinuria) had a poor outcome in 15% of cases versus 41% of those with severe symptoms (nephritic or nephrotic syndrome or nephritic-nephrotic picture) (p=0.011). However, among patients with mild proteinuria at onset, 18% showed a poor prognosis; non-nephrotic proteinuria with a urine albumin/creatinine ratio at a cut-off value of >144 mg/mmol at the 1-year FU was predictive of a poor outcome. Among 59 biopsied patients, 37% of those with advanced histological findings [International Study of Kidney Disease in Children (ISKDC) stages III–V] had a poor outcome compared to none of those with mild findings (ISKDC stages I–II) (p=0.0015). Patients with a poor outcome were older at onset, had more proteinuria, and lower glomerular filtration rate at the 1-year FU compared with patients with a good outcome. Multivariate analysis showed that proteinuria at the 1-year FU and the ISKDC grading score of the renal biopsy were the two most discriminant factors of a poor prognosis.

Keywords Glomerular filtration rate · Outcome · Predictors · Proteinuria · Renal biopsy findings

S. Edström Halling (✉) · U. B. Berg
Department of Clinical Science, Intervention and Technology,
Division of Pediatrics,
Karolinska Institutet, Astrid Lindgren Children´s Hospital,
Karolinska University Hospital Huddinge,
141 86 Stockholm, Sweden
e-mail: stella.edstrom.halling@ki.se

M. P. Söderberg
Department of Laboratory Medicine, Division of Pathology,
Karolinska Institutet–Karolinska University Hospital,
Huddinge, 141 86 Stockholm, Sweden

Introduction

Henoch–Schönlein purpura (HSP) is the most common vasculitis in childhood, with an estimated annual incidence of 10–20/100 000 children [1–3]. It is an acute leucocytoclastic vasculitis, and although it is generally considered to be a self-limiting disease in the majority of cases, the long-term prognosis depends on the severity of renal involvement. Henoch–Schönlein nephritis (HSN) occurs in 35% (range 20–60%) of patients with HSP [2, 4–7]. In unselected cohorts of patients with HSP, the estimated risk of end stage renal disease (ESRD) is around 2% [1, 8]. In contrast, morbidity is low in patients with HSN who have hematuria and mild proteinuria at onset, while it is higher among those with more severe renal disease, as in a nephritic, nephrotic or a nephritic/nephrotic onset [9–12]. The risk of patients selected for treatment at tertiary centres developing ESRD is 5–20% [12–16]. Given the urgent need to reduce disease progression to ESRD in these patients, we retrospectively studied 103 children with HSN with the aim of identifying prognostic factors of a poor outcome.

Materials and methods

Our clinic is one of two referral centers for pediatric renal diseases in Sweden that treats patients with acute and chronic

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renal diseases, including those requiring a renal biopsy. A total of 103 patients (51 boys), mainly referral patients, with a median age 8.9 years (range 2.0–17.1 years) at onset were included in the study which covered the period between January 1995 and March 2008. All patients were investigated at our clinic within 1.5 years (median 0.34 years) from disease onset. Of the 103 patients, 38 were lost to late (>1.5 years) follow-up (FU). An additional 13 patients referred to our clinic later than 1.5 years after disease onset and one patient who reached terminal renal failure within 0.7 years from onset was also included in the late-FU group. Therefore, we had a late study group of 78 patients who were investigated within 5.2±3.2 years from onset; 44 of these patients were also studied 1 year from onset.

The patients were subdivided into five classes according to the renal manifestations at onset [4]: (1) micro- or macroscopic hematuria; (2) persistent mild proteinuria [<1 g/L or urine albumin/urine creatinine ratio (Ua/c) < 200 mg/mmol] ± hematuria; (3) nephritic syndrome, i.e. moderate proteinuria (Ua/c ≥ 200–400 mg/mmol), hematuria, raised serum creatinine and/or hypertension [17]; (4) nephrotic syndrome: urinary albumin excretion >40 mg/h/m^2 or Ua/c > 400 mg/mmol, serum albumin <25 g/L and/or oedema; (5) mixed nephritic/nephrotic syndrome at onset. Classes 1 and 2 were defined as mild clinical features and classes 3–5 as severe.

The glomerular filtration rate (GFR) was determined at admittance and during the FU by renal clearance of inulin during water diuresis [18] or by plasma clearance of iohexol using the slope curve. A group of 50 healthy children aged 10.1±4.6 years served as controls; their mean GFR was 116±11 ml/min/ 1.73 m^2. Blood pressure (BP) was measured with an oscillometric device (Dinamap, Newport, RI). Systolic (SBP), diastolic (DBP) and mean arterial blood pressure (MAP) were measured and then adjusted for gender, age and height. Hypertension was defined according to recommendations from the Working Group of National High Blood Pressure Educational Program [17]. Of the 103 patients, 70 had a renal biopsy performed within 1.4±0.7 years from onset, and 59/78 patients in the late study group were biopsied. The biopsies were all examined by the same pathologist (MPS) and analysed in accordance with the recommendations of the International Study of Kidney Disease in Children (ISKDC) [19]. Thirty of the 78 patients in the late-FU group (38%) were treated: 13 with methylprednisolone pulses followed by oral prednisolone and 14 with additional cyclophosphamide (CYC-P); all 30 received angiotensin converting enzyme inhibitors (ACEi) and/or angiotensin II receptor blockers (ARB). Our treatment indications have been discussed in a previous article [20]. The most severe cases were treated. Compared to the untreated group, the treated patients had higher Ua/c (p=0.0006) and urine immunoglobulin G/c (UIgG/c; p = 0.018) ratios and lower GFR (p=0.029) at onset and a higher ISKDC grading score (p=0.002) in the biopsy. The mean FU time in the treated group was 4.8±3.1 years and in the non-treated group 5.4± 3.3 years. Twenty-one of the 29 patients with crescents were treated: nine with methylprednisolone and oral steroids and 12 with additional CYC-P.

The patients were categorized into four groups based on outcome: A, no or minor urinary abnormalities, i.e. healthy patients and those with microalbuminuria (Ua/c 2.5–25 mg/mmol) with or without hematuria; B, persistent mild proteinuria (Ua/c > 25–200 mg/mmol) and normal GFR; C, active renal disease, i.e. Ua/c > 200 mg/mmol and/or GFR 31–93 ml/min/1.73 m^2 and/or hypertension; D CKD stages IV–V, GFR≤30 ml/min/1.73 m^2 or ESRD requiring dialysis and/or renal transplantation. Categories A and B were defined as good outcome and categories C and D as poor.

The study was approved by the Ethics Committee at the Karolinska Institutet.

Statistical analysis

Statistica 7.0 (Statsoft, Tulsa, OK) was used for all calculations. Variables were expressed in terms of the mean and standard deviation (SD) or, if the data were skewed, as the median and minimum–maximum. The Ua/c was log transformed to obtain normal distributed data. Comparisons between groups were made with Student's t test for normally distributed data and with the Mann–Whitney U test for data with skewed distribution. The chi-square test was used for comparisons of proportions, i.e. categorical variables. Linear regression was used to explore the linear relation between continuous variables and the Spearman correlation between ordinal variables or combination ordinal and continuous variables. Logistic regression (univariate model) was used to explore the discriminators of poor outcome compared to good outcome. The logistic regression model estimates the cut-off level where the odds for a poor outcome exceed 50%. The predictive value of the model against the observed data was evaluated as percentage agreement.

The odds ratios (OR) and their corresponding 95% confidence intervals (CIs) were calculated. A multivariable model was constructed including a combination of the identified predictors. A p value <0.05 was considered to be statistically significant.

Results

The clinical picture at onset in the 103 patients showed that 12% patients had isolated hematuria, 40% had mild

proteinuria ± hematuria, 18% had acute nephritic syndrome, 14% had nephrotic syndrome and 16% had a mixed nephritic–nephrotic picture. Thus, 48% of the patients had severe clinical features at onset. There were no significant sex or age differences between the clinical groups at onset.

Mean GFR at the first investigation (GFR:first) was 100±29 ml/min/1.73 m^2, which was significantly lower than that of controls (p=0.0002). One patient was anuric at onset, and 35% had a GFR:first below −2SD of that of the controls. The GFR:first correlated inversely with age at onset, log Ua/c and UIgG/c at the first investigation, log Ua/c at the 1-year FU (log Ua/c 1y), percentage crescents (CRE%), degree of mesangial proliferation (MP) and interstitial inflammation (II) and the ISKDC grading score (Table 1).

Mean GFR at the last investigation (GFR:last) was 105 ± 27 ml/min/1.73 m^2, which was significantly lower than that of controls (p=0.004). Seventeen patients (22%) had a GFR below −2SD of that of controls, and 26 patients reduced their GFR between the first and last investigation (ΔGFR) −3.4 (range −31 to −0.22) ml/min/1.73 m^2 per year. Two patients reached ESRD after 0.7 and 5 years, respectively, and one had a GFR of 20 ml/min/1.73 m^2 at 5.2 years after onset. No deaths occurred.

The GFR:last correlated inversely with age at onset, log Ua/c and log UIgG/c and systolic hypertension at first investigation, log Ua/c 1y, percentage segmental sclerosis (SGS), degree of MP, II, interstitial fibrosis (IF) and the ISKDC grading score (Table 1). The GFR:last correlated directly with GFR:first (R=0.406, p=0.008) and with GFR at 1 year (R=0.518, p=0.003).

Prognostic factors and clinical findings

The relationship between clinical symptoms at onset and outcome is presented in Table 2, which shows that 58/78 (74%) of the patients had a good outcome (A + B) and that 61% of the patients were healthy or had microalbuminuria (A) at the last follow-up. In the group with mild symptoms at onset, 33/46 (72%) achieved a complete recovery, compared to 15/32 (47%) of those with severe symptoms at onset (p=0.026). Of the 22 patients with nephrotic or nephritic-nephrotic picture, 15 (68%) had a good outcome, with 13 of the 22 (59%) achieving complete recovery. There was no significant difference in outcome between patients with nephrotic versus non-nephrotic proteinuria at onset.

Of the 78 patients, 20 (26%) had a poor outcome (C + D) at the last investigation. Only 1/12 (8%) patients with hematuria at onset had a poor long-term outcome, but the corresponding figure for patients with mild proteinuria was 6/34 (18%, p=nonsignificant). Thirteen of the 32 patients (41%) with severe symptoms at onset had a poor outcome (C + D) compared to 7/46 (15%) of those with mild

Table 1 Correlations between clinical features, biopsy findings and renal function at the first and last investigation

Clincal/biopsy findings	GFR:first				GFR:last			
	n	*r*	*p*	Adjusted r^2	*n*	*r*	*p*	Adjusted r^2
Clinical features[a]								
Age at onset	103	−0.213	0.031	0.045	78	−0.373	0.0008	0.139
Log Ua/c first investigation	100	−0.420	0.00001	0.176	64	−0.295	0.018	0.087
Log UIgG/c first investigation	81	−0.456	0.00002	0.209	52	−0.299	0.031	0.090
Systolic hypertension	30	−0.316	0.089	0.099	22	−0.846	0.000001	0.715
Log Ua/c 1-year FU	62	−0.432	0.0005	0.186	44	−0.495	0.0006	0.245
Biopsy findings								
CRE%[a]	70	−0.334	0.005	0.012	59	−0.231	0.079	0.053
SGS%[a]	70	−0.200	0.097	0.004	59	−0.271	0.038	0.073
MP[b]	70	−0.429	0.0002		59	−0.348	0.007	
II[b]	70	−0.302	0.01		59	−0.262	0.045	
IF[b]	70	−0.109	0.368		59	−0.346	0.007	
ISKDC grading[b]	70	−0.308	0.009		59	−0.334	0.01	

r, Regression coefficient; log Ua/c, log urine albumin/urine creatinine ratio; log UIgG/c, log urine immunoglobulin G/urine creatinine ratio; GFR, glomerular filtration rate; CRE%, percentage of glomeruli with crescents; SGS%, percentage with segmental glomerulosclerosis; MP, mesangial proliferation; II, interstitial inflammation; IF, interstitial fibrosis; ISKDC, International Study of Kidney Disease in Children; FU, follow-up

[a] Linear regression was used between continuous variables i.e. for all clinical variables and CRE% and SGS%

[b] Spearman´s correlation was used between ordinal variables i.e. MP, II, IF and ISKDC grading

Table 2 Outcome in relation clinical features at onset

Symptoms	Categories of patients based on clinical features at onset (n = 78 patients)[a]			
	A	B	C	D
Mild symptoms:				
Hematuria	11	0	1	0
Mild proteinuria ± hematuria	22	6	5	1
Severe symptoms:				
Acute nephritic syndrome	2	2	6	0
Nephrotic syndrome	5	0	3	0
Nephritic–nephrotic syndrome	8	2	2	2
Total	48 (61%)	10 (13%)	17 (22%)	3 (4%)

Ua/c, Urine albumin/urine creatinine ratio; CKD, chronic kidney disease

Number of patients per category per symptom are given

[a] A, Patients had no or minor urinary abnormalities, i.e. healthy patients and those with microalbuminuria (Ua/c 2.5–25 mg/mmol) with or without hematuria; B, patients had persistent mild proteinuria (Ua/c > 25–200 mg/mmol) and normal GFR (≥94 ml/min/1.73 m^2); C, patients with active renal disease, i.e. Ua/c > 200 mg/mmol and/or GFR 31–93 ml/min/1.73 m^2 and/or hypertension; D, patients at CKD stages IV–V

symptoms (p=0.011). Three of the 78 patients (4%) had CKD IV–V (outcome D) at the last visit. Comparisons between mild and severe symptoms at onset in relation to outcome remained significant even if the FU was >3 years (57 patients) (p=0.03), significance was lost when the FU was >5 years (31 patients).

There was no statistical difference in outcome between patients who had normal GFR at onset or 1 year and those with impaired GFR at these same time points. Figure 1 shows GFR:last in relation to FU in patients with different levels of proteinuria at the first investigation. Patients with micro- or no albuminuria had—with few exceptions (three patients)—normal levels of GFR:last, whereas GFR:last was impaired in 14 patients with proteinuria levels above microalbuminuria.

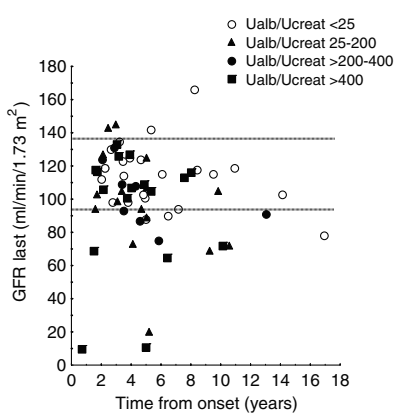

Fig. 1 Glomerular filtration rate at last investigation (*GFR:last*) in relation to time of follow-up and grade of proteinuria at the first investigation. *Ualb/Ucreat* Urine albumin/urine creatinine ratio (mg/mmol). *Ualb/Ucreat <25* Normo- or microalbuminuria, *Ualb/Ucreat 25–200* mild albuminuria, *Ualb/Ucreat >200–400* moderate albuminuria, *Ualb/Ucreat >400* nephrotic albuminuria

At the 1-year FU, patients with a poor outcome were older at disease onset (10.9±3.7 vs. 8.9±3.4 years, p= 0.036) and had a higher Ua/c 1 y [mean 179 (range 0–568) vs. 10 (0–160) mg/mmol, p=0.002] and lower GFR (91±33 vs. 107±17 ml/min/1.73 m^2, p=0.047) than patients with a good outcome. There was no difference in outcome between patients who were normotensive and those who were hypertensive (n=22) at the first investigation.

Prognostic factors and biopsy findings

Signs of acute lesions, such as CRE, were detected in 49% of the biopsies, mesangial matrix expansion (MM) in 58% and MP in 98%; in comparison, chronic lesions, such as global glomerulosclerosis (GGS), were detected in 32% of the biopsies, SGS in 44%, II in 41% and IF in 44%. IF was mild to moderate (grade 1–2), and there were no cases of advanced (grade 3) IF findings. Table 3 presents the outcome in relation to biopsy findings. The time from onset to biopsy did not differ between the outcome groups.

ISKDC III was the most common biopsy finding, diagnosed in 42% of the cases. Advanced findings (III–V) were found in 64% of the patients, of whom 37% had a poor outcome; in comparison, none of those with mild histology (I–II) had a poor outcome (p=0.0015). Comparison between advanced and mild histological findings in relation to outcome remained significant even if the FU was ≥3 or ≥5 years (p=0.002 for both groups). The outcome in relation to individual lesions showed that patients with SGS

Table 3 Outcome in relation to biopsy findings

Histology findings	Categories of patients based on clinical features at onset (n = 59 biopsies)[a]			
	A	B	C	D
Mild histology findings				
ISKDC I	4	2	0	0
ISKDC II	13	2	0	0
Advanced histology findings				
ISKDC III	15	4	5	1
ISKDC IV	4	0	5	1
ISKDC V	0	1	1	1
Total	36 (61%)	9 (15%)	11 (19%)	3 (5%)

[a] For definition of clinical classification of patients at onset, see text and footnotes to Table 2

had a poorer outcome than those without SGS (p=0.018), but a comparison of patients with crescents to those without (p=0.056) or those with GGS to those without (p=0.747) revealed no difference in outcome. Moreover, patients with poor outcome had a higher degree of MM (2–3 vs. 0–1, p=0.005), MP (2–3 vs. 0–1, p=0.002), II (1–2 vs. 0, p=0.007) and IF (1–2 vs. 0, p=0.018) than did those with a good outcome. Log Ua/c at the first investigation correlated to ISKDC grading score (r=0.399, p=0.0008), MM (r=0.363, p=0.0024) and MP (r=0.452, p=0.0001), whereas the log Ua/c 1y correlated to ISKDC grading score (r=0.504, p=0.0003), MP (r=0.512, p=0.0002) and II (r=0.483, p=0.0006).

Of the 30 treated patients, 12 (30%) had a poor outcome compared to 8/48 (16%) of the untreated patients (p=0.02), and no significant difference in outcome was found between the treated and untreated patients with crescents. Neither was there any significant difference in ΔGFR between treated and untreated patients.

Univariate and multivariate analysis of risk factors

The logistic regression univariate analysis identified clinical and morphological predictors of poor outcome (Table 4). Of the clinical variables, age at onset (p=0.044), SBP, clinical features at onset (p=0.017), SBP, serum albumin and log Ua/c 1y (p=0.010) discriminated between a good and poor prognosis. Hypertension at the first investigation or at the 1-year FU was not predictive. Among the morphological lesions, high amounts of MM, MP, II and IF were each identified as individual predictors and the ISKDC grading score was highly predictive (p=0.002).

Based on the results of the univariate analysis, we included four variables into the multivariate model: age at onset, clinical features at onset, log Ua/c 1y and ISKDC grading score. Adding FU-time as a co-dependent variable to each of these four variables did not change their predictive significance. According to the multivariate analysis, the combination of ISKDC grading score and log Ua/c 1y gave the highest agreement to the model (92%). However, these two variables were strongly interrelated, and only the log Ua/c 1y maintained its significance (OR 2.52, 95% CI 1.20–5.27, p=0.015). The observed cut-off level for poor outcome in terms of Ua/c 1y was estimated to be $e^{4.98}$. Thus, a level of Ua/c at 1 year above or below 144 mg/mmol discriminated between poor and good outcome with a sensitivity of 95% and specificity of 40%, (positive predictive value 82%, negative predictive value 73%).

Discussion

In our study, 41% of patients with severe symptoms at onset had a poor outcome, which is a significantly larger proportion than that among those with mild symptoms (15%). Several authors have discussed the relation between clinical symptoms at onset and prognosis [4, 9–16, 21]. Patients with isolated hematuria showed a good prognosis, both in our study and in other studies reported in a review article [11], but there are exceptions [12]. Mir et al. reported a good outcome in 91% of patients with mild proteinuria at onset [21]. In contrast, we found as many as 18% of patients with mild proteinuria at onset with a poor outcome, which is in the same range as that reported by others [10, 22]. In a previous article [23], we reported that advanced morphology can be found in biopsies from patients with mild proteinuria. Thus, the level of proteinuria at onset does not seem to be a reliable predictor. However, proteinuria during FU was of high prognostic significance in our study, which is in agreement with the results reported elsewhere [9, 24]. We found that cut-off Ua/c levels at 1 year as low as 144 mg/mmol were highly predictive of a poor outcome. Special attention was also given to patients with nephrotic syndrome or with a nephritic–nephrotic picture at onset, as such patients are known to be at risk of a poor outcome [10, 14, 19, 21, 25, 26]. Nevertheless, we found a high recovery rate among these patients, which is in accordance with the

Table 4 Logistic regression (univariate analysis) of factors that discriminate between poor and good outcome at the last visit

Clinical variables	*n*	OR (unit)	95% CI	*p*	% agr
At the first investigation					
Age at onset	78	1.17	1.00-1.37	0.044	73
Sex (male vs female)	78	1.5	0.53-4.25	0.436	
SBP, mmHg	64	1.05	1.00-1.10	0.038	80
Hypertension (yes vs no)	64	2.29	0.73-7.14	0.152	
Log Ua/c, mg/mmol	64	1.20	0.95-1.52	0.131	
Log UIgG/c, mg/mmol	52	1.39	0.92-2.11	0.114	
GFR, ml/min/1.73m^2	65	0.99	0.97–1.00	0.109	74
Clinical features at onset (severe vs mild)	78	3.81	1.29–11.31	0.017	
At 1-year follow-up					
SBP, mmHg	44	1.06	1.00–1.13	0.038	64
Hypertension (yes vs no)	44	2.27	0.46–11.29	0.307	
S-albumin, g/L	38	0.89	0.79–0.99	0.047	68
Log Ua/c, mg/mmol	44	1.69	1.14–2.48	0.010	84
Log UIgG/c, mg/mmol	30	1.85	0.84–4.06	0.120	73
GFR, ml/min/1.73m^2	44	0.97	0.94–1.00	0.077	75
Morphological variables (n=59)					
GGS%		1.06	0.98–1.00	0.120	76
SGS%		1.03	0.99–1.0	0.080	78
CRE%		1.03	0.99–1.06	0.089	76
IF 1-2 vs 0		4.53	1.19–17.28	0.027	
II 1-2 vs 0		5.54	1.44–21.33	0.014	
MM 2-3 vs 0-1		6.64	1.57–28.21	0.011	
MP 2-3 vs 0-1		7.20	1.88–27.59	0.005	76
ISKDC grading score		5.1	1.87–13.88	0.002	81

SBP, Systolic blood pressure (age-adjusted); GGS%, percentage global glomerulosclerosis; MM, mesangial matrix expansion; OR, odds ratio; CI, confidence interval, % agr, percentage agreement between observed and expected value

reports of others [15, 27]. We did not find any difference in outcome between patients with nephrotic or non-nephrotic proteinuria at onset. We also found a correlation between GFR:first and GFR:last, which is in agreement with the findings of Schärer et al. [14] who reported an initial renal insufficiency as a predictor of poor outcome. Others did not find low GFR at onset to be a predictor [9, 24], and indeed neither chi-square nor logistic regression analysis could confirm our findings of GFR:first being a predictor of poor prognosis.

We also did not find any difference between sexes in relation to outcome, which is in contrast to the findings published by a number of other authors [9, 10], who found that women ran a markedly greater risk of a poor long-term outcome.

In our study, age at onset was a predictor of poor prognosis in univariate analysis but not in multivariate analysis. Older children tend to have more severe disease at presentation [25], but age at onset has not been shown to be a predictor of poor outcome [10, 15, 19]. Age-adjusted SBP at the first investigation and at the 1-year FU were identified as predictors of a poor prognosis in the univariate analysis, but hypertension was not. Consequently, our findings are in accordance with those of Coppo et al. [9].

We found that the histology grading score was strongly predictive of outcome.

Several authors have discussed the distinction between acute and chronic lesions and the predictive importance thereof. A number of these have stated that glomerular crescents carry a prognostic significance [4, 13, 14, 19, 24, 28, 29]. However, in agreement with others [9, 30, 31], we found no such significance. Instead, the amount of segmental sclerosis correlated inversely to GFR at the last visit (Table 1), and patients with SGS had a poorer prognosis than those without this finding. In our study, the univariate analysis identified both acute lesions (MM, MP) and chronic lesions (II, IF) as predictors of renal outcome. In adult HSN patients, the detection of SGS and the degree of II and IF are predictive of renal outcome [30, 32]. However, advanced IF is a rare finding in pediatric biopsies, and the outcome is therefore more difficult to validate.

In an earlier study [20] we demonstrated that treatment with steroids and CYC-P in combination with ACEi/ARB is effective in reducing proteinuria and improving GFR in severe cases of HSN and IgA nephritis. In this study, we found that a high proportion of treated patients had a poor outcome, mainly due to the fact that treatment was given to

the majority of the severe cases. We did not find crescents to be a factor of poor prognosis, but 21/29 of the patients with crescents were treated, which may have improved the course of the disease. However, treatment was not given according to any protocol and, consequently, no conclusions on efficacy can be made.

Our study has a number of limitations. Advanced histological findings were found in 64% of the cases, which indicates that our cohort was not a random selection of patients; as such, this over- representation of advanced disease might have overestimated the poor prognosis. However, the proportion of severe cases is in the same range as in other reports of the same size [9, 28]. Secondly, the 38 patients who were lost for FU could possibly have had a high recovery rate. However, their clinical features at onset did not differ from those in the late-FU group, and none of the patients are in the Swedish Renal Registry. Thirdly, the 13 patients who were included after >1.5 years of disease duration are likely to have had a late recurrence or a severe form and, therefore, may have increased the proportion of patients with poor outcome in our study. In comparison to rates reported elsewhere [10, 12–16], our rate of CKD stage IV–V/ ESRD is still low (4%), which can be explained by a shorter FU. We conclude that 26% of the patients had a poor outcome after a mean of 5.2 years. Both severe features at onset and advanced biopsy findings were associated with poor outcome. Multivariate analysis showed that the ISKDC grading score and proteinuria at the 1-year follow-up were the best discriminators of a good and poor outcome.

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Original Article

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Predictors of outcome in paediatric IgA nephropathy with regard to clinical and histopathological variables (Oxford classification)

Stella Edström Halling[1], Magnus P. Söderberg[2] and Ulla B. Berg[1]

[1]Division of Paediatrics, Department of Clinical Science, Intervention and Technology, Karolinska Institutet, Karolinska University Hospital Huddinge, Stockholm, Sweden and [2]Division of Pathology, Department of Laboratory Medicine, Karolinska Institutet, Karolinska University Hospital Huddinge, Stockholm, Sweden

Correspondence and offprint requests to: Stella Edström Halling; E-mail: stella.edstrom.halling@ki.se

Abstract

Background. There has been a lack of international consensus on the classification and the predictive value of the histopathology findings in IgA nephropathy (IgAN). Recently, the International IgA Nephropathy Network has developed the Oxford classification in which four histological variables with the most prognostic importance are identified (MEST score). Our objective was to validate these findings and to assess their predictive power in our cohort and to compare them to identified clinical predictors.

Methods. Ninety-nine children with a follow-up time >5 years were included and investigated with clearances of inulin or iohexol for glomerular filtration rate (GFR), proteinuria and blood pressure at biopsy and during follow-up. Biopsies (90/99) were re-evaluated and scored according to the Oxford classification.

Results. Eighteen patients progressed to a poor outcome [end-stage renal disease (ESRD) or GFR reduction >50%]. In the univariate analysis, we found that mesangial hypercellullarity score >0.5, presence of endocapillary hypercellularity or tubular atrophy/interstitial fibrosis of >25% were each associated with a poor outcome, and also presence of cellular or fibrocellular crescents and of global glomerulosclerosis, but segmental glomerulosclerosis did not reach statistical significance. The clinical predictors of a poor outcome were a low GFR, a high mean arterial blood pressure and a high amount of albuminuria (log Ualb/c) at time of biopsy and low GFR and a high log Ualb/c during follow-up.

Conclusion. We found that three of the four histology lesions identified in the Oxford classification, as well as presence of crescents, were valid in predicting a poor outcome in our cohort of patients.

Keywords: glomerular filtration rate; IgA nephropathy; Oxford classification; predictors; proteinuria

Introduction

The natural history of IgA nephropathy (IgAN) shows wide variability in the clinical setting. Long-term studies of paediatric IgAN report different progression rates, with a 10-year renal survival of 87–93% and a 20-year renal survival of 73–89% [1–3]. Several clinical predictors of poor outcome have been discussed, such as age at diagnosis [4], severe proteinuria, hypertension and impaired renal function at presentation [2, 5]. Biopsies show a wide range of different stages of pathology from nearly normal histology, apart from deposits of IgA in the glomeruli, to severe pathology findings with crescentic or sclerotic/necrotic lesions. There has been a lack of international consensus in the validation of previous histopathological classifications [6, 7]. In the new Oxford classification (Oxford MEST score), the International IgA Nephropathy Network has identified four histopathological lesions of independent prognostic importance; mesangial (M) and endocapillary (E) hypercellularity, segmental glomerulosclerosis (S), tubular atrophy and interstitial fibrosis (T). The results were analysed using a systematic approach in order to develop a reproducible classification which could predict clinical outcome [8, 9]. The Oxford classification score has been shown to be valid also in paediatric IgAN [10]. Our aim was to assess the predictability of these findings and clinical variables among our IgAN patients to improve the prognostic evaluation.

Materials and methods

Our clinic is one of two referral centres for children with kidney diseases in Sweden. We studied retrospectively and prospectively 99 consecutive children and adolescents (58 boys) with a follow-up period of >5 years up to 36 (mean 13 ± 8 years, $n = 92$) or had reached end-stage renal disease (ESRD) earlier than after 5 years ($n = 7$). Their mean age was 12 ± 3.6 years at the first investigation. Two patients died due to non-renal causes. Forty-seven patients (47%), 29 boys, were followed for >10 years and 19 patients (19%), 9 boys, for >20 years. The patients were investigated with clearances of inulin during water diuresis or plasma clearance of iohexol to determine glomerular filtration rate (GFR) [11] at time of biopsy and at 1, 3 and 5 years of follow-up and at the last visit. Fifty-nine

healthy children aged 11.7 ± 3.6 years served as controls for GFR. Clinical baseline characteristics included weight, height, serum creatinine, proteinuria and blood pressure measurements adjusted for gender, age and height. Systolic and diastolic hypertension was defined as above the 95th percentile [12]. Proteinuria was measured as the ratio of urine albumin/creatinine (Ualb/c), defined as normoalbuminuric or microalbuminuric if Ualb/c was <25 mg/mmol, mild if the ratio was 25–200 mg/mmol, moderate if ≥200 to <400 mg/mmol and nephrotic if ≥400 mg/mmol.

A renal biopsy was performed in all 99 children during 1974–2007. One-third of the cohort (31/99) was biopsied after the year 2000. Three of the late biopsied patients deteriorated to ESRD within half a year and they were included in the study. Ninety specimens were available for review. The biopsies were performed at a median of 1.6 [interquartile (IQ) range 0.5–4.2] years of disease duration and were examined by one pathologist (M.P.S.), who was blinded to patient outcome at the time of scoring. The biopsies were analysed based on 3 μ Periodic acid Schiff-stained sections. There was a median of 16 (IQ range 10–23) glomeruli in each biopsy and no specimen had <5 glomeruli. The biopsy specimens were classified and standardized according to the Oxford classification [8], in which the number of glomeruli was assessed and their mesangial hypercellullarity scored as ≤0.5 or >0.5, if more or less than half of the glomeruli showed hypercellullarity defined as >4 mesangial cells/mesangial area, endocapillary hypercellullarity (absent or present), segmental glomerulosclerosis (absent or present) and tubular atrophy/interstitial fibrosis (TAIF) (0 = 0–25%, 1 = 26–50% and 2 = >50%). The percentage of glomeruli with cellular and fibrocellular crescents and global glomerulosclerosis (GGS) was calculated and was classified as absent or present.

Treatment was recorded in three different groups: (i) renin angiotensin aldosterone system (RAAS) blockade, indicating treatment with ACEis (angiotensin-converting enzyme inhibitors) or ARBs (angiotensin receptor blockers) or both, $n = 24$, (ii) immunosuppression with corticosteroids in combination with RAAS blockade, $n = 4$ and (iii) immunosuppression with corticosteroids and cyclophosphamide in combination with RAAS blockade, $n = 7$. Seven patients had received treatment with RAAS blockade prior to biopsy at a median of 0.13 years from onset and two of these patients had also received corticosteroids prior to biopsy. Treatment during follow-up was given on clinical indications and did not follow any protocol.

The primary outcome was defined as poor if GFR:last was reduced >50% from GFR:bio or if the patient had reached ESRD.

Statistical analysis

Statistica 9.0 (Statsoft Inc., Tulsa, OK) was used for all statistical analyses. Variables were expressed as the mean and SD for continuous and approximately normally distributed variables and the median and IQ range for skewed data. The Ualb/c was log-(e)-transformed to obtain approximately normally distributed data. To test for differences between groups, Student's *t*-test was used for approximately normally distributed data and the Mann–Whitney *U*-test for data with a skewed distribution. The chi-square test was used to test for differences between proportions, i.e. categorical variables. The Spearman correlation coefficient was used to explore the relationship between the presence of each histological lesion and treatment and between one histological and one clinical variable and the Pearson's correlation coefficient was used for the relationship between two histological lesions (Table 4). The Kaplan–Meier curve was used to illustrate univariate differences between groups of clinical and pathological variables, with respect to time to poor outcome. Cox proportional hazard regression was used to estimate the hazard ratio (HR) between groups (levels) within each factor, by univariate comparisons. Estimates were presented with their corresponding 95% confidence intervals (CIs). Multiple hazard regression was used to estimate a model including a combination of the independent factors of interest and was also used to explore which factors were overall the most important discriminators for time to poor outcome. All tests were two-sided and a P-value <0.05 was considered as statistically significant.

Results

Clinical predictors

GFR at the time of biopsy (GFR:bio) was 100 ± 31 mL/min/1.73m^2, significantly lower than that of controls, GFR 117 ± 10 mL/min/1.73m^2, ($P < 0.001$). Seventy-two patients had a GFR:bio >90 mL/min/1.73m^2 (chronic kidney disease, CKD Stage 1) and 25 patients had a reduced GFR:bio: 16 patients had CKD Stage 2, 4 patients CKD Stage 3, 3 patients CKD Stage 4 and 2 patients had CKD Stage 5 (ESRD) at the time of biopsy. In two cases, the GFR was not measured at the time of biopsy. Thirty patients (30%) were hypertensive at the time of biopsy. Albuminuria (Ualb/c) was measured in 80 patients at biopsy: 12 had nephrotic levels, 25 had mild and moderate levels and 43 were normoalbuminuric or microalbuminuric.

Eighteen patients progressed to poor outcome; 15 (12 boys) developed ESRD after a median of 6 (0.1–14.8) years from onset and an additional 3 patients reduced their GFR by 50% without progressing to ESRD within the follow-up period. Four patients, all with risk factors (hypertension and/or reduced GFR and/or nephrotic proteinuria at biopsy), had a very rapid deterioration and developed ESRD within 6 months from onset. The annual median rate of renal function decline (GFR:slope) was −0.8 (IQR −3.1 to 1.2) mL/min/1.73m^2/year if the four patients with very rapid deterioration were excluded (GFR:slope >50 mL/min/1.73m^2/year).

Table 1 shows the differences in clinical variables between patients with good and poor ($n = 18$) outcomes. Patients with a poor outcome had lower GFR, higher mean arterial blood pressure (MAP) and higher log Ualb/c at the time of biopsy compared to those with a good outcome. GFR remained reduced and proteinuria (log Ualb/c) remained increased during follow-up in the poor versus good outcome group, although the follow-up time was longer in the good outcome group. The proportion of males with poor outcome did not differ from that of females 13/58 (22%) versus 5/41 (12%), $P = 0.19$. The HRs of the predictors verified in the Cox regression univariate analysis are shown in Table 2. MAP was identified as a predictor only at the time of biopsy (HR 1.1, CI 1.05–1.1; $P < 0.001$) and not during follow-up.

The 10-year actuarial renal survival in this study was 86%. Figure 1a–d shows the renal survival with regard to the identified clinical predictors at biopsy. We found that nephrotic proteinuria as well as moderate proteinuria, reduced GFR:bio and hypertension at biopsy were all individual factors of poor renal survival compared with patients without these risk factors. The amount of proteinuria at biopsy increased the risk: Ualb/c >200: HR 23, $P < 0.001$, $n = 17$ and Ualb/c >400: HR 27, $P < 0.001$, $n = 12$ compared to the reference Ualb/c <200 mg/mmol. Proteinuria at follow-up was also shown to be a powerful predictor; renal survival differed between the patients with log Ualb/c >200 at 1 year and those with log Ualb/c <200 (log rank <0.001).

Histopathological predictors

Univariate analysis. Ninety of the 99 biopsies were available for re-analysis based on the new classification. Table 3 shows the predictive power of the different histological lesions in the Cox regression analysis. A mesangial hypercellularity score >0.5 (M1), the presence of endocapillary hypercellularity (E1) and TA/IF of ≥26% (T1–2) as well as presence of cellular and fibrocellular crescents (C1) and of GGS1 were all individual predictors of a poor prognosis in the univariate analysis in which M1, E1, T1–2 and C1 were found to have the highest HRs. S1 was not found to be significantly predictive in the univariate analysis ($P = 0.10$).

Table 1. Comparison of clinical variables at the time of biopsy and during follow-up in patients with good and poor outcomes[a]

	Good outcome			Poor outcome			
				GFR:last reduced >50% of GFR:bio[b] or ESRD			
	n	Mean	SD	*n*	Mean	SD	P
Follow-up time, years	81	13.2	7.9	18	8.4	8.8	<0.05
Age at onset	81	9.4	4.0	18	11.1	3.3	0.10
At time of biopsy							
GFR	80	107	24.7	17	67	37.9	<0.02
MAP	75	83	9.0	17	96	16.7	<0.001
Log Ualb/c	65	1.1	3.6	15	6.1	1.1	<0.001
At 1 year from onset							
GFR	41	114	19.8	9	38	33.6	<0.001
Log Ualb/c	40	1.4	3.0	8	4.5	4.0	0.02
At 3 years from onset							
GFR	39	112.	17.6	10	55	40.8	<0.001
MAP	29	83	10.0	8	99	21.1	0.005
Log Ualb/c	36	0.5	3.9	8	5.0	1.6	0.02
At 5 years from onset							
GFR	64	112	17.3	8	64	43.4	<0.001
Log Ualb/c	57	1.4	3.1	6	4.9	1.1	0.009

[a]GFR, mL/min/1.73m^2; MAP, mmHg; log Ualb/c = log urine albumin/urine creatinine ratio, mg/mmol.
[b] GFR:bio = GFR at the time of biopsy.

Table 2. HRs with 95% CIs of clinical variables that discriminate between good and poor outcomes, at time of biopsy and during follow-up (Cox regression)[a]

	n	At biopsy	*n*	At 1 year	*n*	At 3 years	*n*	At 5 years
GFR	97	0.96*** (0.95–0.98)	50	0.95*** (0.94–0.97)	49	0.97*** (0.96–0.98)	72	0.97*** (0.96–0.98)
Log Ualb/c	80	2.31*** (1.66–3.20)	48	1.44** (1.12–1.85)	44	1.43** (1.15–1.79)	63	1.31* (1.00–1.72)

[a]GFR, mL/min/1.73m^2, log Ualb/c = log urine albumin/urine creatinine ratio, mg/mmol.
***P < 0.001, **P < 0.01, *P < 0.05.

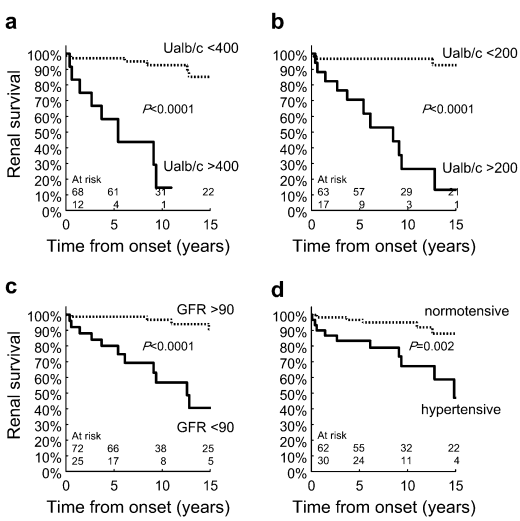

Fig. 1. (**a**–**d**) Renal survival in relation to clinical variables at biopsy Ualb/c = urine albumin/urine creatinine ratio, mg/mmol, GFR, mL/min/1.73m^2.

Figure 2a–f shows the differences in renal survival for presence and absence of the histological findings and Figure 3 illustrates the distribution of the frequencies in each histology lesion. In our cohort, 30% of the patients had biopsies showing M1, 10% E1, 23% S1, 16% T1–2, 17% C1 and 29% GGS. The mesangial hypercellularity score was mean 0.47 ± 0.59. Within the group of patients with fibrocellular/cellular crescents, the mean percentage was 4.5 ± 13.4 and the corresponding figures for segmental glomerulosclerosis% was 3.1 ± 7.5 and for GGS% 6.3 ± 16.3.

Multivariate analysis. Two multivariate models were constructed including one single histological lesion in combination with proteinuria at biopsy (Table 3, Model A) and proteinuria at 1-year follow-up (Model B). We found that E1 was associated with a significant risk of a poor outcome in both models, but for all other histological lesions except S1, the risk of a poor prognosis was significant only in Model B. When two histological variables were included, without any clinical variable, the combination of M1/E1 gave the highest risk and when two histological variables were added in Model A, the combination M1/T1–2 showed equally high risk as the combination E1/T1–2. In Model B, the combination of E1/T1–2 showed the highest risk. In the multivariate model, no combination with S1 added any predictive information.

Table 3. Cox regression analysis of histological variables that discriminate between good and poor outcomes[a]

	Univariate			Multivariate					
	$n = 90$	HR (95% CI)	P	$n = 74$	Model A HR (95% CI)	P	$n = 44$	Model B HR (95% CI)	P
Mesangial hypercellularity score									
≤0.5 (M0)	62	1	<0.001	49	1		27	1	
>0.5 (M1)	28	**7.07 (2.25–22.22)**		25	0.63 (0.13–3.00)	0.565	17	**4.98 (1.43–17.34)**	**0.012**
Endocapillary hypercellularity									
Absent (E0)	81	1		66	1		39	1	
Present (E1)	9	**7.15 (2.21–23.13)**	**<0.001**	8	2.52 (0.69–9.16)	0.16	5	**6.62 (1.98–22.42)**	**0.002**
Segmental glomerulosclerosis									
Absent (S0)	69	1		54	1		31	1	
Present (S1)	21	2.38 (0.85–6.70)	0.101	20	0.77 (0.24–2.48)	0.663	13	2.25(0.79–6.38)	0.127
TA/IF									
0–25% (T0)	76	1		61	1		37	1	
26–50% (T1)	11	**5.92 (2.14–16.37)**	**<0.001**	13	2.08 (0.72–6.01)	0.175	7	**4.22 (1.48–12.03)**	**0.007**
>50% (T2)	3								
Cellular or fibrocellular crescents									
Absent (C0)	74	1		58	1		32	1	
Present (C1)	16	**6.62 (2.14–20.45)**	**<0.001**	16	1.42 (0.42–4.83)	0.575	12	**6.10 (1.93–19.03)**	**0.002**
GGS									
Absent (GGS0)	64	1		50	1		30	1	
Present (GGS1)	26	**5.23 (1.79–15.3)**	**0.003**	24	2.50 (0.83–7.53)	0.103	14	**3.96 (1.33–11.80)**	**0.014**

[a]The HRs showing the predictive power of the individual histology lesions in the univariate analysis and in combination with proteinuria (log Ualb/c) at the time of biopsy (Model A) and at 1-year follow-up (Model B) in the multivariate analysis.

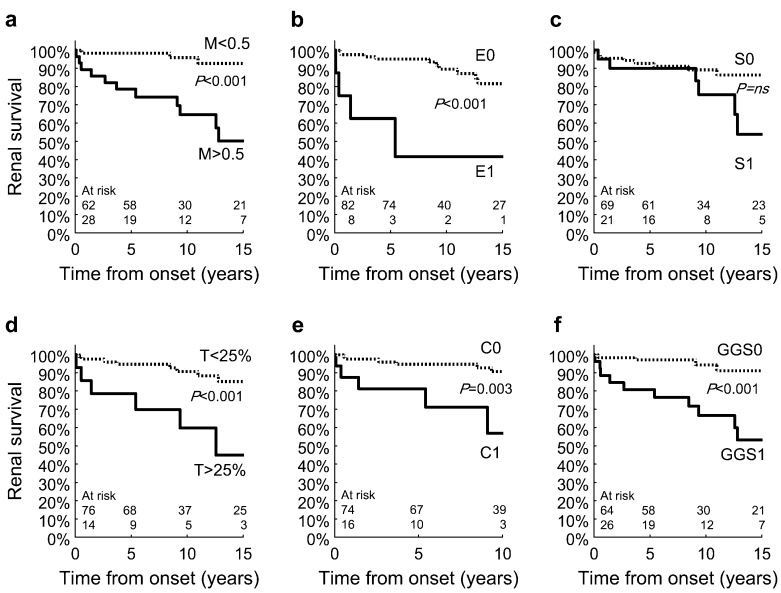

Fig. 2. (**a**–**f**) Renal survival in relation to histological variables M<0.5 = mesangial hypercellullarity score ≤0.5 (M0), M>0.5 = mesangial hypercellullarity score >0.5 (M1); E0 = no endocapillary hypercellullarity, E1 = endocapillary hypercellullarity present; S0 = no segmental glomerulosclerosis, S1 = segmental glomerulosclerosis present; T<25% = TA/IF 0–25% (T0), T>25% = TA/IF ≥26% (T1-2); C0 = no crescents, C1 = crescents present; GGS0 = no GGS, GGS1 = GGS present.

Correlations between different histological variables. We found positive correlations between several histological lesions shown in Table 4. The strongest correlation of all histological combinations was found between TA/IF and GGS ($r = 0.83$). TA/IF correlated to all other histology lesions. We also found strong correlations between M and E and between C and E. The strongest correlations among clinical variables with regard to histology were found between log Ualb/c at biopsy and M1 and log Ualb/c at biopsy and C1 ($r = 0.47$, respectively). GFR:bio correlated inversely with all histological variables (data not shown).

Associations between clinical and histologic variables. Table 5 shows the association between the histological lesions at the time of biopsy and at the last visit. All histological lesions (M1, E1, S1, T1–2, C1 and GGS1) were associated individually with a reduced GFR:bio and a high log Ualb/c at the time of biopsy. At the last visit, all histological lesions except S1 were associated with reduced GFR:last and all except C1 also with a high log Ualb/c last.

The annual GFR reduction (GFR slope) was more prominent among patients with T1–2 findings than among patients without this lesion (P = 0.016), but no significant difference in GFR slope was found when comparing presence (M1, E1, S1, C1, GGS1) with absence (M0, E0, S0, C0, GGS0) of any other histologic lesion.

Treatment

Thirty-four per cent of the patients had received treatment from Groups 1–3 (see Materials and methods section). Only the severe cases were treated. Patients with nephrotic proteinuria and/or hypertension at biopsy occurred more frequently among treated than non-treated patients (P = 0.002 and P = 0.02, respectively). In the different histology groups, we found associations to treatment among patients with T1–2 ($r = 0.32$, Spearman correlation) and to E1 and C1 ($r = 0.22$ and $r = 0.26$, respectively). Immunosuppressive drugs (IS) were more common among patients with crescents than among those without (P < 0.001). Treatment with ACEi/ARB was given more frequently to patients with TA/IF than to those without this lesion (P = 0.007). The GFR slope did not differ significantly before (-0.8 ± 2.6 mL/min/1.73m^2) versus after (-2.0 ± 3.4 mL/min/1.73m^2) treatment with ACEi/ARB.

When treatment (IS and ACEi/ARB, respectively) was added as a clinical variable in the multivariate analysis the prognostic significance of the histology variables M1, E1, T1–2, C1 and GGS1 still remained significant.

Discussion

The two main objectives of this study were to identify the clinical and histopathologic predictors of a poor prognosis in IgAN and to compare their prognostic power. Many authors have emphasized the predictive value of the clinical variables at onset and during follow-up [1, 2], but the controversial issue is whether the histological findings have an individual impact on the prognostic assessment, independently of clinical variables [8, 9, 13, 14]. In this study, GFR:bio, MAP and log Ualb/c at biopsy and GFR and log Ualb/c at follow-up were found to be clinical predictors of a poor outcome (Table 2 and Figure 1), with log Ualb/c at biopsy being the stronger predictor. Our findings that nephrotic as well as moderate proteinuria and hypertension at the time of biopsy and proteinuria at follow-up were predictive factors of a poor outcome (Table 2, Figure 1) are in agreement with several other authors [1, 2, 13–19].

Table 4. Correlations between the different histologic variables[a]

	E	S	T	C	GGS
M	0.54***	0.27**	0.49***	0.36***	0.47***
E		n.s.	0.36***	0.58***	0.27**
S			0.27**	n.s.	0.22*
T				0.24*	0.83***

[a]M = mesangial hypercellullarity score, E = endocapillary hypercellullarity in percentage of glomeruli, S = segmental glomerulosclerosis in percentage of glomeruli, T = TA/IF Grades 1–2, C = cellular or fibrocellular crescents in percentage of glomeruli, GGS in percentage of glomeruli, n.s. = not significant.
*P ≤ 0.05, **P ≤ 0.01, ***P < 0.001.

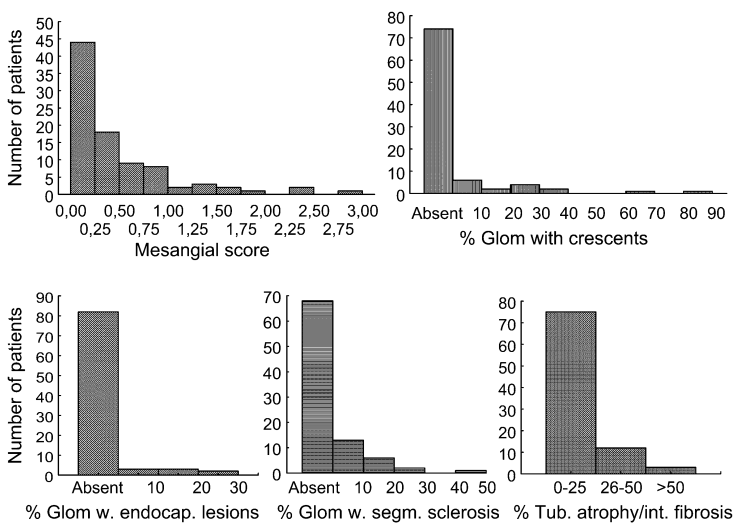

Fig. 3. Distribution of the level of pathology within each group of histology lesions.

Table 5. Relations between clinical and histological variables at the time of biopsy and at the last visit[a]

		At the time of biopsy		At the last visit	
		GFR	Log Ualb/c	GFR	Log Ualb/c
	M0	106 ± 25	0.9 ± 3.7	101 ± 30	0.1 ± 3.4
	M1	84 ± 38	4.4 ± 2.7	67 ± 47	3.0 ± 3.1
P		**0.002**	**<0.001**	**<0.001**	**<0.001**
	E0	104 ± 28	1.6 ± 3.7	94 ± 36	0.7 ± 3.5
	E1	63 ± 35	5.1 ± 2.4	53 ± 54	3.5 ± 4.0
P		**<0.001**	**0.01**	**0.003**	**0.05**
	S0	106 ± 28	1.5 ± 3.8	94 ± 36	0.5 ± 3.6
	S1	82 ± 33	3.5 ± 3.3	78 ± 47	2.5 ± 3.0
P		**0.002**	**0.04**	n.s.	**0.03**
	T0	107 ± 22	1.4 ± 3.8	97 ± 33	0.6 ± 3.6
	T1–2	65 ± 45	4.8 ± 1.7	55 ± 53	3.2 ± 2.8
P		**<0.001**	**0.002**	**<0.001**	**0.02**
	C0	105 ± 24	1.2 ± 3.8	94 ± 36	0.61±3.57
	C1	74 ± 44	4.9 ± 2.0	71 ± 50	2.26 ± 3.49
P		**0.001**	**<0.001**	**0.03**	n.s.
	GGS0	105 ± 27	1.2 ± 4.0	99 ± 30	0.5 ± 3.4
	GGS1	88 ± 38	3.9 ± 2.3	67 ± 51	2.3 ± 3.7
P		**0.02**	**0.003**	**<0.001**	**0.04**

[a]Differences in GFR and proteinuria at the time of biopsy and at the last visit in the different histology groups. GFR, mL/min/1.73m^2, log Ualb/c = log urine albumin/urine creatinine ratio, mg/mmol, n.s. = not significant. Histologic abbreviations as in Table 3.

We also found a low GFR at biopsy and at follow-up indicating an unfavourable prognosis in the univariate analysis but not in the multivariate analysis (Models A and B). In studies reviewed by Coppo and D'Amico [1, 2], impaired GFR at diagnosis was not found to indicate a poor long-term prognosis among children.

Our results show that each one of the histologic findings M1, E1, T1–2, C1 and GGS1 was associated with a poor prognosis, but we did not find S1 to be of statistical prognostic significance (Table 3, Figure 2). However, the low number of patients might explain why we did not reach significance with regard to S.

Taking into account the relatively small sample size, endocapillary proliferation (E) was shown to be a strong predictor in this study, although we found a weak association to treatment. Oxford classification study and other studies have shown that the amount of E is more frequent, and the amount of T is less frequent among paediatric patients compared to adults [8, 10, 20]. In the Oxford classification study, E was not significantly predictive of a poor prognosis, and the authors show a strong association to treatment and therefore E was excluded in their multivariate model.

In our study, special attention was given to patients with cellular or fibrocellular crescents (C) as they had a high predictive value (HR 6.6, CI 2.1–20.5) in the univariate analysis, although it did not maintain its significance in the multivariate analysis (Model A). This observation is shared with authors of several other studies reviewed by D'Amico [2]. Cellular or fibrocellular crescents were not predictive in the Oxford classification study, due to a low prevalence. In our study, a higher percentage of patients with C were treated with immunosuppression and/or ACEi than patients without this lesion, but treatment did not seem to prevent a poor outcome, which might be due to the interactive effect of co-existing chronic lesions in the biopsy specimens with cellular or fibrocellular crescents. We have discussed our experiences of treatment in the most severe cases of IgAN and their effect on outcome in a previous paper [21].

TA/IF >26% was found in 16% of the cases and had high prognostic importance in this study (HR 7.3, CI 2.7–20.2). In a previous paper from our group, we showed that the segmental glomerulosclerosis and tubulointerstitial damage were associated with a low GFR [22]. TA/IF has been shown to be a strong independent predictor in several studies reviewed by D'Amico [2], and in a recent paper [23], the authors showed that even a small amount of IF increases the risk of ESRD. As in the Oxford study, we identified a correlation between GGS and TA/IF ($r = 0.83$).

When comparing our results with those of the international study, certain differences in the design must be observed. In the Oxford classification study, the patients with initial GFR <30 mL/min/1.73m^2 and a urine albumin excretion <0.5 g/24 h/1.73m^2 in children were excluded, and those with a follow-up of more than a year were included. When we excluded the first pre-requisite, our results for renal survival still remained significant for all histological lesions except crescents (log rank 0.066). Moreover, we did not exclude patients with urine albumine excretion <0.5 g/24 h/1.73m^2 and we also had different inclusion criteria regarding disease duration. We selected patients who have had the disease for >5 years (except for four patients with very rapid deterioration reaching ESRD within 5 years), in order to enhance the opportunity of finding independent prognostic factors of a poor prognosis. In contrast, the original study included cases after 12 months of disease duration, to minimize the impact of the most acute and rapidly progressive cases. In our study of 90 children, the frequency of patients reaching the end point (GFR reduction >50% or ESRD) was lower than in the Oxford study of 209 adults and 59 children (18 versus 35%, respectively), which might be due to the different selections of included patients.

Our Models A and B, including one clinical and one histological variable, differ from those in the Oxford classification study in which three clinical and three histological variables were included. When we replaced log Ualb/c with GFR bio/GFR 1 year in Models A and B, we did not obtain any further prognostic information. Due to the low number of events ($n = 18$), we could not expand our models.

Our actuarial 10-year renal survival of 86% is in the lower range of results reported from other centres [1–4] and poorer than in the Japanese studies (96.4%) [24]. These results might reflect different indications for renal biopsy in Europe, in the USA and in Japan [3] and also the different traditions of treatment over time and between the countries. Our 10-year renal survival could also be explained by the fact that our cohort shows a high proportion of advanced disease, due to referral patients.

In summary, all histological variables except S were each highly discriminative of a poor prognosis in the univariate analysis. However, in the multivariate analysis, we found that the discriminative ability was reduced when histology was combined with proteinuria at biopsy (Table 3, Model A), whereas the ability was more pronounced when histology was combined with proteinuria at 1-year follow-up (Model B). The observation might be due to the selection of patients with a more severe disease in Model B and the smaller sample size.

The low discriminative power of histological variables in our Model A is due to the impact of albuminuria at biopsy, as only two variables can be included in the multivariate model. Although a limited number of patients with a poor outcome in our study could lead to conclusions in the analysis of the subgroups as underpowered, we still reached some noteworthy results. The strengths of this study are the total sample size and the long follow-up time (mean 13 years with 50% of the patients with follow-up time of >10 years) and the accuracy of the renal function investigations with GFR being measured by clearance studies. The limitation is that the study cohort mainly consists of referral patients which might overestimate the poor prognosis.

In conclusion, we found that the Oxford classification score is a valuable tool for prognostic purposes. We also found in our paediatric population that the presence of cellular/fibrocellular crescents was of prognostic significance as well as albuminuria at biopsy and during follow-up.

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