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Molecular Pathology Research Group, Institute of Biomedicine and Translational Medicine, University of Tartu.

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ABBREVIATIONS

AKI	acute kidney injury
AMH	asymptomatic microscopic haematuria
ANCA	anti-neutrophil cytoplasmic antibody
BMI	body mass index
BP	blood pressure
C3	complement component 3
C1q	part of the complement system
CCB	calcium channel blockers
CI	confidence intervall
CKD	chronic kidney disease
CKD-EPI	chronic kidney disease-epidemiology
CS	corticosteroids
CRP	C-reactive proteiin
eGFR	estimated glomerular filtration rate
ESKD	end-stage kidney disease
GBM	glomerular basement membrane
GFR	glomerular filtration rate
GN	glomerulonephritis
Hb	haemoglobin
Hct	haematocrit
HD	haemodialysis
IF	immunofluorescence
Ig	immunoglobulin
IgA	immunoglobulin A
IgG	immunoglobulin G
IgM	immunoglobulin M
IgAN	immunoglobulin A nephropathy
LGP	isolated non-nephrotic proteinuria
MAP	mean arterial pressure
MEST	**m**esangial hypercellularity, **e**ndocapillary hypercellularity, **s**egmental glomerulosclerosis/adhesion, and **t**ubular atrophy/interstitial fibrosis
mIgA	serum monomeric IgA
NS	nephrotic syndrome
Ns	non-significant
S-Creat	serum creatinine
SIgA	secretory IgA
SLE	systemic lupus erythematosus
RASb	renin angiotensin blockers
RRT	renal replacement therapy

1. INTRODUCTION

Most glomerulopathies, even the more common types, are rare diseases. However, they are important since they frequently affect young people, often cannot be cured, and can lead to chronic kidney disease with associated morbidity and cost (Floege, Amann 2016). IgA nephropathy (IgAN) remains the most common primary glomerulonephritis worldwide (Berger, Hinglais 1968, D'Amico 1987, Levy, Berger 1988, Glassock 2008). Beside diabetic nephropathy, IgAN is another important health-care issue in nephrology as it often affects young adults, and the nephropathy keeps a slow but relentless clinical course. The clinical progression in IgAN varies, and consequent end-stage kidney disease (ESKD) occurs in 15% of patients within 10 years (D'Amico 2000), in 30% to 40% of patients within 20 to 30 years after the first clinical presentation (Rychlik et al. 1999, Lai et al. 2016) and in about 50% (or less) of patients within 25 years after the diagnosis according to Glassock's data (Glassock 2008). The kidney is a target of injury in IgAN, yet the primary defect originates from a systemic aberrant glycosylation of *O*-linked glycans in the hinge region of IgA1, resulting in the increased serum levels of galactose-deficient IgA1 (Gd-IgA1). As the immunochemical abnormality of IgA is not corrected by renal transplantation, not surprisingly IgAN can frequently recur in allograft. An effective and specific treatment for IgAN is still lacking (Yu et al. 2011, Lai et al. 2016).

Immunoglobulin A nephropathy was first described by Berger and Hinglais (Berger, Hinglais 1968). However, slow progress in the understanding of disease over the 50 decades urged the development of renal biopsy techniques, after that the number of publications grew dramatically, and Berger's original report in IgAN investigation is currently listed as a citation classic by the Institute for Scientific Information (Emancipator 1998).

Clinically, Berger described the acute and chronic forms of IgAN. The acute form, arisen at the peak of infection, was clinically manifested by haematuria, without azotemia, oedema and hypertension. Once in many cases resolved, haematuria persisted in a few patients, and the episodes of infection exacerbated the disease. The chronic form of IgAN showed the persistent haematuria and proteinuria. The disease is pathologically characterized by a focal glomerulonephritis with predominant IgA in glomeruli (Berger, Hinglais 1968, Emancipator 1998). Since then, the so-called Berger's disease has been found to be a common glomerulonephritis all over the world, and, in many countries, it is the most frequent renal biopsy finding among glomerulonephritic patients (Ots 1998, Rifai et al. 2008).

IgA depositions in glomeruli are not only found in primary glomerulonephritis. Berger (Berger, Hinglais 1968) discovered that IgA depositions in glomeruli formed an important part also in a systemic form of IgAN, the so-called Henoch-Schönlein Purpura, and composed a part of Ig depositions in lupus nephritis as well. The secondary IgAN, which is uncommon, is also

described. Liver cirrhosis, HIV infection, and celiac disease are all associated with a high frequency of glomerular IgA deposition. Also, IgAN has been infrequently associated with a variety of many other diseases (Berger et al. 1977, Abramowsky et al. 1985, Bene et at. 1988, Katz et al. 1979, Helin et al. 1983, da Silva et al. 2015).

Since many features of the pathogenesis of IgA nephropathy are still obscure, specific treatment is not yet available (Tomino 2014, Lai et al. 2016).

General aim of the study was to focus on the characteristics of primary IgAN in the Estonian population. The occurrence, clinical manifestations, histopathological findings according to the Oxford classification, clinicopathological correlations with age-related differences, clinical and morphological risk factors, long-term outcome with different antihypertensive drug treatment regimens were investigated. Significance of both clinical and morphological prognostic risk factors in IgA nephropathy progression was assessed.

2. REVIEW OF LITERATURE

2.1. IgA Nephropathy

2.1.1. Definition

Glomerulonephritis characterized by the predominance of IgA among Ig deposits in glomeruli, in the absence of systemic disease or other nonrenal disease, is considered primary, or idiopathic, IgAN (Emancipator 1998). Morphologically, the disease is defined by the predominance of diffuse, mainly mesangial, granular deposition of IgA, identified by immunofluorescence or immunohistochemistry and by a variable degree of glomerular damage by light microscopy (Parai, Ghose 1985, Emancipator 1998, Roberts 2014). IgAN has a wide spectrum of clinical presentations, varying from isolated hematuria to rapidly progressive GN. Its clinical presentation and progression in individual patients is variable and its course is generally benign in cases without proteinuria, hypertension or reduced glomerular filtration rate (GFR) (Rifai, Dworkin 2008). Mesangial IgA deposition might be present in about 5–15% of healthy individuals, but only about 1 in 50 people with IgA deposits present with a clinical disease (Glassock 2008).

2.1.2. Etiology and Pathogenesis

The single diagnostic feature of IgAN is the finding of IgA immune deposits in the glomerular mesangium on renal biopsy. An explanation for this finding, and the clinical complications with which it is associated, have been the subject of considerable investigation. Despite the advance in the understanding of IgA immune system in health and the identification of a number of key changes in IgA biology in IgAN, no unifying pathological mechanism has been found to explain the development of IgAN (Emancipator 1998, Yu et al. 2011, Boyd et al. 2012, Lai et al. 2016).

Synthesis of IgA and immune system. IgA is the most abundant human immunoglobulin, a protein, having the synthetic rate approximately 2.7 mg/kg per hour. The majority of IgA is produced in mucosal tissues and secreted as secretory IgA (SIgA), with a little entering the circulation. A small amount of IgA is produced in the bone marrow by plasma cells and enters circulation, most in monomeric form (mIgA). The mucosal and systemic compartments are tightly linked and regulated, the so-called “mucosa–bone marrow axis” and two compartments function dependent on each other (Suzuki, Tomino 2007, Yu et al. 2011). IgA is divided into two subclasses, IgA1 and IgA2, which differ by the absence of an 18-amino acid sequence in the hinge region of IgA2 (Narita, Gejyo 2008, Yu et al. 2011). This difference explains the resistance of IgA2 against degradation by bacterial proteases in mucosal surface. IgA1 comprises 85% of total serum IgA. IgA produced by plasma

cells are mainly of IgA1 subclass, 93% in the spleen, peripheral lymph nodes, tonsils, nasal mucosa, 60–80% in bronchial mucosa, exocrine glands, duodenal, and gastric mucosa, in contrast to a dominance of IgA2 (64%) in the colon (Brandtzaeg, Johansen 2005, Yu et al. 2011). Despite the presence of significant amounts of IgA2, IgA1 still predominates in the mucosae derived from the embryonic foregut, including airways (Emancipator 1998, Yu et al. 2011).

The IgA system is different in human and mice. Murine IgA lacks a hinge region with *O*-glycosylation sites. Serum IgA in mice is exclusive polymeric in nature. A homologue of IgA receptor FcαRI is not present in mice. These differences have limited the development of animal models for IgAN. The ddY mouse is a well-known model of spontaneous IgAN. These mice develop glomerulonephritis with IgA deposition in the mesangium (Tomino Y 2010).

Biological function of circulating IgA and IgA receptors. The biological activities and physiological functions of circulating IgA are still poorly understood. It seems that circulatory IgA plays an obscure but important role in immune regulation. The major role of serum monomeric IgA has a powerful anti-inflammatory effect, such as the down-regulation of IgG-mediated phagocytosis, chemotaxis, bactericidal activity, and cytokine release (Monteinro 2010, Yu et al. 2011). There are five types of IgA receptors: FcαR, Fcα/μ receptor, pIgR, the hepatic asialoglycoprotein receptor (ASGPR), and transferrin receptor (CD71). FcαRI (CD89) is expressed on Kupffer cells in liver, neutrophils, monocytes, and eosinophils. FcαRI plays an essential anti-inflammatory role in physiology by the transmission of inhibitory signals following the binding of mIgA to FcRγ-associated FcαRI. However, in pathology, FcRγ-associated FcαRI favors the pro-inflammatory role. The polymeric IgA complexes result in enhanced IgA binding to FcαRI on blood monocytes, which activate monocytes. This also leads to the generation of soluble IgA–FcαRI complexes due to the cleavage of the FcαRI extracellular domain and then the release of IgA–FcαRI complexes into circulation. Soluble IgA–FcαRI complexes deposit in tissue such as renal mesangium through binding of IgA1 to the other receptors such as CD71 and initiate inflammation (Monteinro 2010, Yu et al. 2011).

Importance of glomerular mesangium. Together with the mesangial matrix, the mesangial cells compose the mesangium which form a stalk of a glomerular tuft (Emancipator 1998). The mesangial cells are considered to be smooth muscle like cells containing contractile microfilaments (Kreisberg et al. 1985, Emancipator 1998). The mesangial cells are coupled by gap junctions to each other and, in series, to the cells of the extraglomerular mesangium of the juxtaglomerular apparatus. They elaborate a lot of vasoactive agents or hormones, including prostanoids and platelet activating factor and are thought to respond by contraction or relaxation to a number of vasoactive hormones, such as angiotensin II and atrial natriuretic peptide, for which they have receptors (Kreisberg, Venkatachalam 1986).

The mesangial cell is an important target cell in many glomerular immune or non-immune diseases and characteristically responds by injury, repair and proliferation. In this connection, it has been recognized that the mesangial cells do not only respond to a number of growth factors and inflammatory mediators but can also produce them in an autocrine fashion (Emancipator 1998). These processes are responsible for initiating and sustaining the mesangial cell proliferation following injury (Emancipator 1998). Being not a typical phagocyte, the mesangial cells can endocytose colloids, macromolecules, protein aggregates, and immune complexes and in this way clear extraneous material that finds its way into the mesangial matrix from the circulation (Emancipator 1998).

In glomerular disease, the mesangial cell is thought to play a role in the sequestration and disposal of circulating immune complexes. The mesangial cells are responsible for the production and degradation of the mesangial matrix which is frequently increased in glomerular disease. A minority of mesangial cells (~2% of total glomerular cells) are phagocytic (Schreiner, Cotran1982).

The mesangial matrix fills irregular spaces between the mesangial cells and contains a large number of common extracellular matrix proteins, including several types of collagens (III, IV, V, and VI) as well as several components of microfibrillar proteins, also several glycoproteins (fibronectin is the most densely accumulated) and several types of proteoglycans (Couchman et al. 1994).

Mechanism of glomerular injury in IgAN. The pathophysiology of IgAN remains in part unsolved but it is primarily recognized as a mesangiopathic and an immune complex disease. The elevated serum levels of IgA were observed in about half of the patients with IgAN (Galla 1995, Yu et al. 2011). Increasing evidence supports the fact that the underglycosylated IgA-containing immune-complexes, including IgG antibodies against the glycans of the hinge region of IgA1, are key factors for the mesangial deposition and then trigger inflammation and glomerular injury. The polymeric IgA is produced after aberrant mucosal IgA response. The displacement of mucosal B cells to systemic lymphoid organs and bone marrow may arise from the abnormal trafficking of lymphocytes along the mucosa–bone marrow axis involving the changes of chemokines and adhesion molecules (Yu et al. 2011). Then, the formed glomerular deposits of immune complexes containing undergalactosylated IgA1 activate the mesangial cells, leading to the local overproduction of cytokines, chemokines and complement. Emerging data indicate that mesangial-derived mediators that are released following the mesangial deposition of IgA1 lead to a podocyte and tubulointerstitial injury via humoral crosstalk (Lai et al. 2016).

The links between IgAN and the mucosa have been recognized since the 1970s. In particular, the observation of visible haematuria induced by respiratory infections in the patients with IgAN and the association of IgAN with the diseases in which the mucosa plays a part have been taken as the evidence of a mucosa-kidney axis (Floege, Amann 2016).

2.1.3. Epidemiology and demographic characteristics

IgA nephropathy, or Berger's disease, is recognized as the most widespread type of glomerulonephritis worldwide and one of the main causes of chronic kidney disease (CKD) (Emancipator 1998, Glassock 2008, Rifai, Dworkin 2008, Cattran et al. 2009, Roberts et al. 2009). The prevalence rate varies across different geographical regions. Typically, it is 30–35% of all primary glomerular diseases in Asia, but can be up to 45% (Li et al 2002). In Europe, this is about 30–40% (Ots 1998, Simon et al. 2004, Rychlik et al. 2004, Covic et al. 2006, Carvalho et al. 2006, Beitnaraite et al. 2007, Wirta et al. 2008, Werner et al. 2009, Braun et al. 2011). Recently in the USA, IgAN was also reported to be the most common primary glomerulopathy in young adult Caucasians (Nair, Walker 2006, Wyatt, Julian 2013). Primary IgAN occurs at any age (the range is 4 to 80 years), but it is particularly common in younger individuals (Simon et al. 2004). The peak incidence of IgAN is between 20 and 40 years of life with a gradual decline over the remaining years (Nair, Walker 2006). The overall mean age is 38 years. Gender is important in epidemiology. Virtually all studies show a male predominance of at least 2:1 (Schena 1990, Galla 1995). Series of Asian patients show nearly equal involvement in males and females. IgAN patients have been reported from all over the world, and the disease is observed in all races (Emancipator 1998, Glassock 2008, Rifai, Dworkin 2008, Cattran et al. 2009, Roberts et al. 2009).

2.1.4. Clinical Expression

2.1.4.1. Clinical Findings

In the early stages of the disease, many patients have no obvious symptoms and have no complaints (D'Amico 1987). In these patients, IgA nephropathy may be suspected only during a routine screening or investigation of another condition. However, some patients may present with aggressive disease (Donadio et al. 2002). Patients with IgAN typically present in one of five ways: isolated microhematuria (Gutiérrez et al. 2012, Chan, Gale 2015), one or recurrent episodes of visible haematuria, microscopic haematuria and usually mild proteinuria, and rare occurring nephrotic syndrome or acute/or chronic renal failure (Galla 1995, Donadio et al. 2002).

Haematuria. Haematuria can result from bleeding anywhere from the glomerulus to urethra, and can be divided into glomerular or non-glomerular causes. Non-glomerular causes include malignancy, stones, papillary necrosis and metabolic disorders. Older adults (especially smokers) should initially be investigated to exclude urothelial or renal malignancy, whereas non-neoplastic kidney disease is more likely in those under 40. Microhematuria or macrohematuria is the most consistent clinical manifestation of IgAN as the presenting symptom or sign in 88% and 43% of all patients, respectively (Cornell 2011). Microscopic or non-visible haematuria is defined as two or more red cells per

high-power field present in a mid-stream urine sample on more than one occasion, and unrelated to exercise, trauma or menstruation. Prevalence ranges between 0.18 and 16.1% depending on the population (Cohen, Brown 2003, Chan, Gale 2015). Microscopic haematuria of glomerular origin can occur with proteinuria, hypertension or renal dysfunction, which indicate kidney damage.

Isolated microscopic haematuria refers to haematuria in the absence of proteinuria, hypertension or renal dysfunction. For many years, isolated microscopic haematuria was regarded as 'benign' but, while the short-term risk of kidney failure is undoubtedly small, it is now known that the increased risk of developing ESKD is significantly higher than that of the general population (Vivante et al. 2011, Gutiérrez et al. 2012, Chan, Gale 2015). The most common cause of microscopic haematuria of glomerular origin is probably IgA nephropathy; frequencies by reporters vary from 70% (Rychlik et al. 2004) to 88% (Cornell 2011). However, some genetic disorders can also present similarly, including Alport syndrome and thin basement membrane nephropathy.

About 40 to 50% of patients present with one or recurrent episodes of visible or *macroscopic haematuria*, usually following an upper respiratory infection (Schena 1990, Galla 1995, Cornell 2011). This has sometimes been called "synpharyngitic haematuria". These episodes can be provoked by bacterial tonsillitis, or by other viral upper respiratory infections or less often gastrointestinal and urinary tract infections (Galla 1995); they may occur in individuals who have already undergone tonsillectomy. It is presumed, although not proven, that the first episode represents the onset of the disease. Patients may complain of flank pain during acute episodes, which usually reflects the acute swelling of the kidney. Low-grade fever may also be present. Most patients have only a few episodes of macrohematuria and episodes usually recur for a few years at most (Galla 1995, Donadio, Grande 2002).

Assessment of kidney function. The key outcome measures for the management of GN include the assessment of kidney function, particularly the measurement of proteinuria and glomerular filtration rate (GFR).

- **Proteinuria.** Proteinuria, which is established in urinalysis, is a frequent sign of IgAN, encountered to some degree in nearly 57% of patients (Cornell 2011). The 24-hour protein excretion remains the reference (''gold standard'') method for the quantification of proteinuria in patients with GN. According to reports, 52% of patients have <1g/d proteinuria, 33% of patients have >1g/d proteinuria and 5% (Galla 1995) to 10% (Emancipator 1998, Cornell 2011) of patients have proteinuria in the nephrotic range, which is an uncommon presentation. If present, proteinuria is usually accompanied by haematuria and constitutes the other common initial presentation in 30–40% of patients – microscopic haematuria and proteinuria (Galla 1995). Isolated proteinuria is reported as unusual and rare finding in IgAN (Berg et al. 1991).
- **GFR.** Glomerular filtration is the process by which the kidneys filter the blood, removing excess wastes and fluids. Glomerular filtration rate (GFR) is a calculation that determines how well the blood is filtered by the kidneys,

which is one way to measure the remaining kidney function. GFR is also used to find the stage of chronic kidney disease. Glomerular filtration rate is usually calculated using a mathematical formula that compares a person's size, age, sex, and race to serum creatinine levels. This number is an estimated GFR (eGFR) (KDIGO 2013). A eGFR under 60 mL/min/1.73 m² may mean chronic kidney disease, and eGFR under 15 mL/min/1.73 m² means kidney failure. eGFR may not be a good measure of kidney health in some people, such as the very old or very young, obese people, or the persons with amputated limbs. Rarely, IgAN patients develop *acute kidney injury* with or without oliguria. This may be due to crescentic IgA nephropathy, or to heavy glomerular haematuria leading to tubular occlusion and/or damage by red cells (Gutiérrez et al. 2007). Up to 20% of patients with IgA nephropathy present with severe azotemia that is a *long-standing disease*, either because the patients' condition did not come to early medical attention or because the patients were referred late without an established diagnosis (Donadio, Grande 2002).

Hypertension. As with most forms of glomerulonephritis, hypertension is common. It occurs infrequently at the time of initial presentations (5 to 10%), but more commonly as the course of the disease lengthens (30 to 40%) or when IgAN presents beyond the fourth decade of life (Galla 1995).

2.1.4.2. Serological and Immunological Findings

Serum immunoglobulins and complement. The concentration of total serum IgA is elevated in 33 to 50% of adults with IgAN and in a somewhat higher percentage in children (D'Amico 1986, Galla 1995). However, other immunoglobulins (IgG, IgM) and complement levels are not elevated (Kim et al 2012).

Tests of limited utility. A number of other tests have been proposed for the evaluation of possible IgAN presence, but none of them are recommended being tests of limited utility (Barrat, Feehally 2016). These tests include searching for several circulating autoantibodies or immune complexes.

- Although circulating autoantibodies including anti-gliadin (Sategna-Guidetti et al. 1992, Ots et al. 1998) or anti-endothelin (Barrat, Feehally 2016) antibodies have been reported in IgAN, none appear to be disease specific.
  - Circulating IgA-rheumatoid factors and IgA-immune complexes have been considered as diagnostic markers but are not diagnostically useful, nor can they be reliably correlated with the disease activity.
  - Circulating IgA-fibronectin complexes were proposed as a diagnostic test since their presence carried the implication that increased reactivity between patient IgA and fibronectin within the glomerular mesangium might provide an explanation for the mesangial IgA deposition. However, it is now clear that the assays developed

for this purpose do not reliably distinguish between IgA complexed to fibronectin and free IgA (Cederholm et al.1998).

- Skin biopsy, looking for IgA deposition in the dermal capillaries, has not proven to be sufficiently predictive in IgA nephropathy (Hasbargen, Copley 1985).
- Plasma polymeric IgA1 levels are elevated in 30 to 50 percent of cases, but this suggestive finding is not sufficiently specific to establish the diagnosis (Hastings et al 2013).
- The measurement of the proportion of poorly galactosylated IgA1 *O*-glycoforms in the serum with or without measurement of poorly galactosylated IgA1-specific IgG antibodies has been proposed as a clinically useful diagnostic test (Moldoveanu et al. 2007, Suzuki et al. 2009, Glassock 2009). However, the utility of both assays is unclear since neither has been evaluated in patients who do not have IgA nephropathy but who present similarly to those with IgA nephropathy, such as those with haematuria or renal failure (Roos, Kooten 2007).
- MicroRNAs (miRNAs) are endogenous small (18 to 24 nucleotides long) noncoding single-stranded RNAs that regulate gene expression at the posttranscriptional level (Szeto, Li 2014, Trionfini et al. 2015). Certain miRNAs, including miR-148b and let-7b, can affect O-galactosylation of IgA1. These two miRNAs were measured in sera from 533 patients with or without IgA nephropathy to test the diagnostic utility of these potential biomarkers (Serino et al. 20016). Both miRNAs were elevated in the patients with IgAN, and a diagnostic rule that used them in combination had a sensitivity of 64 percent and a specificity of 74 percent. The positive and negative predictive values for identifying the patients with IgAN were 84 and 47 percent, respectively. Thus, these miRNAs are of no utility as diagnostic biomarkers (Serino et al. 2016).

2.1.4.3. Diagnosis

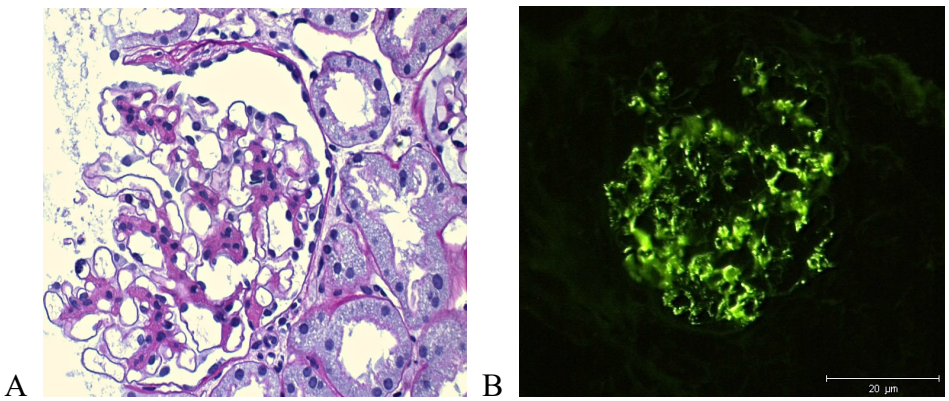
At present the suspicion of a diagnosis of IgAN is generally based upon the clinical history and laboratory data. The diagnosis can be confirmed only by the kidney biopsy with immunofluorescence or immunoperoxidase studies for IgA deposits (Galla 1995, Roberts et al. 2009, Emancipator 1998, Barratt, Feehally 2016).

2.1.5. Histopathology of Renal Biopsy

2.1.5.1. Light Microscopy (LM)

The morphological expression on renal injury in IgAN is, like the clinical presentation, wide ranging and variable. However, much of this variability reflects different combinations of glomerular, tubular, vascular, and interstitial lesions (Emancipator 1998, Roberts et al. 2009).

Glomeruli. All the patterns of immune glomeruli injury are observed in IgAN (Emancipator 1998, Roberts et al. 2009). The most common alteration associated with IgAN identified by LM is the focal or diffuse expansion of mesangial regions, with cells and matrix (Donadio et al. 2002). However, the mesangial cell and matrix expansion is not specific for IgAN and can be observed in a number of other renal diseases: diabethic nephropathy, focal segmental glomerulosclerosis, and a variety of glomerular lesions associated with a systemic disease (Emancipator 1998, Cornell 2011, Roberts et al. 2009). Moreover, a wide variety of lesions, such as diffuse endocapillary proliferation, segmental sclerosis, segmental necrosis, and cellular crescent formation may be seen in the patients with IgAN (Emancipator 1998, Cornell 2011, Roberts et al. 2009).



A – Light microscopy, PAS stain, x400: the glomerulus shows mesangial hypercellularity and expanded mesangial matrix.
B – Immunofluorescence shows prominent mesangial IgA deposits, x400.

Figure 1. Typical morphological alterations in IgA nephropathy

Interstitium and tubules. In addition to the glomerular alterations, a variety of tubulointerstitial changes may be found in the patients with IgAN. Such changes are: interstitial fibrosis, tubular atrophy, interstitial inflammation, and red and/or proteinogenous casts within the tubules. These features may be seen in progressive renal disease of any course (Emancipator 1998, Donadio, Grande 2002, Cornell 2011). Nevertheless, the assessment of these features provides an important prognostic information for the patients with IgAN (Roberts et al. 2009).

Vessels. Vessels do not express any specific features characteristic of IgAN. The alteration of vessels are rather associated with hypertension (arteriolohyalinosis), secondary or induced by the underlying disease, atherosclerosis in older patients (sclerotic arteriopathy), rarely vasculitis (the inflammation of vessel's wall) (Emancipator 1998, Donadio, Grande 2002).

2.1.5.2. Immunofluorescence Microscopy (IF)

IgAN in the native kidney is defined as a predominant staining with IgA in glomeruli by immunofluorsescence or immunoperoxidase. Not all glomeruli need to show this positivity. SLE-related nephritis should be excluded. The intensity of IgA staining should be more than a trace. The distribution of IgA staining should include the presence in the mesangium, with or without capillary loop staining, excluding a pure membranous, diffuse, global granular GBM staining pattern or a linear GBM staining pattern. IgG and IgM may be present, but not in greater intensity than IgA, except that IgM may be prominent in sclerotic areas. Complement 3 (C3) may be present. The presence of C1q staining in more than trace intensity should bring up the consideration of lupus nephritis (Roberts et al. 2009, Bellur et al. 2011).

2.1.5.3. Electron Microscopy (EM)

Amorphous electron-dense deposits in the mesangium and paramesangium (100%), subendothelial deposits (11%), subepithelial deposits (6%), sometimes intramembranous deposits (2%) are observed. As additional points GMB abnormalities often present, also extensive foot processes effacement when proteinuria is present are reported. Increased hypercellularity and the increased matrix in mesangium are found (Dickersin et al. 2000, Valaitis 2002).

2.2. Natural History and Prognosis of IgA Nephropathy

2.2.1. Course and Progression

The idiopathic type of IgAN has only recently attracted the attention of nephrologists, since it is becoming evident that it is the most frequent primary glomerular disease in the world (D'Amico 1987). Originally it was thought that IgAN was a benign disease, but it is now known that approximately one third of patients develop a progressive renal disease after a diagnosis which progresses to ESKD (Rychlik et al. 1999). Even more, its variable and often long natural history makes it difficult to predict an outcome (Bartosik et al. 2001). Two main reasons can explain why this disease has long been neglected: (1) its diagnosis is based on immunohistological examination which became a routine practice in the majority of institutions only at the end of the 60s; (2) the disease is characterized in more than half of patients by almost no clinical symptoms, so that biopsy is considered unnecessary by many nephrologists (D'Amico 1987). Long-term natural history studies have demonstrated that the rate of progression has an extremely wide range, from 5 to 25% after 10 years and 25–50% after 20 years, and a complete remission is reported in 5 to 30% of cases (Coppo, D'Amico 2005).

2.2.2. Prognostic Indices, Clinical

The impairment of renal function, sustained hypertension, and marked proteinuria at the time of diagnosis are the strongest clinical predictors of an unfavorable renal outcome (Radford et al. 1997, Mustonen et al. 2001, Glassock 2008, Reich et al. 2007, Le et al. 2011, Wyatt, Julian 2013). Proteinuria at diagnosis has been the focus in many studies (Reich et al. 2007, Moriyama et al. 2012, Le et al. 2011, and Wyatt, Julian 2013). Particularly, patients with time-averaged urinary protein excretion >1.0 g/day have a risk of ESKD that is 46 times the risk among patients with values of <0.5 g/day (Wyatt, Julian 2013). Furthermore, the renal outcome is better with a value <0.5 g/day rather then with a value 0.5 to 1.0 g/day (Wyatt, Julian 2013). Also, in IgAN, overweight/ obesity, present at diagnosis, is associated with an increase in the major risk factors (hypertension, proteinuria and severe renal lesions) which translate into a worse final outcome (Berthoux et al. 2013).

2.2.3. Prognostic Indices, Morphological

Although the rate of progression is very slow, about 50% (or less) of the patients with IgAN progress to ESKD within 25 years (Rychlik et al. 1999, Coppo, D'Amico 2005). Beside the clinical prognostic factors of the disease progression, pathology studies of IgAN looked for the morphological prognostic indices helping to predict a renal outcome. Some of these indices were already known in the 80s-90s, such as glomerular sclerosis, interstitial fibrosis, and the involvement of the glomerular capillary wall – all they predict a poor outcome (D'Amico 1992, Galla 1995).

The Oxford classification System (2009) – a gold standard for IgAN prognostication

The Oxford classification, which was presented by an international consensus group, renewed the interest in the prognostic value of the histologic features of the diagnostic renal biopsy (Coppo et al. 2010, Wyatt, Julian 2013). The goal of this new system was to identify specific pathological features that more accurately predict the risk of progression of a renal disease in IgAN, thus enabling both clinicians and pathologists to improve the prognostication of an individual patient. In order to develop this classification, clinical data and renal biopsies were obtained from 265 patients who were followed for a median of five years (Cattran et al. 2009). The entry criteria for the Oxford study excluded patients with and estimated GFR (eGFR) of less than 30 ml per minute per 1.73 m^2 of body-surface area (excluding the patients with stage 4 or 5 CKD), and the outcome measure was the progression to ESKD or the decrease in the eGFR of more than 50% from the rate at study entry (Cattran et al. 2009, Wyatt, Julian 2013). Four histologic features showed an independent value for predicting the outcome of renal function: mesangial hypercellularity, endocapillary hyper-

cellularity, segmental glomerulosclerosis, and tubular atrophy/interstitial fibrosis – MEST (Roberts et al. 2009, Cattran et al. 2009). The predictive value of each of these variables appears to be similar in adults and children (Coppo et al. 2010). A recent review of 13 Oxford validation studies confirmed the independent prognostic value of tubular atrophy and interstitial fibrosis in 10 studies, mesangial hypercellularity in 4 studies, and segmental sclerosis in 4 stadies (Roberts 2013, Wyatt, Julian 2013, Karoui et al. 2011, Herzenberg et al. 2011, Shi et al. 2011, Alamartine et al. 2011, Tanaka et al. 2013, Lee et al. 2012). Based on these data, the consensus recommendation is that every biopsy report of IgAN includes the numerical scores based upon the presence or absence of these variables summarised in the MEST score. The pathological definitions for grading different renal lesions are summarised in the paper „The Oxford classification of IgA nephropathy: pathology definitions, correlations, and reproducibility“ (Roberts et al. 2009).

2.2.4. Clinicopathological Correlations

Clinicopathological correlations have been the goal for many researchers, e.g. Mustonen 1984, Kawamura 2004, who tried to find independent prognostic values for IgAN. The Special IgA Nephropathy Study Group of the Progressive Renal Diseases organized by the Ministry of Health, Labor and Welfare in Japan conducted a multicenter retrospective case-control study on IgA nephropathy in 2004 to develop an evidence- and lumped-system-based clinicopathological classification of IgAN for predicting the long-term risk of progression to ESKD. The investigators reported that a cellular/fibrocellular crescent and glomerular density were found to be the predictors of progression in multivariate analyses (Kawamura et al. 2013).

The recent Oxford classification study of IgA nephropathy published in 2009 became the gold standard of clinicopathological correlations in the IgAN investigation. The correlations between the pathology lesions and clinical presentation at renal biopsy in the Oxford study showed that mesangial score, segmental glomerulosclerosis, endocapillary hypercellularity, and extracapillary proliferation were strongly correlated with proteinuria at the time of biopsy. Segmental glomerulosclerosis was correlated with reduced eGFR and higher MAP at the time of biopsy. Tubular atrophy/interstitial fibrosis were correlated with reduced initial eGFR and higher initial MAP and proteinuria. Arterial disease was strongly correlated with initial blood pressure and eGFR but had no relation with initial proteinuria (Cattran et al. 2009).

Correlations between pathology lesions and outcome in the same study also have been presented. These correlations have shown that the above mentioned pathology features have a value independent of the patients' clinical parameters in predicting the outcome in IgAN. The Oxford study used three widely accepted clinical outcomes in the models that assessed the independent relevance of these variables (Cattran et al. 2009).

2.2.5. Remission

Suzuki and collaborators (Suzuki et al. 2014) proposed the following criteria of clinical remission. In the case where the criteria (urinary sediment < 5 red blood cells/HPF, the amount of proteinuria < 0,3g/24h) are met for 3 consecutive times or more over at least for 6 months, the patients are classified as being in the "haematuria remission" or "proteinuria remission," and both the haematuria and the proteinuria remission are defined as the "clinical remission." The haematuria or proteinuria remission alone is designated as "partial remission." In addition, the first date on which the remission criteria are met is considered as the remission date.

2.3. Secondary IgAN

Most cases of IgA nephropathy are clinically limited to the kidney (Emancipator 1998, Galla 1995, Wyatt, Julian 2013), but IgA nephropathy may be associated with other conditions developing into secondary IgAN. An association between a glomerular disease and cirrhosis has been known since the 1950s (Pouria, Feehally 1999). The studies from the 1970s progressed when IF came into use and confirmed that mesangial IgA deposition in glomeruli was the commonest pattern seen in the patients with liver cirrhosis (Newell 1987, Pouria, Feehally 1999).

Cirrhosis, HIV infection, and celiac disease are all associated with a high frequency of glomerular IgA deposition (Pouria, Barratt 2008). However, most patients have little or no evidence of glomerular disease. These observations indicate that a high circulating load of polyclonal IgA is not in itself adequate to promote the nephritis characteristic of IgAN and that other abnormalities of IgA and its metabolism are necessary for IgA deposition to translate into mesangial activation and glomerular injury. It is also important to note that the reported incidence of mesangial IgA deposition in apparently healthy individuals ranges from 3 to 16 percent (Barrat , Feehally 2016).

IgAN has been infrequently associated with a variety of other diseases, including dermatitis herpetiformis, seronegative arthritis (particularly ankylosing spondylitis), small-cell carcinoma, lymphoma (Hodgkin lymphoma and T-cell lymphomas, including mycosis fungoides), disseminated tuberculosis, bronchiolitis obliterans, and inflammatory bowel disease (Crohn's disease and ulcerative colitis). These are usually clinically evident at the time of biopsy (Galla 1995, Pouria, Barratt 2008).

2.4. Treatment and the Prevention of the Progression of IgA Nephropathy

2.4.1. General Management of the Patients with IgA Nephropathy

IgA nephropathy is the most common human glomerulonephritis worldwide but there is no specific therapy. During 40 years since IgA nephropathy was first reported, the cause of this disease has never been clarified. One of the main reasons for this was the lack of an appropriate animal model. Since many features of the pathogenesis of IgAN are still obscure, specific treatment is not yet available. However, efforts by investigators around the world have gradually clarified different aspects of the pathogenesis and treatment of IgA nephropathy. Current data implicate overproduction of aberrantly glycosylated IgA1 as being pivotal in the induction of renal injury. New therapeutic approaches will be developed after the pathogenesis of the disease is better understood (Tomino 2014, Lai et al. 2016).

The patients with minor urine abnormalities, normal blood pressure and normal GFR usually do well and require only periodic monitoring, such as biennial clinic visits. For other patients, the therapeutic options are limited and include nonspecific treatment to reduce proteinuria by RAAS blockade and non-specific control of inflammation using fish oil and medications such as corticosteroids, cytotoxic agents, anti-metabolite, and immunomodulatory drugs (Lai et al. 2016).

The evidence base for the treatment of IgAN is gradually increasing both in the number and quality of Publisher trials. There is the consensus that the supportive treatment with RAAS blockade and tight BP control should be the initial treatments. A part of patients who have persistent proteinuria during supportive therapy are still at higher risk for disease progression. There is still no consensus if corticosteroids or other immunosuppressive agents soften the risk of progression with acceptable toxicity (Boyd et al. 2012). A paucity of high-quality clinical trials means that the evaluation of additional therapies, particularly immunosuppressive regimens, is difficult and a great deal of confusion over the optimal treatment of patients with a high risk for progression to chronic kidney disease remains.

2.4.2. Treatment and the Prevention of Progression

Glucocorticoids. Uncertainty exists regarding immunosuppression in patients with IgA nephropathy who are at risk of progressive disease (Floege, Eitner 2011). There is a consensus no longer to give immunosuppression to the patients with a GFR below 30 mL/min at presentation unless they already have a rapidly progressive glomerulonephritis course. Although glucocorticoids are effective for the treatment of the IgAN patients with minor to moderate

glomerular injuries, it is necessary to use large doses of the drug for long periods. This treatment regimen is associated with severe adverse effects such as diabetes, peptic ulcer and aseptic necrosis of the bones. Japanese researches reported (Kobayashi et al. 1996) the efficacy of prednisolone (PSL) treatment in a 10-year follow-up of the IgAN patients in the early stage (proteinuria of 1–2 g/day, Ccr of more than 70 mL/min and a histological severity score of more than seven) of progressive IgA nephropathy. Tomino and collaborators also performed a multicenter trial on PSL in the Japanese patients with IgA nephropathy (Tomino et al. 2004) with good results. However, in a meta-analysis of the role of corticosteroids in IgAN, the findings from all studies showed reductions in proteinuria, but several researchers detected no benefits for GFR (Lv et al. 2009). In particular, the question of whether corticosteroids still bring a benefit if added after the optimisation of supportive measures, including intense RAS blockade, remains unresolved. The sequence of the first optimising supportive measures for 3–6 months before considering corticosteroids in patients with persistent proteinuria above 1 g/day and GFR greater than 50 mL/min has been suggested by the guidelines (KDIGO).

Angiotensin-converting enzyme (ACE) inhibitors and/or angiotensin-receptor blockers (ARBs) induce a marked renoprotective effect in patients with IgA nephropathy. Combination therapy with an ACE inhibitors and ARBs was reported to induce a more pronounced decrease in the progression of proteinuria in normotensive patients with IgAN. However, caution should be exercised to avoid too large a decrease in BP. RAS blockers are often prescribed for the patients with IgAN and proteinuria. In a meta-analysis of 585 patients from 11 randomized clinical trials (RCTs) significant renoprotection and reduction of proteinuria were achieved with an ACEIs or ARBs versus control (Cheng et al. 2009).

Fish oil. The possible benefit of fish oil containing omega-3 polyunsaturated fatty acid in the treatment of IgAN rests on reducing intra-renal inflammation by mitigating inflammatory cytokines and eicosanoids. However, the published reports failed to show convincing benefits. In the original Mayo Clinic multicenter study with 106 subjects (Donadio et al. 1994), fewer patients randomly assigned for fish oil treatment reached the end-point of at least a 50% rise in serum creatinine. In particular, neither this original study nor a subsequent trial showed a reduction of proteinuria. Proteinuria is a key therapeutic target because it may itself cause renal injury, and its reduction correlates with the preservation of renal function. A recent trial of 30 patients suggested that a RAS blocker combined with polyunsaturated fatty acids reduced proteinuria more than a RAS blocker alone (Ferraro et al. 2009). The KDIGO 2012 Clinical Practice Guidelines (Radhakrishnan 2012) suggest an optional use of fish oil in the treatment of patients with persistent proteinuria of more than 1 g/day, despite 3 to 6 months of optimized supportive care including ACEI or ARBs and blood pressure control. Yet, the long-term benefits on preventing ESRD are uncertain (Lai et al. 2016).

Calcium channel blockers (CCBs). CCBs such as amlodipine and nifedipine, which selectively block L-type calcium channels, can dilate afferent arterioles but not efferent arterioles in the glomeruli. Therefore, these drugs elevate the glomerular pressure, even though they increase the renal blood flow, and thereby induce exacerbation of renal dysfunction. Several subtypes of calcium channels, such as L, T and N channels, have been identified. Recent studies have revealed that inhibitory action on N- and T-type calcium channels is useful in the treatment of various renal diseases. CCBs capable of inhibiting N- and T-type calcium channels dilate the efferent arterioles. Possible factors involved in this effect include the suppression of sympathetic activity mediated by the inhibition of N-type calcium channels and the correction of renal hemodynamics mediated by the inhibition of T-type calcium channels (Takenaka at al. 2009). Benidipine inhibits not only the L-type, but also the T-type and N-type calcium channels. Previous clinical studies involving hypertensive CKD patients have shown that benidipine has a more potent renoprotective effect compared to nifedipine and amlodipine which are known to inhibit L-type calcium channels (Hayashi et al. 2007). Clinical and preclinical studies have demonstrated that benidipine dilates both afferent and efferent renal arterioles, leading to the reduction in the glomerular pressure and the alleviation of proteinuria. The long-term antiproteinuric effect of benidipine has not been evaluated in detail in hypertensive CKD patients including those with IgA nephropathy (Tomino et al. 2011).

Tonsillectomy with steroid pulse therapy The tonsils are mucosa-associated lymphoid tissues that come in close contact with extrinsic antigens, especially infectious antigens, and are the sites of the initiation of immune responses. In recent years, there has been much focus on tonsils. Macroscopic haematuria is occasionally observed after acute tonsillitis and/or pharyngitis in patients with IgA nephropathy. The clinical effects of tonsillectomy with steroid pulse therapy for the treatment of proteinuria and hematuria in patients with IgAN have been reported mainly in Japan (Miura et al. 2009).

Outside Japan, the benefits of tonsillectomy have not been documented until 2016 when Feehally and others (Feehally et al. 2016) recently showed that in the large VALIGA cohort of the European subjects with IgAN, no significant correlation was found between tonsillectomy and the decline of renal function.

2.5. Experimental Models

2.5.1. IgAN Experimental Models

The availability of adequate animal models may speed up the discovery of the biomarkers for the disease staging and the individualization of therapy as well as the design and testing of novel therapeutic strategies. One of the main reasons for the clarification of the cause of IgAN was the lack of an appropriate animal model. In 1979, a passive transfer of dinitrophenol conjugated to BSA

was reported to cause mesangial IgA deposition, mesangial matrix expansion and haematuria in rats (Rifai et al.1979). Murine models include oral xeno-immunization resulting inglomerular IgA deposition (Emancipator et al.1983); spontaneous IgA nephropathy in a non-inbred dd-stock named ddY mice (Imai et al.1985). Since then, the findings in the ddY mouse have been extrapolated towards the pathogenesis and treatment of patients with IgA nephropathy (Tomino et al. 2014). In 1985, Imai (Imai et al. 1985) first reported that the ddY (Deutschland, Denken, Yoken) strain of mouse could serve as a spontaneous animal model for the human IgA nephropathy. Imported from Germany before 1920, ddY mice have since then been maintained in Japan. These ddY mice exhibit mild proteinuria without haematuria and mesangioproliferative glomerulonephritis with glomerular IgA deposits. These immunopathological findings appear when the mice are over 40 weeks of age. Although the incidence of IgA nephropathy in ddY mice is highly variable, it appears that the clinicopathological aberrations besides haematuria in ddY mice resemble those in the IgA nephropathy patients (Tomino et al. 2014). In Tomino research division, sequential renal biopsies were performed on more than 360 ddY mice (Tomino et al. 2014). IgA nephropathy occurred in about 30% of the mice by 20 weeks of age (early onset group) and in about 30% of the mice at 40 weeks of age (late onset group). IgA nephropathy did not occur in the remaining mice (the quiescent group) (Suzuki et al.2005). When an “association study” on onset was performed on the early onset and the quiescent groups of mice, multiple disease receptor gene loci were observed (Suzuki et al. 2005). Since one of the loci was found to be homologous with the gene locus reported for the human familial IgA nephropathy, at least some of these mice appear to be subject to the same genetic regulation as human IgA nephropathy. Therefore, ddY mice were considered to be useful as an animal model (Suzuki et al. 2005, Okazaki et al. 2012).

Murine models include finally, the advent of transgenic, knockout, and knock-in models. However, none of these models allowed the identification of the factors that control the transition between the disease onset and progression to end-stage renal disease. Thus, good models of IgA nephropathy leading to kidney failure that mimic the pathogenesis of the human disease are needed. (Ortiz et al. 2015).

2.5.2. CKD Experimental Models

Many longstanding animal models have failed to result in therapeutic advances in the clinical setting, such as the kidney ischemia-reperfusion injury and diabetic nephropathy models. In this regard, most models for diabetic nephropathy are unsatisfactory because they do not evolve into renal failure. Satisfactory models for additional nephropathies including IgAN are urgently needed (Ortiz et al. 2015).

However, CKD models may be used to study the pathogenesis and therapy of kidney diseases or to study the systemic consequences of decreased kidney function. Subtotal (5/6) nephrectomy (the remnant kidney) has been a mainstay of studies of progressive CKD. This non-immunological model is the equivalent to the humans having lost a part of the functional mass of the kidney and progressing despite the removal of the original cause of the kidney injury. Both in rats and mice, unilateral nephrectomy and either partial infarction or the amputation of the poles of the remaining kidney result in the progressive glomerular and tubulo-interstitial injury, the loss of remnant nephrons and the development of systemic and glomerular hypertension. It is also associated with the progressive intrarenal capillary loss, inflammation and glomerulosclerosis. (Ortiz et al. 2015). The "remnant kidney" chronic kidney disease (CKD) progression theory based on hemodynamic, proteinuric and inflammatory mechanisms consequent to nephron loss has not been confirmed in a human disease. Bazzi and collaborators aimed to evaluate whether some of these mechanisms are present in IgA nephropathy and predict a functional outcome. And finally, they found that in IgAN, progressive nephron loss is associated with an increase of proteinuric markers of glomerular and tubular damage. Fractional excretion of IgG/SG is the best outcome predictor. These data represent the first validation in a human disease of some pathophysiological mechanisms of CKD progression theory (Bazzi et al. 2012).

Taken together, the remnant kidney experimental model remained a mainstay of the studies of progressive CKD.

2.6 Summary of the literature

IgA nephropathy (IgAN) remains the most common primary glomerulonephritis worldwide (Berger, Hinglais 1968, D'Amico 1987, Levy, Berger 1988, Glassock 2008). Beside diabetic nephropathy, IgAN is another important health-care issue in nephrology as it often affects young adults, and the nephropathy keeps a slow but relentless clinical course. The clinical progression in IgAN varies, and consequent end-stage kidney disease (ESKD) occurs in about 50% (or less) of patients within 25 years after the diagnosis according to Glassock's data (Glassock 2008). The kidney is a target of injury in IgAN, yet the primary defect originates from a systemic aberrant glycosylation of *O*-linked glycans in the hinge region of IgA1, resulting in the increased serum levels of galactose-deficient IgA1 (Gd-IgA1). As the immunochemical abnormality of IgA is not corrected by renal transplantation, IgAN can frequently recur in allograft.

At present the suspicion of a diagnosis of IgAN is generally based on the clinical history and laboratory data. The diagnosis can be confirmed only by the kidney biopsy with immunofluorescence or immunoperoxidase studies for IgA deposits (Galla 1995, Roberts et al. 2009, Emancipator 1998, Barratt, Feehally 2016). The impairment of renal function, sustained hypertension, and marked proteinuria at the time of diagnosis are the strongest clinical predictors of an

unfavourable renal outcome (Radford et al. 1997, Mustonen et al. 2001, Glassock 2008, Reich et al. 2007, Le et al. 2011, Wyatt, Julian 2013). Proteinuria at diagnosis has been the focus in many studies (Reich et al. 2007, Moriyama et al. 2012, Le et al. 2011, and Wyatt, Julian 2013). Particularly, patients with time-averaged urinary protein excretion >1.0 g/day have a risk of ESKD that is 46 times the risk among patients with values of <0.5 g/day (Wyatt, Julian 2013). Also, in IgAN, overweight/obesity, present at diagnosis, is associated with an increase in the major risk factors (hypertension, proteinuria and severe renal lesions) which translate into a worse final outcome (Berthoux et al. 2013). Beside the clinical prognostic factors of the disease progression, pathology studies of IgAN looked for the morphological prognostic indices helping to predict a renal outcome. The Oxford classification, which was presented by an international consensus group in 2009, is a gold standard for IgAN prognostication**.** Four histologic features showed an independent value for predicting the outcome of renal function: **m**esangial hypercellularity, **e**ndocapillary hypercellularity, **s**egmental glomerulosclerosis, and **t**ubular atrophy/ interstitial fibrosis – MEST (Roberts et al. 2009, Cattran et al. 2009). The consensus recommendation is that every biopsy report of IgAN includes the numerical scores based on the presence or absence of these variables summarised in the **MEST** score. Also, the recent Oxford classification study of IgA nephropathy published in 2009 became the gold standard of clinicopathological correlations in the IgAN investigation. The correlations between the pathology lesions and clinical presentation at renal biopsy in the Oxford study showed that mesangial score, segmental glomerulosclerosis, endocapillary hypercellularity, and extracapillary proliferation were strongly correlated with proteinuria at the time of biopsy. Segmental glomerulosclerosis was correlated with reduced eGFR and higher MAP at the time of biopsy. Tubular atrophy/interstitial fibrosis were correlated with reduced initial eGFR and higher initial MAP and proteinuria.

An effective and specific treatment for IgAN is still lacking (Yu et al. 2011, Lai et al. 2016).

3. AIMS OF THE STUDY

The general aim of the present study was to analyse the single-centre kidney biopsy material evaluating the occurrence of IgA nephropathy and assessing its clinicomorphological correlations according to the Oxford classification. We hypothesized, firstly, that the occurrence of IgA nephropathy has not significantly changed in the course of time in comparison with the historically conducted study at the same centre; secondly, that potential gender-related differences regarding the IgA nephropathy progression exist, and finally, that renoprotection has a benefit in the outcome of IgA nephropathy patients.

AIMS OF THE STUDY

1. To find the occurrence of various glomerulopathies, including IgA nephropathy, in the native kidney biopsy material at the Tartu University Hospital.
2. To identify the clinical presentation and the morphological patterns of IgAN at the time of biopsy according to the Oxford classification.
3. To assess the clinical and morphological data correlations in IgAN patients and to define their gender-related differences.
4. To assess clinical and morphological prognostic risk factors and their long-term significance in the IgA nephropathy progression.
5. To explore a benefit of renoprotection in the outcome of IgA nephropathy patients.

4. PATIENTS AND METHODS

4.1. Ethical Considerations

Paper I. The study is a part of the epidemiology project on chronic kidney disease in Estonia (Ethics Committee of the University of Tartu, Minutes nr 141/30, 19.09.2005 and164/T-0, 22.10.2007).
Papers II and III. The Research Ethics Committee of the University of Tartu has approved this study (Minutes No. 212/T-13, 2012).

4.2. Patients

The patients were selected according to the native kidney biopsy material at the Tartu University Hospital. Over the 10-year study (2001–2010), a total of 578 native kidney biopsies were performed and retrospectively reviewed. 31 cases were excluded from the study I because of insufficient or inadequate kidney biopsy samples. In total, 547 kidney biopsies of the patients in the study I were included (340 men, 238 women; mean age 39.9 ± 17.9 years). Five percent of the patients were children where female predominance was noted. The biopsy material, for the investigation by the pathology department, was sent from 4 separate Estonian hospitals (11 different departments). The exact population data were obtained from the database of Statistics Estonia for the calculations of biopsy rate and incidence of glomerulopathies in the country. In 2010, the kidney biopsy material covered the whole of the Estonian population and the biopsy rate calculated for that year was 8.1 per 10x5 of population. (Paper I). A total of 88 cases of IgAN, which was defined as glomerulonephritis with the predominance of IgA among Ig deposits in glomeruli in the absence of systemic disease or other nonrenal disease, during 10 years were registered (Papers II, III). 15 patients were excluded from the study II because of insufficient renal tissue (less than 8 glomeruli) and the cohort of 73 IgAN patients for study II was formed. To allow a comparison with the previous reports, we defined the children as ≤15, the younger adults as 16–40, the older adults as 41–65 and the elderly as >65 years of age in all studies. The patients' characteristics of different studies are shown in Table 1.

IgAN patients' cohort of 64 IgAN patients was formed for the FU study (59% males, 41% females). Nine patients were lost to follow-up: 3 patients died from trauma or other diseases, 1 patient had terminal renal failure at the time of diagnosis, and 5 patients did not complete the necessary control programs and protocols.

Table 1. The patients' characteristics in different studies (Papers I-III)

Study	Total number of cases, n	Total number of study cases, n	Total excluded from the study cases, n	Gender M/F	M/F ratio	Age (years), range and mean age
I	578	547	31	340/238	3:2	4–79 39.9 ± 17.9
II–III	88	73	15	45/28	3:2	16–76 33.7 ± 13.6

4.3. Clinical and Laboratory Findings

The clinical data (presenting clinical syndromes) for study I were collected from the records of the Tartu University Hospital. The baseline clinical data were collected within 3 months from the kidney biopsy and at the end of the follow-up (FU) for studies II–III: weight (kg), height (cm), body mass index (BMI, calculated), smoking history, presenting clinical syndrome at the time of biopsy, systolic and diastolic blood pressure (mmHg), serum creatinine (μmol/L), serum albumin (g/L), serum cholesterol (mmol/L) and serum triglycerides levels (mmol/L). Proteinuria was expressed in grams (g) per 24hrs/1.73m^2 in children and in grams per 24hrs in adults and was ranked at the intervals < 1 g/24hrs, 1–3.49 g/24hrs and >3.5 g/24hrs; microhematuria was ranked at the intervals of <75, 76–150, >150 erythrocytes/μL. eGFR (ml/min/1.73 m²) was calculated using the modified MDRD formula for adults and the Schwartz formula for children (Schwartz et al. 2009). Patients were defined as overweight/obese when their BMI was >25/30. ESKD was defined as eGFR <15 ml/min/1.73m² and as the transition to dialysis or transplantation. According to the Oxford classification study, 50% reduction of renal function from baseline eGFR was defined as an endpoint. The mean rate of renal function decline was expressed as a slope of eGFR during the follow-up. The mean arterial pressure (MAP) was defined as diastolic pressure plus a third of the pulse pressure (according to the Oxford classification quidelines).

Clinical syndromes:

- **Asymptomatic microhematuria** was defined as two or more red cells per high-power field presented in a mid-stream urine sample on more than one occasion, and unrelated to exercise, trauma or menstruation.
- **Macroscopic haematuria** was defined as visible haematuria; stones and malignancy were excluded.
- **Asymptomatic microhematuria and proteinuria** was defined as greater than 3 red blood cells per 1 high-power field by microscopic examination and as the increase of the excretion of albumin, the range normally detect-

able by standard dipsticks, which is not accompanied by clinical manifestation.

- **Nephrotic Syndrome (NS)** was defined as a combination of nephrotic-range proteinuria (> 3.5 g/24hrs) with a low serum albumin level and oedema.
- **Acute renal failure** was defined as an abrupt loss of kidney function that develops within 7 days.
- **Chronic renal failure** was defined as abnormalities of kidney function, present for more than 3 months, with implications for health and decreased GFR below 60 ml/min/1.73 m2 according to KDIGO and this corresponds with the eGFR categories G3a-G5 (KDIGO 2013).

4.4. Renal Biopsy

4.4.1. Kidney Biopsy Procedure

All renal biopsies were performed percutaneously under ultrasound guidance. A 14- or 16-gauge automated biopsy needle was used for the procedure. Two kidney samples were taken per case: 1 sample for light and 1 sample for immunofluorescence microscopy and in selected cases a sample of fresh kidney tissue was divided also for electron microscopy. Kidney biopsy complications are extremely low and no serious complications were seen in our studied patients' cohort.

Indications for kidney biopsy: isolated microhematuria, isolated non-nephrotic proteinuria, nephrotic syndrome, acute nephritic syndrome, unexplained acute renal failure.

4.4.2. Kidney Biopsy Material

The routine evaluation of a percutaneous renal biopsy involves the examination of the tissue under light and immunofluorescence microscopy (or immunoperoxidase in some laboratories (Mölne et al. 2005)). Each component of the evaluation can provide important diagnostic information (Whittier et al. 2016).

Light microscopy (LM). One sample of renal tissue was fixed in 10% buffered formalin for light microscopy and after the fixation it was embedded into parafin, using routine procedures. The kidney tissue sections were cut at 3 μm thickness and stained with hematoxylin-eosin, periodic acid Schiff (PAS), silver methamine and for elastic fibers (Verhoeff elastic stain). The kidney tissue samples were examined, using a Zeiss microscope (Axioskop 40).

Immunofluorescence microscopy (IF). Another sample of renal tissue was snap-frozen or for selected cases it was divided into 2 pieces: one piece for IF microscopy with subsequent snap-frozing and one piece for electron microscopic examination. For IF, tissue samples were snap-frozen with isopentane in liquid nitrogen and were subsequently cut by cryostat at 4 μm thickness. IF was

based on the use of antisera conjugated with fluorescein directed against human antigens such as IgA, IgG, IgM, complement fractions (C3, C1q), and kappa and lambda light chains of immunoglobulins (DAKO products). The kidney tissue samples were examined, using a Zeiss microscope (Axioskop 40) equipped with the epi-fluorescence illuminator (HBO 100).

4.4.3. Microscopic Examination

Light microscopy (LM). Each biopsy was evaluated according to the clinical data of the case and sorted according to the classification of kidney and glomerular diseases. Kidney diseases were divided into four categories: (i) primary glomerulopathies; (ii) secondary glomerulopathies; (iii) tubulointerstitial diseases; and (iv) other conditions. A diagnosis of primary glomerulopathy was considered if at the time of biopsy there was no evidence of multisystemic disease. Primary glomerulopathies were divided into nine groups: mesangioproliferative glomerulonephritis (MesGN), some other than IgA nephropathy or lupus nephritis; immunoglobulin A nephropathy (IgAN); membranoproliferative glomerulonephritis (MPGN); focal and segmental glomerulosclerosis (FSGS); minimal change disease (MCD); crescentic/necrotic glomerulonephritis (CGN) without the criteria of systemic disease; membranous glomerulonephritis (MGN); diffuse endocapillary proliferative glomerulonephritis (DEPGN); and sclerosing glomerulonephritis (SGN). Secondary glomerulopathies were classified according to their association with systemic diseases, such as systemic lupus erythematosus, amyloidosis, diabetes mellitus, vasculitis, glomerulonephritis associated with infectious diseases (endocarditis) and hereditary disorders. (Paper I). Each biopsy, selected for studies II and III, was scored by two independent pathologists according to the Oxford Classification. A simplified score sheet of the Oxford classification of IgA nephropathy study was used (Roberts et al. 2009): total number of glomeruli, mesangial hypercellularity, M0/M1 (< or equivalent to 50% />50% of glomeruli showing >4 mesangial cells in one area); endocapillary proliferation, E0/E1 (present/ absent), segmental glomerulosclerosis/adhesion, S0/ S1 (present/ absent); glomerular membrane duplication, necrosis, cellular/fibrocellular crescent were categorized as present or absent; tubular atrophy/interstitial fibrosis, according to the Oxford classification, tubular atrophy/interstitial fibrosis is measured as percentage of cortical area involved by the tubular atrophy or interstitial fibrosis, whichever is greater. T0/T1/T2 was categorized as absent/ mild (0%–25%), moderate (26%–50%) or severe (>50%). Arteriosclerosis (the worst arterial vessel was scored) A0/A1/A2 was categorized as absent, < than media of arterial vessel and > than media of arterial vessel; arteriolar hyalinosis was categorized as absent or present. A0–A2 and arteriolar hyalinosis are not parts of the Oxford classification and the items were included in investigation as an important morphological features associated with hypertension. Interstitial fibrosis and tubular atrophy were taken as one item.

Immunofluorescence microscopy (IF). The IF findings were evaluated according to guidelines of the Oxford Classification. IgAN in the native kidney is defined as dominant or co-dominant staining with IgA in glomeruli by immunofluorescence. Not all glomeruli show this positivity. SLE-nephritis should be excluded. The intensity of IgA staining should be more than a trace. The distribution of IgA staining should include the presence of granular deposits in the mesangium, with or without capillary loop staining, a pure membranous, diffuse, global granular GBM staining pattern or a linear GBM staining pattern should be excluded. IgG and IgM in minority cases could present, but not in greater intensity than IgA; IgM might be prominent in sclerotic areas. C3 could be present.

4.5. Treatment of IgAN Patients (Papers II, III)

A drug treatment was prescribed to the patients who had lower eGFR, higher proteinuria and more severe histological lesions, while the patients with minimal clinical symptoms and the ones with near-normal kidney function remained without drug treatment. The treatment information included antihypertensive, immunosuppressive, fish oil and statins medications as well as tonsillectomy. Therapy with corticosteroids was not consistent and it has not been analysed separately in the current study. The data of the antihypertensive drug treatment were detailed, showing the number of medications and the classes of antihypertensives: the treatment with renin-angiotensin system blockers (RASb): angiotensin converting enzyme inhibitors or angiotensin receptor blockers and the treatment with calcium channel blockers (CCB). 45.8% of the patients did not receive any antihypertensive and immunosuppressive drug treatment. Thus, the patients were divided into two main study groups according to their drug-treatment: drug-treated and untreated patients' groups. Two subgroups among the patients receiving two different antihypertensive drugs were formed and statistically analysed: RASb- and CCB-receiving patients. Also, patients' subgroups with and without the presence of clinical and/or morphological risk factors were used for statistical analysis.

4.6. Clinicomorphological Correlations

Various correlations between the clinical and pathological variables were examined: between MAP, proteinuria, eGFR and the parts of MEST, and arteriosclerosis as well; between different levels of proteinuria and eGFR; between clinical and morphological risk factors, different treatmen regimens and eGFR(FU) (Papers II, III).

4.7. Follow-up Study

The duration of follow-up was the period from the histological diagnosis to the last check.

Most patients of the present study are continuously under the control at the departments of nephrology at the Tartu University Hospital or the hospitals of Tallinn. The clinical data were collected by the nephrologists at the end of the follow-up using a standard form of follow-up dataset. The mean follow-up of the cohort (64 patients) was 4.1 years (1–12 years) (Papers II–III).

4.8. Statistical Analysis

All the data were collected in a standard Excel spreadsheet and stored in a standard Excel database. The statistical analyses was used the Statistica 12.0 programme. Descriptive statistics was used to characterize the cohort. The qualitative data were presented as absolute and relative (percentage) frequencies. The quantitative data were expressed as the standard deviation intervals (means ± SD). A *P*-value <0.05 was considered to be statistically significant in all analyses (Papers I, II, III).

The means of the groups were compared using the Student's T-test. The annual incidence was defined as the number of new cases per year in relation to the total population, and expressed per 10x5 of population (Paper I).

The Spearman Rank Order Correlations were used to assess the bivariate relationships. The rate of the renal function decline, expressed as the slope of eGFR at the end of the follow-up as a clinical outcome, was analysed (Paper II).

The medians were compared using the Mann–Whitney U test. The continuous variables were compared using the Student's t-test for independent samples after verifying the normality of distribution using the Kolmogorov-Smirnov test or by the analysis of variance (ANOVA) when more patients' groups were compared. The differences of eGFR decrease between the study groups were assessed, using the Wilcoxon rank sum test for continuous variables. The univariate and multivariate logistic regressions were used to examine the correlations between independent and dependent variables. The independent variables included: age, gender, BMI, MAP, eGFR, proteinuria, smoking history. Spearman Rank Order Correlations were used to assess the bivariate relationships between clinical variables and morphological scores (Paper III).

5. RESULTS

5.1. Occurrence of Glomerulopathies in Estonia (Paper I)

Primary glomerulopathies (248 cases) comprised the main part (45.4%) of all informative kidney biopsies (total number 547) and IgAN formed the main part of them (88 cases, 35.5%). The information about the distribution of glomerular and tubulointerstitial diseases is summarized in **Table 2**. Among primary glomerulopathies, inflammatory damage of glomeruli dominated (63.4%). MesGN was the most common type of glomerular damage (41.2%) and IgAN formed the main part of it (n = 88, 35.5%), followed by DEPGN (n = 28, 11.3%) and CGN (n = 8, 3.2%), comprised of "pauci-immune" (ANCA-positive) glomerulonephritis (n = 2), pauci-immune (ANCA-negative) glomerulonephritis (n = 1), anti-glomerular basement membraane nephritis (n = 2) and non-classified CGN cases (n = 3). MPGN was a rare type of glomerular damage in Estonia (7.7%). Noninflammatory diseases of glomeruli (MCD, MGN and FSGS) comprised 34.6% of all primary glomerulopathy cases (50 males, 36 females), and 0.9% of cases formed SGN. FSGS was the most frequent disease (n = 40, 16.1%, mean age 49.7 ± 16.7 years), followed by MCD (n = 35, 14.1%, mean age 31.4 ± 18.8 years) and membranous nephropathy (n = 11, 4.4%, mean age 42.5 ± 11.8 years). Secondary glomerulopathies in Estonia composed 22.3% (n = 122) of all informative biopsies.

Table 2. Distribution of glomerular and tubulointerstitial diseases

Group of diseases	Number of patients, n	Percentage, %
Primary glomerulopathies	**248**	**45.4**
Mesangioproliferative GN	14	2.6
IgA nephropathy	88	16.1
Membranoproliferative GN	19	3.5
Focal and segmental glomerulosclerosis	40	7.3
Minimal change disease	35	6.4
Crescentic/necrotic GN	8	1.5
Membranous GN	11	2.0
Diffuse endocapillary proliferative GN	28	5.1
Sclerosing GN	5	0.9
Secondary glomerulonephritis	**122**	**22.3**
Lupus nephritis	41	7.5
Henoch-Schönlein purpura	3	0.5
Systemic vasculitis	9	1.6
Granulomatosis with polyangiitis	9	1.6
GN associated with infectious diseases	2	0.4
Renal amyloidosis	26	4.8
Diabetic glomerulosclerosis	8	1.5
Alport syndrome	2	0.4
Other hereditary disorders	22	4.0
Tubulointerstitial diseases	**45**	**8.2**
Other conditions	**132**	**24.1**
Total	**547**	**100.0**

GN – glomerulonephritis

Gender and age-related differencies of the cohort are shown in the original article (Paper I, Tables 2 and 3).

5.2. IgAN Study in the Estonian Cohort (Papers II, III)

5.2.1. Patients

Between January 2001 and December 2010, a total of 88 patients were diagnosed with primary IgAN by renal biopsy. A total of 73 biopsy-proven IgAN cases were selected and analysed in the IgAN study: 56 male and 32 female patients. The patients' mean age at the presentation was 33.7 years (16–76 years): 32.0 for males and 36.4 for females. This glomerulopathy was absent in the children's group (≤15 years) and only two cases (0.8%) of IgA nephropathy were registered in the elderly group. The mean age of the patients and gender distribution in the cohort is shown in the original article (Paper II, Table 1).

5.2.2. Clinical and Laboratory Findings

The clinical findings and laboratory data are presented in the original article II (Table 1).

The tabel shows the data of the whole cohort and separately the data of male and female patients. Te average of time between the onset and the first clinical presentation was 2.6 years (range 0.1–21.2 years). In general, the male patients at the time of diagnosis had relatively higher eGFR and nobody of male patients had immunosupression because of the proteinuria of secondary diseases.

For the whole cohort, asymptomatic microhematuria and asymptomatic microhematuria with proteinuria were the main presenting clinical syndromes, comprising 48% and 39% of all cases, respectively. Nephrotic syndrome and macroscopic haematuria, which were associated with acute respiratory infection, were rare at presentation (7% and 4%, respectively). There was only one patient with acute renal failure and chronic renal failure (eGFR<60 ml/min/1.73m²) in each group.

The MAP at the time of biopsy was 94.5±16.7 mmHg and 7% of the patients were hypertensive and/or were having an antihypertensive treatment.

The mean proteinuria at the time of biopsy was similar in both male and female patients, 0.95 g/24hrs and 0.86 g/24hrs, respectively; whereas 81% of the patients had mild proteinuria <1 g/24hrs.

The mean eGFR for all cases was 94.9±30.7 ml/min/1.73m², which was 100.5±32.7 ml/min/1.73m² and 85.8±25.3 ml/min/1.73m² for the male and female groups, respectively. To assess the CKD staging the eGFR data were used. The distribution of patients to the CKD G1, G2 and G3 stages at presentation was 55%, 34% and 10%, respectively (men 66%, 23%, 10% and women 37%, 52%, 11%), while 1 patient in the male group was within the G5 stage, and no cases within the G4 stage were registered. The distribution of

patients to the CKD G1, G2, G3 and G4 at the end of follow-up in whole cohort was 52%, 29%, 16% and 3%.

Other clinical data, such as BMI, smoking and tonsillectomy, showed little differences in presentation between the males and females: the mean BMI was higher than normal in both genders and the overweight/obese patients in the male and female groups composed 58% and 65%, respectively.

5.2.3. Pathomorphological Peculiarities

5.2.3.1. Light Microscopy (LM)

The frequency of pathological findings in 73 kidney biopsies is shown in **Figure 2.**

All cases. The typical finding in glomeruli was the expanding of mesangial area and the mesangial cell proliferation (73% of cases). These changes were mostly global and diffuse and varied in severity from mild to severe. Segmental glomerulosclerosis/adhesion was found in 52% of kidney biopsies. Endocapillary cellularity was registered in 32% of specimens. Crescents were found only in a few cases. Tubular atrophy/interstitial fibrosis were found in 10% of kidney biopsies; however, arteriosclerotic lesions were registered in 35% of all cases.

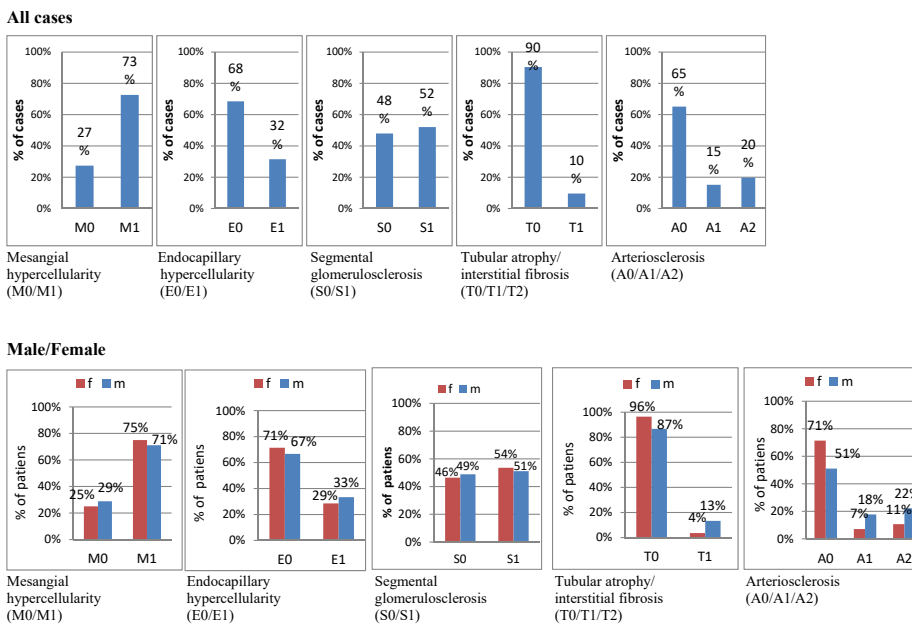


Figure 2. The frequency of pathological findings in 73 kidney biopsies

5.2.3.2. Immunofluorescence Microscopy (IF)

The IF findings were evaluated according to guidelines of the Oxford Classification. IgAN in the native kidney was defined as a dominant or codominant staining with IgA in glomeruli by immunofluorescence. Not all glomeruli showed this positivity. SLE-nephritis was excluded. The intensity of IgA staining was more than a trace (moderate (++) or marked (+++/++++)). The distribution of IgA staining included the presence of granular deposits in the mesangium, with or without capillary loop staining, a pure membranous, diffuse, global granular GBM staining pattern or a linear GBM staining pattern was excluded. IgM in minority cases was present, but not in greater intensity than IgA, and IgM was prominent in sclerotic areas. C3 was present in one third of cases.

5.2.3.3. Gender-related Differences

The gender related differences of pathomorphological findings in 73 kidney biopsies are shown in **Figure 2.** There were not significant differences in the glomerular lesions of the male and female groups. The differences appeared in tubular atrophy/interstitial fibrosis and arteriosclerosis categories with the male prevalence more than twice in both of them (male-female: 13%-4% and 40%-18%, respectively).

5.2.4. Follow-up Study (FU)

5.2.4.1. Patients' Clinical Characteristics at the End of the FU

Nine patients were lost to the follow-up. For the rest of 64 patients (59% male and 41% female), the mean follow-up was 4.1 years. The initial and FU clinical data are presented in the original article II, Table 1. The final comparison of the initial and the FU data were performed only in the cases where both periods were available for analysis (64 patients in total).

A drug treatment was prescribed for 58% of the patients. The patients in the drug-treated group were significantly older (37.5 *vs* 28.8, p = 0.04), more likely to be overweight (27.5 *vs* 25.2, p = 0.02), with higher serum creatinine level (106.1 *vs* 77.2, p = 0.02) and had lower eGFR than the patients in the untreated patients' group (76.5 *vs* 98.8, p = 0.01, respectively) (Paper III, Table 1). Table 2 in Paper III shows clinical picture and drugs prescription to the patients according to the clinical syndrome. RASb were prescribed to the patients having better kidney function and lower blood pressure. The lowest kidney function was noticed in the patients receiving CCBs (Paper III, Table 3).

At the end of the FU the presenting clinical syndromes in all studied patients were similar to the initial clinical presentation (Paper III, Table 2). Macrohematuria (11.1%), microhematuria (59.3%) and asymptomatic microhematuria with proteinuria (29.6%) were the leading syndromes among untreated patients whereas among drug-treated patients no macrohematuria was presented and less

microhematuria was registered (34.2%) but more microhematuria with proteinuria (50.0%) was found.

5.2.4.2. Clinicomorphological Correlations

The correlations between renal pathology and clinical findings at the time of biopsy

The frequency of the MEST findings was similar in males and females, except arteriosclerosis that was more frequent in the male patients (P=0.004). No correlation between the parts of the MEST score or arteriolosclerosis as well as the levels of MAP in the whole cohort and in both genders was found (Paper II, Table 2). However, M1, E1, S1, T1 and A2 were correlated with the levels of eGFR and proteinuria. The pathology scoring of the same categories revealed the differences in males and females as follows: a statistically significant correlation in males was observed between the parts of the MEST score and the eGFR, arteriosclerosis and proteinuria as well, except the E score, which didn't reveal significant correlation with proteinuria. In females, no significant statistical correlation between the parts of the MEST score and the eGFR was found, except the correlation between proteinuria and E, and S. The M did not reveal a correlation with eGFR; the arteriosclerosis score correlation with proteinuria in females was also statistically insignificant.

The lowest MEST score was present in the patients' group having macroscopic haematuria. Those patients did not receive treatment. The IgAN patients with nephrotic syndrome had the highest MEST score, the prominent clinical symptoms and, therefore, all of them received the continuous treatment with antihypertensives and temporary treatment with corticosteroids. A higher MEST score was also found in patients with asymptomatic microhematuria and proteinuria whereas two thirds (71%) of those patients received the continuous treatment with antihypertensives (Paper III, Table 2).

Correlations between a clinical presentation and an outcome

The decline of eGFR in males was faster than in females within a shorter follow-up time (**Figure 3**). The mean rate of renal function decline in the cohort was -2.6 ml/min/1.73 m² per year, P<0.05 (-3.4 ml/min/1.73 m² per year in males, P<0.05, and -0.7 ml/min/1.73 m² per year in females). The end point was reached in 3% of the cases (CKD G4 in two males and in one female) and no ESKD cases were registered (Paper II).

After analysing the patients in two study groups (drug-treated and untreated), many statistically significant correlations disappeared in the clinicopathological correlation analysis of the drug-treated patients' group and only a few correlations remained: M correlation between U-Prot (FU); E correlation with U-Prot (FU); S correlation with eGFR (FU) and U-Prot as well as with U-Prot (FU); T correlation with S-Creat and eGFR as well as with eGFR(FU). Thus, M1, E1, S1, T1 and A2 scores were correlated with the levels of MAP, eGFR

and proteinuria. A higher level of arteriosclerosis and the MEST parts was noticed in those patients whose proteinuria was higher and eGFR was lower at the end of the follow-up.

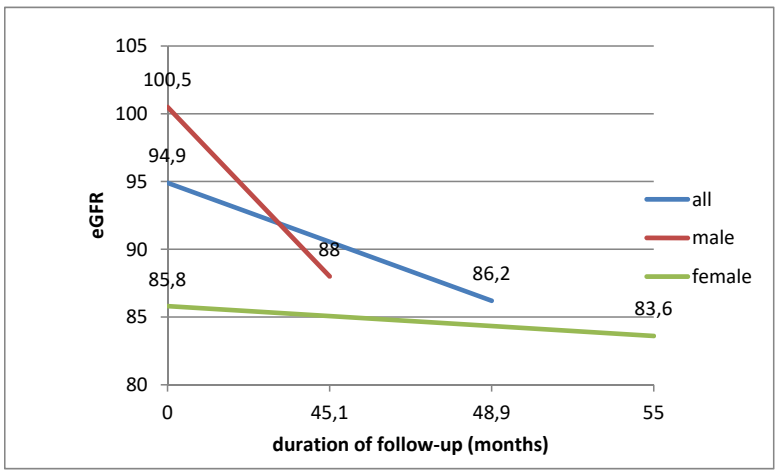


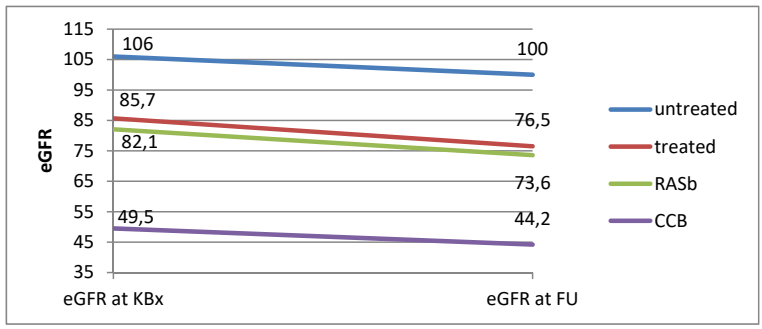
Figure 3. IgA nephropathy male and female patients' outcome showing eGFR decline during the follow-up.

In the untreated patients' group, only one statistically significant correlation between the pathomorphological and clinical risk factors was found: between arteriosclerosis and microhematuria (r_s = -0.4, p = 0.04).

5.2.4.3. IgAN Progression Risk Factors

The decline of mean eGFR was found in all study groups. (**Figure 4, A**). The changes of eGFR in IgAN patients were found as follows: eGFR decline was noticed in 54.8% of patients, in 12.3% of the patients eGFR remained at the same level and in 30.2% of the patients it slightly rose. By ANOVA, the decline of eGFR was noticed generally in all patients' subgroups notwithstanding which treatment they received or what kind of risk factors, clinical or morphological, they had. (**Figure 4, B**). The trend of eGFR decline was found among the subgroups of patients having clinical, morphological or both risk factors which statistically was insignificant. Using Mann–Whitney U comparison tests we analysed the IgAN treatment subgroups. The following statistically significant correlations in IgAN cohort were found: in patients with lower kidney function (eGFR <60 ml/min/1.73 m²), higher MAP ($p< 0.01$) and proteinuria irrespectively of the fact whether patients received ($p< 0.01$) or did not receive RASb ($p< 0.01$).

A



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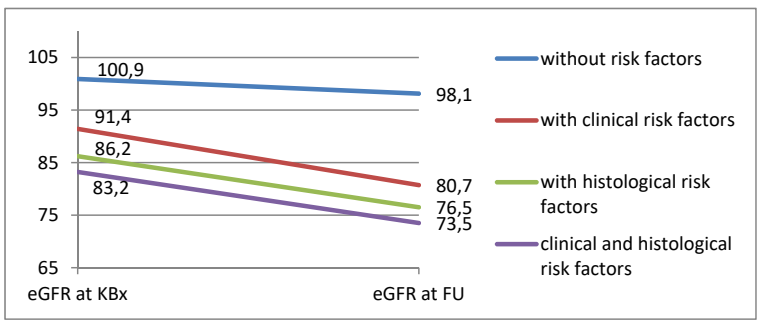

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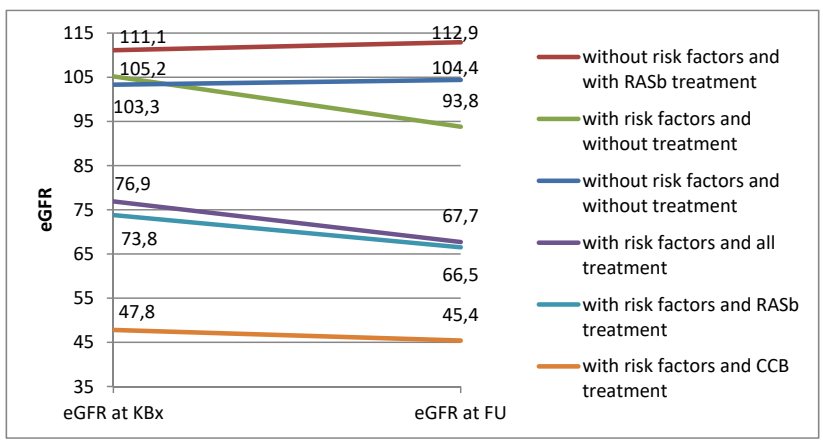

A – eGFR decline in the IgAN patients' subgroup according to treatment regimens: untreated (N = 27), all drug-treated (N = 38) and RASb- (N = 31) and CCB-treated (N=6).
B – eGFR decline in the IgAN patients' subgroups according to clinical and pathomorphological IgAN progression risk factors: without risk factors (N = 17), with clinical risk factors (N = 45), with histological risk factors (N = 38), with clinical and histological risk factors (N = 27).
C – eGFR in the IgAN patients' subgroups according to risk factors and the presence of treatment.
eGFR – estimated Glomerular Filtration Rate, KBx – kidney biopsy, FU – follow-up

Figure 4. eGFR decline according to treatment regimens and IgAN progression risk factors

The dispersion analysis showed that BP affected significantly the dispersal of eGFR ($p < 0.01$), especially when we estimated BP concurrence with smoking ($p = 0.01$). After the FU eGFR was lower in both study groups and more significantly among the patients with clinical and morphological risk factors (ANOVA). The biggest significant eGFR decline by the Wilcoxon rank sum test was found among the patients who had risk factors and received treatment. The result was confirmed by a post hoc analysis and did depend on the presence of treatment. In the investigation of subgroup receiving RASb we found that the decrease of eGFR did depend on the presence of clinical and morphological risk factors. Finally, we assessed the study subgroups separately according only to major clinical risk factors (overweight/obesity, hypertension, smoking and proteinuria), and the following results were found: in patients with major clinical risk factors and without drug treatment the decline of eGFR was 13% from initial but in those who had major clinical risk factors and received RASb it was 10.5%. (**Figure 4, C**). However, we did not find statistically significant reliability for these differences. Thus, there was not progression of IgA nephropathy in the patients groups without risk factors and without treatment, and without risk factors and with RASb treatment.

6. GENERAL DISCUSSION

6.1. Occurrence of Glomerulopathies

6.1.1. Indications for Renal Biopsy

The occurrence of glomerulopathies in biopsy material depends on the indications for a biopsy, which only indirectly reflect the spectrum of chronic kidney disease (CKD) in the population. The indications for performing a renal biopsy vary among nephrologists, stated largely by the presenting signs and symptoms. This is an important issue since there is a number of settings in which a renal biopsy is not indicated, thus avoiding the potential risk of bleeding and other complications associated with renal biopsy.

The overall rate of native kidney biopsy (in number of procedures per million population (pmp) varies from over 250 pmp in Australia to less than 75 pmp in the United States (Briganti et al. 2001). The renal biopsy rate is higher in adults than in children. These differences in the renal biopsy rate are not driven by the differences in the spectrum of renal pathology, but rather by the opinions regarding the value of the procedure in diagnosis, prognosis, and therapy. In many academic medical centers including ours, the biopsies of the transplanted kidney exceed those performed to diagnose a disease in the native kidney.

In the patients with **asymptomatic microscopic haematuria (AMH)** (ie, persistent microscopic haematuria with dysmorphic red blood cells, negative "dipstick" for proteinuria, normal serum creatinine concentration, and normal blood pressure), as well as **isolated non-nephrotic proteinuria (LGP)** (less than 0,5–1 g/24 h, ie, the absence of glomerular haematuria, normal renal function, and the absence of clinical or serologic evidence of a systemic disease that can cause glomerulonephritis), the renal biopsy may not alter therapy, as these patients generally have a good prognosis. So, in these cases a renal biopsy is generally not performed. When biopsies are performed, they typically demonstrate either a normal kidney biopsy or one of three disorders in persistent microscopic haematuria: IgAN, hereditary nephritis (Alport syndrome), or thin basement membrane disease; isolated non-nephrotic proteinuria may be present in e.g. systemic lupus erythematosus, vasculitis, or a paraproteinemia. Most patients with IgAN and thin basement membrane disease without proteinuria have a good long-term prognosis and, other than angiotensin-converting enzyme inhibitors, there is no clear effective therapy for any of these conditions. Some of the patients with isolated non-nephrotic proteinuria will have mild primary focal segmental glomerulosclerosis, IgA nephropathy, or membranous nephropathy (Hall et al. 2004); however, immunosuppressive therapy would not be indicated in this setting, since the prognosis with non-nephrotic proteinuria is often excellent.

As a result, a renal biopsy is not routinely performed in many medical centres accross the world for isolated asymptomatic microscopic haematuria or

isolated non-nephrotic proteinuria to establish a specific diagnosis, at least in the United States, unless there is coexisting proteinuria or the evidence of renal insufficiency. The biopsy is indicated for coexisting AMH and LGP identifying major and potentially progressive nephropathies in 70% of patients who should be managed by nephrologists (Richards et al. 1994, Hall et al. 2004, Coppo et al. 2010). However, these data suggest that the knowledge of renal histology is essential in the management of the patients with a renal disease. This is particularly true with IgA nephropathy since the majority of patients who are first seen with isolated microhematuria have a progressive disease (Szeto et al 2001). According to Coppo and collaborators, mild cases of IgAN are surely missed as screening programs do not exist, with the exception of some Asian countries, including Japan, Korea and Taiwan (Coppo et al. 2010).

In this light, in some European medical centres including ours and also in Japan, the patients with asymptomatic persistent microscopic haematuria are included in the kidney biopsy procedure which gives a diagnosis as well as a chronicity level to clinicians for the further management of the patients with early and mild IgAN disease or allows excluding hereditary disorders. And, this is also the purpose of the Oxford classification – to find out a MEST score which may help in the management of patients during a long-lasting disease course.

6.1.2. Occurrence of Glomerulopathies in the Estonian Population

Glomerulonephritis is a relatively rare disease with numerous subtypes which are possible to identify by renal biopsy. Our study presents the epidemiological data and the patterns of primary glomerulopathies diagnosed by renal biopsy over a 10-year period in Estonia. IgA nephropathy was the most common glomerular disease in our renal biopsy material (35.5%), comprising 34.7% of primary glomerulopathies in adult groups. The occurrence of IgAN in Estonia, as in most European countries, such as the Czech Republic, Italy, France, Romania, Portugal, Lithuania, Finland and Germany (Simon et al. 2004, Rychlik et al. 2004, Covic et al. 2006, Carvalho et al. 2006, Beitnaraite et al. 2007, Wirta et al. 2008, Werner et al. 2009, Braun et al. 2011), and some South American countries, e.g. Brazil (Polito et al. 2009) and in China as well (Li, Liu 2004) is the most common, with percentages of 28.9–46% typically found. Its incidence in the USA is around 22% (Pesce, Schena 2009). The low occurrence of IgAN in the USA may be explained by different indications and low frequency of renal biopsy. According to the 2009 IgA nephropathy study, IgAN frequency mostly depends on an ethnic group, and it is less frequent in some Asian countries; for example, in India and Saudi Arabia, and the IgA nephropathy percentages in South Africa were only 5.8–8.6% (Narasimhan et al. 2006, Jalalah 2009, Okpechi et al. 2010). It is possible that the differences of

occurrence in the black and white people in the USA and South Africa may be influenced by different indications for renal biopsy.

An important finding in our study was the fact that the occurrence of MPGN in Estonia was not high (n = 19, 7.7%, mean age 50.2 ± 18.0 years). The occurrence of MPGN is much lower than in the earlier period (11.7%) (Ots 1998). Infection with hepatitis C virus and human immunodeficiency virus (HIV) are recognized as an important cause and consequence of CKD (Izzedine et al. 2009), and the decreased prevalence of MPGN is probably associated with the better control of chronic persistent viral and bacterial infections. A low frequency of MPGN has also been reported in the other developed European countries (Polenakovic et al. 2003, Rychlik et al. 2004,Werner et al. 2009,Braun et al. 2011), in contrast with Romania where the frequency of MPGN has been shown to be still high, 29.4% (Covic et al. 2006).

Non-inflammatory glomerulopathies (FSGS, MCD and MGN) which usually manifest themselves with nephrotic syndrome, were also frequent in the biopsy material (34.6%). Comparing the data of 1991–1994 with those of 2001–2010, the increase in these glomerulopathies from 12% to 34.6% was reported (Ots 1998). In the present study FSGS was the most frequent disease (16.1%), followed by MCD (14.1%) and membranous nephropathy (4.4%). In the children's group, the changes towards non-inflammatory glomerulopathies were noticed. There were no cases of FSGS in the children's group (≤15 years old); however, MCD was the main non-inflammatory primary glomerulopathy in this group. Comparing the data of 1991–1994 with those of 2001–2010, the frequency of MCD in the children's group increased by 53.8% (n = 7 cases, previously n = 0 cases). This trend is probably related to the changes in the indication for renal biopsy, along with more active work by paediatric nephrologists in the intensive diagnostics of idiopathic nephrotic syndrome. The occurrence of MCD in adult groups was higher than in the children's group (n = 27, 11%). FSGS was the most frequent disease in the older adult group (n = 20, 8.0%), and 9 cases of FSGS were diagnosed in the elderly group. 11 cases of MGN were registered (five males and six females). The occurrence of non-inflammatory glomerulopathies in Estonia (34.6%) is comparable with the other studies performed in Western Germany (43%, Werner et al. 2009), Northern Germany (40%, Braun et al. 2011), Finland (20.5%, Wirta et al. 2008), the Czech Republic (31.2%, Rychlik et al. 2004), Lithuania (31.9%, Beitnaraite et al. 2007), Macedonia (30.6%, Polenakovic et al. 2003), Russia (26.8%, Dzhanaliev et al. 2002), Romania (31.2%, Covic et al. 2006), Iran (55.3%, Mohammadhoseiniakbari et al. 2009), Saudi Arabia (49–52.4%, Jalalah 2009) and India (38.4%, Narasimhan et al. 2006). The increase in the occurrence of primary FSGS, as observed in non-European countries such as Jamaica, Saudi Arabia, India and Brazil (Narasimhan et al. 2006, Jalalah 2009, Polito et al. 2009, Soyibo et al. 2009), could not be demonstrated in the Western and Central European countries and neither in Estonia.

The frequency of secondary glomerulopathies in Estonia (n = 122, 22.3%) was lower than in the other European countries (25.4–29.4%) and some South

American countries (55.5%), but similar or lower than in Asian (17.2%) and some non-European countries (Rychlik et al. 2004, Beitnaraite et al. 2007, Braun et al. 2011). Official databases clearly show that a policy in the renal biopsy practice plays an important role (Pesce, Schena 2009). The annual kidney biopsy rate per 10^5 of the population in Estonia (8.1) is comparable with the other European countries, exept Finland (17.6) (Wirta et al. 2008), reflecting a good biopsy indication policy. The annual incidence of biopsy-proven glomerulonephritis (any, primary or secondary) was 4.5 which is medium compared with the data of the other European centres (3.4–9.3) (Wirta et al. 2008). The Estonian population is very small (1.34 million) and therefore some rare diseases of glomeruli, such as cast nephropathy or paraproteinic deposition disease, and some hereditary diseases were found only in limited numbers. A kidney biopsy was not generally performed in patients with diabetes mellitus.

Our study contains the largest continuous single-centre renal biopsy material data in Estonia and reflects the demographic picture of the occurrence of glomerular diseases in the country.

6.2. IgAN Study in the Estonian Cohort

6.2.1. Patients

The study presents the long-term follow-up data of IgAN patients with the kidney biopsy diagnosis performed at the Tartu University Hospital. We applied the Oxford classification of IgAN to re-classify all cases. The main demographic, clinical and pathology data as well as the treatment of 64 Estonian IgAN patients were evaluated. The patients were followed for an average of 4.1 years similarly to some other IgAN studies (Cattran et al. 2009, Maixnerova et al. 2011, Le et al. 2011, Alamartine et al. 2011, Tanaka et al. 2013), representing the whole spectrum of the cases appearing in the local clinical practice.

Probably, the most interesting characteristics of the cohort were that more than half of the patients had only persistant microscopic haematuria presenting very early or a mild disease.

According to Coppo and collaborators, mild cases of IgAN are surely missed in many medical centres as screening programs do not exist (Coppo et al. 2010). Our IgAN patients' cohort contained main part (55%) of the patients having normal initial eGFR and only 11% of the patients having eGFR <60 ml/min/1.73 m² at the time of biopsy unlike other similar studies where clinicomorphological correlations, using the Oxford classification, have recently been investigated (Barbour et al. 2016). The mean eGFR of the patients of the Oxford original study (Cattran et al. 2009), North American validation study (Herzenberg et al. 2011) and VALIGA study (Coppo et al. 2014) was 68.4 ml/min/1.73 m² but in our cohort the mean eGFR was 94.9 ml/min/1.73 m² which means that the main part of our patients were biopsied in a clinically asymptomatic stage of the disease. However, this difference may give some

advantages like a duly identification and the subsequent mild disease, allowing to assess the impact of clinical and morphological risk factors on the renal function decline in the untreated IgAN patients and the patients with different treatment regimes.

6.2.2. Clinical and Morphological Data Correlations and Gender-related Differences in IgAN Patients

Our main findings were: first, as in the Oxford classification study (Roberts et al. 2009, Cattran et al. 2009), the Estonian cohort's pattern of mesangial hypercellularity, endocapillary hypercellularity, segmental sclerosis/adhesion and tubular atrophy/interstitial fibrosis (MEST) confirmed having a correlation with the clinical data as their higher score was linked to the eGFR decline; second, we found a statistically significant correlation between the M1 and the eGFR decline in males, but not in females. This finding shows that gender-related differences have a prognostic value of M1 in males.

Although gender-related differences have someway been observed or even reported in other papers (Cattran et al. 2009, Maixnerova et al. 2011, Le et al. 2011, Tanaka et al. 2013, Zeng et al. 2012), this investigation, in our opinion, is the first study directly addressing these differences and finding such an considerable result.

According to our study, higher mesangial score (M1) did not correlate with the eGFR decline in female patients, furthermore, it did not have an impact on an unfavourable IgAN outcome, showing little eGFR changes during the follow-up and highlighting the difference of the disease progression in males and females.

In the pathology baseline data we noticed a relatively better starting position in the male patients' group compared to females. According to the Oxford classification, M0E0S0T0 in male and female groups was 21% and 15%, respectively. However, among higher scores, female patients had more cases of higher mesangial scores (M1E0S0T0, 27% and 16% in female and male groups, respectively) and more cases of complex higher scores (M1E1S1T0 was 31% and 26%, respectively). Segmental sclerosis/adhesion cases in both groups were registered to a similar extent; however, the complex score M1S1 in males occurred more frequently (M1E0S1T0 score was observed in 37% males and 27% females) as well as the cases with T1 (13% and 4% in males and females, respectively). On the other hand, the baseline clinical data in males showed a correlation to the M1, whereas in females the M1 did not show any correlations with the eGFR (eGFR in females at M0 was 83.5±15.0 ml/min per 1.73 m² and at M1 was 86.6±28.1 ml/min per 1.73 m²). On the contrary, M1 was correlated with the eGFR in the whole cohort and in males. The other – S1 – was correlated with the proteinuria level and eGFR at the time of renal biopsy in both genders, T1 lesions with eGFR and proteinuria in males, E1 in females did not show correlation with eGFR, but showed correlation with proteinuria.

Some chronic glomerulonephritis studies have reported that females have a more favourable outcome in chronic glomerular disease, including IgAN, compared with males (Neugarten et al. 2000, Eriksen, Ingebretsen 2006) but other investigators have found either no gender-related differences or have observed women to be at a greater risk of a progressive loss of renal function (D'Amico 1992, Jafar et al. 2003). According to Cattran and collaborators, the IgAN outcome for female patients was reported as contrary results pointing to a worse disease outcome (Cattran et al. 2008). Cattran and collaborators, in their chronic glomerulonephritis study, found that a better outcome of IgAN disease is mostly mediated through both, lower proteinuria and blood pressure at presentation and throughout the follow-up, although the study reported that females did have an independent advantage at higher levels of proteinuria (Cattran et al. 2008). Our study confirms that proteinuria above 1.0 g/24hrs, which was associated with the E1 and S1 pathology scores, has a direct impact on the disease progression and on an unfavourable disease outcome in females. However, for the patients with proteinuria levels lower than 1.0 g/24hrs, the disease progression was faster in males. It is possible that the M1 score correlation with the eGFR decline plays an important role here, greatly affecting the progression of the disease in males.

Similarly to the others (Cattran et al. 2009, Maixnerova et al. 2011, Le et al. 2011, Tanaka et al. 2013), our results confirm the higher MEST parts (in the whole cohort and in the male group) being correlated with the disease progression. However, this was not a rule for female patients, where the M1 did not show any correlation with the eGFR decline. There is previously-published evidence available proving that the male gender is associated with a more rapid rate of progression of non-diabetic chronic kidney disease (Neugarten et al. 2000, Eriksen, Ingebretsen 2006, Silbiger, Neugarten 2008), either independently or through the modulation of other known risk factors. Our study confirms this in IgAN patients where we found a more rapid disease progression in males than in females although the renal biopsy revealed a milder pathomorphological picture.

This study also provides a novel insight into the importance of arteriosclerosis as well as microhematuria. Namely, we observed arteriosclerosis being correlated only with microhematuria in untreated patients. Whereas this morphological lesion had no significant correlation in the drug-treated study group and microhematuria has generally been considered as a benign clinical symptom, we speculate that, in the long term, microhematuria may have much bigger importance than expected because the decline of renal function was still found in the untreated study group patients. Recent findings suggest a pathogenic role for glomerular haematuria in kidney disease (Yuste et al. 2015). Although the majority of studies rule out a repercussion of haematuria on the renal outcome of patients with the IgAN, the true role of haematuria on a renal outcome remains uncertain (Moreno et al. 2016).

6.2.3. Clinical and Morphological Prognostic Risk Factors of Long-term Significance in the IgAN Progression

To summarise, our study confirmed that the most significant IgAN prognostic morphological risk factors are segmental glomerulosclerosis and tubular atrophy/interstitial fibrosis and the clinical ones – proteinuria and hypertension. In addition, in IgAN, overweight/obesity, present at diagnosis, is associated with an increase in the major risk factors (hypertension, proteinuria and severe renal lesions) which translate into a worse final outcome (Berthoux et al. 2013).

6.2.4. Long-term Outcome in IgAN Patients with Different Drug Treatment Regimes

In our study, drug treatment was prescribed to the patients who had lower eGFR, higher proteinuria and more severe histological lesions. The decline of eGFR was noticed in all study subgroups and changing CKD group into higher grade was noticed among 17% of the patients. As mentioned above, the mean eGFR was normal in the whole IgAN cohort and, although it declined at the end of the follow-up, it still stayed at a near-normal level (85.8 ml/min/1.73 m^2). The IgAN patients with less clinical symptoms (lower MAP, without proteinuria, with normal kidney function) stayed without treatment but the patients with severe clinical symptoms (higher MAP, microhematuria with proteinuria), lower kidney function and serious morphological lesions (S1/T1, 2) were treated. A similar management of IgAN where the drug-treatment is mostly indicated for more serious cases has also been described in many other studies (Barbour et al. 2016). The optimal approach to the treatment of IgAN is still uncertain but renoprotection is a well-known approach in the management of various chronic kidney diseases today following basic experimental (Anderson et al. 1985, Kato et al. 1999) and clinical landmark studies (Lewis et al. 1993, Brenner et al. 2001, Jafar et al. 2001). Our study revealed that RASb were generally effective in arresting the IgAN progression and diminishing proteinuria but RASb were not effective in patients with clinical and morphological risk factors present. When we assessed the study subgroups separately only according to clinical risk factors, the decline of eGFR was the lowest in the RASb treated patients with clinical risk factors. Thus, maximal renoprotection can be achieved when patients' blood pressure is normal, they do not smoke and keep their body weight within normal range. We were able to demonstrate that RASb were only effective in preventing the progression when clinical risk factors were modest or missing. The same question was raised by other authors but they have failed to find similar differences in the subgroups based on treatment exposures (Barbour et al. 2016).

Another aspect deserves to be pointed out. Similarly to RASb usage, CSs were also prescribed to the IgAN patients who had proteinuria, more serious morphological lesions and lower kidney function. In our cohort, the lowest

kidney function was noticed in the patients receiving CCBs or CSs. Interestingly, since the KDIGO guideline now recommends CSs to the patients with eGFR >50 ml/min/1.73 m², we have historically used these drugs in many patients below a recommended level. Similar findings have also been reported by Coppo et al. in VALIGA study where immunosuppression was more frequently used in the patients with lower eGFR (Coppo et al. 2014). These findings demonstrate nephrologists' aggressive therapeutic attitude in treating IgAN patients with more severe CKD and, on the other hand, leaving the patients without severe clinical symptoms untreated.

7. CONCLUSIONS

1. According to the study, we can conclude that during the period of 2001–2010, inflammatory glomerulopathies dominated in the spectrum of primary glomerulopathies in the Estonian population. Comparing this data with the data of the period of 1991–1994, the change towards non-inflammatory glomerulopathies was noticed. The distribution pattern of various glomerulopathies largely corresponds to the distribution described in the other European centres. IgA nephropathy has been the most common primary glomerulonephritis in our population and it has not changed over the time (Paper I).
2. Asymptomatic microhematuria and asymptomatic microhematuria with proteinuria were the main presenting clinical syndromes in IgAN patients, comprising 48% and 39% of all cases, respectively. Nephrotic syndrome and macroscopic haematuria, which were associated with acute respiratory infection, were rare at presentation (7% and 4%, respectively). The typical finding in glomeruli was the expanding of mesangial area and the mesangial cell proliferation (73% of cases). Segmental glomerulosclerosis/adhesion and tubular atrophy/interstitial fibrosis were found in 52% and 10% of kidney biopsies, respectively. (Paper II).
3. Mesangial hypercellularity, endocapillary hypercellularity, segmental sclerosis/adhesion and tubular atrophy/interstitial fibrosis (the MEST parts) have a correlation with the clinical data as their higher score was linked to the eGFR. We found a statistically significant correlation between the M1 and the eGFR in males, but not in females. This finding shows that gender-related differences have a prognostic value of M1 in males. The other – S1 – was correlated with the proteinuria level and eGFR at the time of renal biopsy in both genders, T1 lesions with eGFR and proteinuria in males, E1 in females did not show correlation with eGFR, but showed correlation with proteinuria. (Paper II).
4. The biggest eGFR decrease after the follow-up was found among the patients who had clinical and morphological risk factors (hypertension, proteinuria, overweight/obesity, and segmental glomerulosclerosis and tubular atrophy/interstitial fibrosis). Clinical risk factors had important influence on long-term outcome. More rapid IgAN progression occurred in males compared with females through faster eGFR decline that makes prognosis worse (Papers II–III).
5. Progression of IgA nephropathy was not found in the patients groups without risk factors and without treatment, and without risk factors and with RASb treatment. (Paper III).

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9. SUMMARY IN ESTONIAN

IgA nefropaatia uuring Oxford'i klassifikatsiooni järgi: IgA nefropaatia kliinilis-morfoloogilised korrelatsioonid, haiguse progresseerumise ja renoprotektiivse ravi efekti uuringud

Sissejuhatus

Enamik glomerulonefriite, isegi kõige sagedasemad, on harva esinevad haigused. Sellele vaatamata on nad kliinilises praktikas olulised, kuna patsiendid ei parane ja areneb progresseeruv krooniline neeruhaigus (KNH). (Floege et al. 2016). Immuunoglobuliin A nefropaatia (IgAN) on kõige sagedasem glomerulonefriit maailmas (Berger, Hinglais 1968, D'Amico 1987, Levy, Berger 1988, Glassock 2008). Kroonilise glomerulonefriidi haigeid on Eestis ligikaudu 30% lõpp-staadiumi neerupuudulikkuse haigete seas ja IgAN on olnud nende hulgas kõige sagedasem sarnaselt teistes maades läbiviidud uuringutele. Diabeetilise nefropaatia ja neerukahjustusega kõrgvererõhktõve kõrval on IgAN jäänud järgmiseks oluliseks tervishoiuprobleemiks nefroloogia valdkonnas, sest haigestuvad peamiselt just noored inimesed ja haigus areneb pidevalt aeglaselt edasi. Kliiniliselt progresseerub IgAN erineva kiirusega ja lõpp-staadiumi neeruhaigus tekib ligikaudu 15% patsientidest 10 aasta jooksul (D'Amico 2000), 30% kuni 40% patsientidest 20–30 aasta jooksul esimeste kliiniliste nähtude avaldumise järgselt (Lai KNe tal. 2016) ning umbes 50% patsientidest 25 aasta jooksul esmasest diagnoosist (Glassock 2008). IgA nefropaatia korral on neerud kahjustuse sihtmärgiks, kuigi esmane defekt pärineb süsteemsest aberrantsest O-seotud glükaanide glükosüleerimisest IgA1 ühenduspiirkonnas, mis põhjustab suurenenud galaktoosi-defitsiitset IgA1 (Gd-IgA1) taset vereseerumis. Immuunokeemilist hälvet ei ole võimalik neerutransplantatsiooniga korrigeerida, mis tähendab seda, et IgAN taasteke neerutransplantaadis on sage. Efektiivne ja spetsiifiline IgAN ravi tänapäevani puudub (Hsin-Hu YU et al. 2011, Lai et al. 2016).

Enamike KNH, sealhulgas IgA nefropaatia korral alaneb neerufunktsioon kuude või aastate jooksul, mis põhjustab progresseeruva neerukahjustuse ja enamikel juhtudel lõpp-staadiumi neerupuudulikkuse. KNH peamisteks põhjusteks on mitmesugused glomerulopaatiad, eelkõige glomerulonefriidid, suhkur- ja kõrgvererõhktõbi. Enamikel juhtudel kulgeb neerufunktsiooni süvenemine varjatult, väheste kliiniliste nähtudega. Peamiste KNH progresseerumise riskitegurite hulka kuuluvad hüpertensioon, proteinuuria, ülekaalulisus ning morfoloogiliselt avastatud kroonilisuse näitajad nagu segmentaarne glomeruloskleroos ja interstiitsiumi fibroos/tuubulite atroofia. Erinevates maades on kümnete tuhandete inimeste sõeluuringute käigus uriini ja neerufunktsiooni näitajate põhjal avastatud, et ligikaudu igal kümnendal inimesel esineb neerukahjustus: mõnel inimesel kergem, mõnel tõsisem neerukahjustus, mis lõpptulemusena võib vajada neerude tööd asendavat ravi – dialüüsi või neeru siirdamist. Need

ravimeetodid aga kuuluvad ühiskonnas kulukaimate ravimeetodite hulka, mida saavad endale lubada vaid arenenud riigid. Seetõttu pööratakse suurt tähelepanu KNH varajasele avastamisele ja progresseerumise riskitegurite kõrvaldamisele. Kahjuks kuulub ka IgAN nende KNH hulka, mille kulg on varjatud, sest algstaadiumides esineb enamikel juhtudel kliiniliselt vaid mikrohematuuria.

Päsmakestes esinev immuunpõletik areneb aeglaselt põhjustades aastate või aastakümnete jooksul ulatusliku neerukahjustuse, mis avaldub kliiniliselt neerupuudulikkuse sümptoomidena ja ureemia nähtudega. IgA nefropaatia varajaseks avastamiseks peavad pere- ja üldarstid olema väga tähelepanelikud uriini analüüsi interpreteerimisel ning hematuuria esinemisel kahtlustama IgAN esinemise võimalust. Kahjuks jõuavad nii meil kui mujal maailmas IgAN haiged nefroloogi juurde sageli hilinenult väljendunud kliiniliste ja morfoloogiliste neeruhaiguse progresseerumise riskitegurite esinemisega.

Nimetatud asjaolud on olnud aluseks rahvusvahelise IgAN töögrupi projektile ja hiljuti avaldati uus IgAN klassifikatsioon, mis põhines kliinilis-morfoloogilistele uuringutele (nn. Oxfordi klassifikatsioon). Selle klassifikatsiooni valideerimise projektis (VALIGA) osales ka käesoleva uurimistöö autor. Teaduskirjanduse andmetel on viimastel aastatel nüüd ilmunud mitmeid IgAN uurimusi, kus on kasutatud uut Oxfordi klassifikatsiooni. Kuid samas, arvestades IgAN leviku ulatust, võimalikke regionaalseid iseärasusi, neerubiopsia kättesaadavust erinevates keskustes, KNH progresseerumise riskitegurite varieeruvust, on veel uue klassifikatsiooni kasutuse ja kliinilis-morfoloogiliste korrelatsioonide kohta läbi viidud vähe uuringuid.

Käesoleva uurimustöö üldine eesmärk oli analüüsida ühe keskuse-põhist neerubiopsia materjali, leida IgA nefropaatia esinemissagedus ja võrrelda erinevat ravi saanud haigete kliinis-morfoloogilisi seoseid. Käesolevas dissertatsioonis hüpotiseerisime: 1) et IgA nefropaatia esinemissagedus ei ole oluliselt muutunud võrreldes varasemate sama keskuse uuringute tulemustega; 2) IgA nefropaatia progresseerumine on erinev meestel ja naistel; 3) renoprotektsioon omab IgA nefropaatia haigetel head efekti.

Uuringu eesmärgid

1. Leida erinevate glomerulopaatiate jaotuvus sealhulgas IgAN osakaal glomerulopaatiate seas neerubiopsia materjali põhjal Tartu Ülikooli Kliinikumis.
2. Analüüsida IgAN kliinilist leidu ja haiguse morfoloogiat biopteerimise ajal vastavalt Oxfordi klassifikatsioonile.
3. Hinnata kliiniliste ja morfoloogiliste andmete korrelatsioone IgAN patsientidel ja võrrelda nende soolisi erinevusi.
4. Hinnata kliinilisi ja morfoloogilisi riskitegureid ja nende pikaajalist mõju IgAN progresseerumisele.
5. Analüüsida renoprotektsiooni mõju IgAN patsientidel jälgimisperioodi järgselt.

Uuritavad ja uurimismeetodid

Kliinilis-morfoloogilised retrospektiivsed uuringud viidi läbi Tartu Ülikooli Kliinikumis (TÜK). Uuriti 2001–2010 kõikide neerubiopsiate materjal, kokku 578 juhtu. 31 juhtu arvati välja ebapiisava biopsia materjali tõttu. Kokku oli 547 patsientide neerubiopsia andmed lisatud uuringuse I (340 mest, 238 naist; keskmine vanus 39.9 ± 17.9 aastat). Viis protsenti patsientidest olid lapsed. Täpsed rahvaarvu andmed saadi Eesti Statistika andmebaasist neerubiopsia sageduse ja glomerulopaatiate esmaste juhtude levimuse arvutamiseks. 2010 aastal saabus neerubiopsia materjal TÜK Patoloogiateenistusse kõikidest Eesti haiglatest, mis võimaldas arvutad neerubiopsia sageduse, milleks oli 8.1 *per* 10^5 rahvastikust. (Artikkel I). Kümne aasta jooksul registreeriti 88 IgA nefropaatia juhtu (Artiklid II, III). Uuringust II–III arvati välja 15 patsienti, sest nende neerubiopsia materjal sisaldas vähem kui 8 päsmakest ja 73 IgAN patsiendist kohort oli moodustatud. Teiste maade uuringute andmetega võrdlemiseks määrasime patsientide vanusegrupid järgmiselt: lapsed ≤15, noored täiskasvanud 16–40, vanemad täiskasvanud 41–65 ja eakad >65 aasta vanused inimesed kõikides uuringutes.

Järeluuringu kohorti modustas 64 IgAN patsienti (59% mehed, 41% naised). Üheksa patsiendi andmeid ei saanud järeluuringus kasutada: 3 patsienti surid trauma või teiste haiguste tõttu, 1 patsiendil oli terminaalne neerupuudulikkus haiguse diagnoosimise ajal, 5 patsientdl ei olnud teostatud vajalikke uuringuid.

Uuringul on Tartu Ülikooli inimuuringute eetikakomitee luba.

Kliinilised ja laboratoorsed andmed koguti haiguslugude põhjal neerubiopsia teostamise ajal ja jälgimisperioodi lõpus. Uuringusse kaasati patsiendid kõikidest vanuse rühmadest. Koguti järgmisi kliinilisi andmeid: kliiniline sündroom, vererõhk, kehakaal, pikkus, suitsetamine, laboratoorsed analüüsid (kreatiniin, kolesterool, albumiin, valk uriinis), kasutatud ravimid. Seerumi kreatiniini taseme alusel arvutati glomerulaarfiltratsiooni kiirus (arvutuslik ehk *estimated*, eGFR) ning kehakaalu ja pikkuse alusel arvutati kehamassiindeks (KMI), kus KMI üle 25/30 peeti ülekaalulisteks/rasvunuteks . KNH jaotati vastavalt kaasaegsele ravijuhendile viide raskuskategooriasse (G1-G5) (KDIGO 2013). Kliinilised sündroomid IgAN esmase diagnoosi korral olid järgmised: asümptomaatiline mikrohematuuria, makrohematuuria, asümptomaatiline mikrohematuuria koos proteinuuriaga, nefrootiline sündroom, äge neerupuudulikkus, krooniline neerupuudulikkus.

Patomorfoloogilised andmed on kõik hinnatud ja klassifitseeritud käesoleva uurimistöö autori poolt. Iga bioptaat uuriti valgusmikroskoopiliselt ja immuunfluorestsents (IF) meetodi abil. Kogu uuritud neerubiopsia materjali põhjal klassifitseeriti neeruhaigused, süvendatult uuriti IgAN morfoloogiat lisaks teise, sõltumatu patoloogi poolt.

Käesolevas töös on kasutatud uue Oxfordi klassifikatsiooni töölehte, kus hinnati morfoloogilisi parameetreid järgnevalt: mesangiaalne hüpertsellulaarsus (M), endokapillaarne proliferatsioon (E), segmentaarne glomerulosklerоos (S) ja interstiitsiumi fibroos/tuubulite atroofia (T) – nn. MEST skoor. Iga IgAN

neerubiopsia juhu analüüsimisel hinnati erinevaid neerukoe piirkondi järgnevalt: totaalne päsmakeste arv, mesangiaalne hüpertsellulaarsus, M0/M1 (< või ekvivalentne 50%/ >50% päsmakestes rohkem kui 4 mesangiumi rakku ühes piirkonnas); endokapillaarne proliferatsioon, E0/E1 (esinemine/puudumine), segmentaarne glomeruloskleroos/adhesioon, S0/S1 (esinemine/puudumine); päsmakeste perifeersete kapillaarilingude membraanide duplikatsioon, nekroos, tsellulaarsed/fibrotsellulaarsed poolkuud (esinemine/puudumine). Interstitsiaalset fibroosi ja tuubulite atroofiat käsitleti ühe tunnusena. Vastavalt Oxfordi klassifikatsioonile, hinnati tuubulite atroofiat või interstitsiaalset fibroosi neerukoore osas, mis oli protsentuaalselt suurem. Tubulaarne atroofia/interstitisaalne fibroos, 0–25% (T0), 26–50% (T1), >50% (T2). Lisaks kategoriseeriti veresoonte muutused järgnevalt: arterioskleroos: puudumine (A0), mõõdukas (A1) (intima fibroos < kui veresoone seina lihaskest) või raskekujuline (A2) (intima fibroos > kui veresoone seina lihaskest); arteriolohüalinoos (esinemine/puudumine). IF leidu hinnati vastavalt Oxfordi klassifikatsiooni juhistele.

Statistiline analüüs. Kõik andmed oli kogutud ja salvestatud andmebaasis Excel. Kogutud andmete analüüsimiseks kasutati statistikaprogrammi Statistica 12.0, mille alusel on tehtud kirjeldav statistika kogutud morfoloogiliste ja kliiniliste andmete kohta (keskväärtused, standardhälbed) soo- ja vanusgruppide kaupa kohordis. Kvalitatiivsed andmed olid esitatud kui absoluutsed ja relatiivsed sagedused. Kvantitatiivsed andmed olid väljendatud kui standardhälvete intervallid, lubatud statistilise vea piiriks oli 5% ($p \leq 0.05$).

Erinevate uuritavate gruppide tunnuste keskväärtuste hindamisel ja varasemate andmetega võrdlemiseks kasutati Student's t-testi. Ühe aasta neerubiopsia esinemissagedus oli defineeritud kui neerubiopsiate arv aastas 10^5 rahvastikust.

Spearmani korrelatsiooni kasutati kahepoolsete seoste hindamiseks. Neerufunktsiooni vähenemise määr oli väljendatud kui eGFR kõver jälgimisperioodi lõpus, mida hinnati kui kliinilist tulemust. Mediaanid võrreldi kasutades Mann–Whitney U testi. Pidevad muutujad võrreldi kasutades Student t-testi sõltumatute valimite jaoks pärast normaaljaotuse kontrollimist. Kolmogorov-Smirnovi testi või dispersioonanalüüsi (ANOVA) kasutati, kui oli tarvis rohkemaid patsientide rühmi võrrelda. Hinnati eGFR languse erinevusi uuritud rühmade vahel kasutades Wilcoxoni summa testi pidevmuutujate erinevuste hindamiseks. Ühe- ja mitme muutujaga logistilist regressiooni kasutati uurimaks seoseid sõltumatu ja sõltuvate muutujate vahel. Sõltumatud muutujad olid: vanus, sugu, KMI, MAP, eGFR, proteinuuria, vererõhk, suitsetamine. Spearman korrelatsiooni kordajat kasutati kahepoolsete kliinilise muutujate ja morfoloogilised skooride vahel seoste hindamiseks.

Tulemused

1. Glomerulopaatiate esinemissagedus Eestis (Artikkel I)

Primaarsed glomerulopaatiad (248 juhtu) moodustasid peamise rühma (45,4%) kõikidest informatiivsetest neerubiopsiatest (547) ja IgAN moodustas nendest põhiosa (88 juhtu, 35,5%). Glomerulaar- ja tubulointerstitsiaalsete haiguste jaotus neerubiopsia materjali põhjal on esitatud artiklis I, Tabel 1. Primaarsete glomerulopaatiate seas põletikulised glomerulopaatiad domineerusid (63.4%). Mesangioproliferatiivne GN oli kõige sagedasem päsmakeste kahjustus (41.2%) ja IgAN moodustas kõige suurema osa sellest (n = 88, 35.5%), millele järgnes difuusne endokapillaarne proliferatiivne GN (n = 28, 11.3%) ja poolkuude tekkega GN (n = 8, 3.2%). Membranoproliferatiivne GN oli harva esinev päsmakeste kahjustus Eestis (7.7%). Mittepõletikulised glomerulopaatiad (minimaalsete muutustega haigus (MCD), membranoproliferatiivne GN ja fokaalsegmentaarne glomeruloskleroos (FSGS) moodustasid 34.6% kõikidest primaarsete glomerulopaatiate juhtudest (50 meest, 36 naist), ja 0.9% juhtudest moodustas skleroseeriv GN. FSGS oli kõige sagedasem (n = 40, 16.1%, keskmine vanus 49.7 ± 16.7 aastat), millele järgnes MCD (n = 35, 14.1%, keskmine vanus 31.4 ± 18.8 aastat) ja membranoosne GN (n = 11, 4.4%, keskmine vanus 42.5 ± 11.8 aastat). Sekundaarsed glomerulopatiad moodustasid 22.3% (n = 122) kõikidest informatiivsetest neerubiopsiatest.

Soo ja vanusega seotud erinevused kohordis on esitatud originaalartiklis (Artikkel I, Tabelid 2 ja 3).

2. IgAN uuring Eesti kohordis (Artiklid II, III)

Patsiendid

IgAN uurimuse jaoks selekteeriti 73 biopsiapõhist IgAN juhtu, mille moodustasid 62% mees- ja 38% naispatsiendid. Uuringust lülitati välja 15 patsienti ebapiisava neerukoe hulga tõttu (vähem kui 8 päsmakest). Patsientide keskmine vanus esmaste kliiniliste nähtude ajal oli keskmiselt 33,7 aastat (16–76 aastat): meestel 32,0 ja naistel 36,4 aastat. IgAN ei esinenud laste rühmas (≤15 aastat) ja ainult 2 juhtu oli registreeritud eakate rühmas (0.8%).

Kliinilised ja laboratoorsed andmed

Kliinilise ja laboratoorsed andmed on esitatud artiklis II, Tabel 1. Keskmine ajavahemik haiguse algusest ja esimesestest kliinilistest nähtudest oli 2,6 aastat. Üldiselt, diagnoosimise ajal oli meestel kõrgem eGFR ja mitte keegi neist ei olnud saanud immuunsupressiivset ravi sekundaarsete haiguste tõttu. Kõige sagedasemateks kliinilisteks sündroomideks olid IgAN korral mikrohematuuria ja asümptomaatiline mikrohematuuria proteinuuriaga, vastavalt 48% ja 39% juhtudest. Nefrootiline sündroom (NS) moodustas 7%, makrohematuuria 4% ja patsiente ägeda või kroonilise neerupuudulikkusega (eGFR<60 ml/min/1,73m²) oli kummaski rühmas üks haigusjuht. MAP neerubiopsia ajal oli 94,5± 16,7 mmHg ja 7% patsientidest olid hüpertensiivsed ja/või vajasid antihüperten-

siivset ravi. Keskmine proteinuuria neerubiopsia ajal meestel ja naistel oli sarnane, vastavalt 0,95 g/ööp ja 0,86 g/ööp ning 81% patsientidest esines vähene proteinuuria <1 g/ööp. Keskmine eGFR oli 94,9 ± 30,7 ml/min/1,73m² ja eraldi meestel 100,5 ± 32,7 ml/min/1,73m² ning naistel 85,8 ± 25,3 ml/min/ 1,73m². KNH raskuskategooria määramiseks kasutati eGFR andmeid: G1, G2 ja G3 kategooria esines vastavalt 55%, 34% ja 10% patsientidest kliinilise leiu avaldumise ajal, ühel patsiendil oli G4 raskuskategooria ja G5 raskuskategooria haigusjuhte ei olnud. Jälgimisperioodi lõpus oli patsientide jaotuvus KNH raskuskategooria järgi järgmine: G1 – 52%, G2 – 29%, G3 – 16% ja G4 – 3%.
Teiste kliiniliste andmete osas nagu KMI, suitsetamine või tonsillektoomiaei esinenud olulisi erinevusi meeste ja naiste vahel: KMI oli normist kõrgem mõlema soo patsientidel ja ülekaalulised/rasvunud patsiendid moodustasid meeste ja naiste rühmades vastavalt 58% ja 65%.

Patomorfoloogilised iseärasused

Tüüpiline päsmakeste leid oli mesangiaalse maatriksi hulga suurenemine ja mesangiaalsete rakkude proliferatsioon (73% juhtudest). Enamikel juhtudest olid need muutused globaalsed ja difuussed ning varieerusid kergest raskekujuliseni. Segmentaarset glomerulosklerоosi/adhesiooni leiti 52% neeru bioptaadis, endokapillaarset tsellulaarsust 32% proovides, üksikuid parietaalse nefroteeli poolkuid leiti mõnedes bioptaatides, tuubulite atroofiat/interstitsiaalset fibroosi esines 10% neeru bioptaatides ning arteriosklerootilist kahjustust esines 35% kõikidest juhtudest. A0-A2 ja arteriolohüalinoos ei ole Oxfordi klassifikatsiooni osa, kuid lülitati antud uuringusse kui oluline morfoloogiline tunnus, mis on seotud hüpertensiooni ja tõenäoliselt ka hematuuriaga. Viimasele sümptoomile ei ole varasemalt olulist tähtsust IgAN progresseerumisele pööratud. Päsmakeste kahjustuse mustris ei olnud olulisi muutusi meeste ja naiste gruppide vahel.

Muutused ilmnesid aga tuubulite atroofia/interstitsiaalse fibroosi ja arterioskleroosi kategooriates meeste prevaleerumisega rohkem kui kaks korda mõlemas neist, mehed-naised: vastavalt 13%–4% ja 40%–18%.

Järeluuring (FU)

Üheksa patsienti andmeid ei saanud jälgimisperioodi (*follow-up*, FU) lõpul kasutada, kuid ülejäänud 64 patsiendi jaoks oli FU 4,1 aastat. Esialgsed ja FU kliinilised andmed on esitatud artiklis II, Tabel 1. Lõplik esmaste ja FU andmete võrdlus teostati ainult nendel juhtudel, mil mõlema perioodi andmed olid analüüsi jaoks kättesaadavad (kokku 64 patsienti). Medikamentoosset ravi sai 58% patsientidest. Ravi saanute rühma patsiendid olid märkimisväärselt vanemad (37,5 vs 28,8, p = 0,04), ülekaalulisemad (27,5 vs 25,2, p = 0,02), kõrgema seerumi kreatiniini tasemega (106,1 vs 77,2, p = 0,02) ja nendel oli madalam eGFR kui ravi mittesaanud patsientidel (vastavalt 76,5 vs 98,8, p = 0,01) (Artikkel II, Tabel 1). Sarnane kliiniline pilt ja ravimite määramine leiti vastavalt kliinilisele sündroomile moodustatud patsientide rühmades (Artikkel III, Tabel 2). RASb oli määratud patsientidele, kellel oli parem neeru funktsioon ja

madalam vererõhk. Madalam neerufunktsioon esines aga kaltsiumikanali blokaatoreid (*calcium channel blockers*, CCB) saanud patsientidel (Artikkel III, Tabel 3).

FU lõpus esinenud kliinilised sündroomid IgAN haigetel oli üldiselt sarnased esmasele haiguse avaldumisele (Artikkel III, Tabel 2). Juhtivateks sündroomideks mitteravitute seas olid makrohematuuria (11,1%), mikrohematuuria (59,3%) ja asümptomaatiline mikrohematuuria proteinuuriaga (29,6%) ning ravitud patsientide seas makrohematuuria haigusjuhte ei registreeritud, vähem oli mikrohematuuria juhte (34,2%) ja rohkem proteinuuriaga mikrohematuuria haigusjuhte (50,0%).

Korrelatsioonid patomorfoloogiliste ja kliiniliste leidude vahel neerubiopsia ajal

MEST sagedus meestel ja naistel oli sarnane, välja arvatud arterioskleroos, mis oli sagedasem meespatsientidel (p=0,004). Korrelatsioone MEST osade ja arterioloskleroosi, samuti MAP taseme vahel kohordis ja mõlemas meeste ja naiste rühmas eraldi ei esinenud (Artikkel II, Tabel 2). Siiski, M1, E1, S1, T1 ja A2 korreleerusid eGFR ja proteinuuria tasemetega. Samade patomorfoloogiliste kategooriate korrelatsioonid selgitas välja mees- ja naispatsientide vahel järgmised erinevused: statistiliselt oluline korrelatsioon esines meeshaigete rühmas MEST osade ja eGFR ning arterioskleroosi ja proteinuuria vahel, E1 ei esinenud märkimisväärset korrelatsiooni proteinuuriaga. Naishaigete rühmas ei olnud usutavat korrelatsiooni MEST osade ja eGFR vahel, välja arvatud korrelatsioon proteinuuria ja E1 ning S1 vahel, M1 osas korrelatsiooni eGFRga ei esinenud.

Kõige madalam MEST skoor oli patsientidel makrohematuuriaga, kellel kliiniline leid oli vähene ja kes ei saanud ravi. IgAN patsientidel NSga oli kõige kõrgem MEST skoor ja kõige drastilisem kliiniline presentatsioon. Patsiendid NSga said kestvat antihüpertensiivset ravi ja ajutist ravi glükokortikosteroididega. Kõrgem MEST skoor leiti ka patsientidel asümptomaatilise mikrohematuuria ja proteinuuriaga, kusjuures 2/3 nendest patsientidest (71%) said kestvat antihüpertensiivset ravi (Artikkel III, Tabel 2). Ravi oli määratud patsientidele, kellel oli madalam eGFR, kõrgem proteinuuria ja raskem morfoloogiline leid. Minimaalse kliinilise kuluga patsientidele ei olnud ravi määratud.

Korrelatsioonid kliinilise presentatsiooni ja tulemuse vahel

Neerufunktsiooni alanemine eGFR alusel oli meestel kiirem kui naistel isegi lühemas FU perioodis (Artikkel II, Joonis 1). Neeru funktsioon alanes keskmiselt -2,6 ml/min/1,73 m² aastas, p<0,05 (-3,4 ml/min/1,73 m² meestel, p<0,05, ja -0,7 ml/min/1,73 m² naistel). Uuringu lõpus esines 3% juhtudest G4 raskuskategooria ja G5 juhte ei registreeritud (Artikkel II).

Ravitute ja mitteravitute haigete gruppide võrdlemisel leiti arvukalt statistiliselt olulisi korrelatsioone. Ravitud haigete uuringurühmas algselt esinenud paljud kliinilis-morfoloogilised olulised korrelatsioonid kadusid ja ainult mõned neist jäid nagu M korrelatsioon proteinuuriaga (FU), E korrelatsioon

proteinuuriaga (FU); S korrelatsioon eGFRga (FU) ja S korrelatsioon U-Prot (FU), mis oli olemas ka esimesel uuringul; T korrelatsioon kreatiniiniga ja eGFRga (FU). Seega, M1, E1, S1, T1 ja A2 korreleerusid MAP, eGFR ja proteinuuriaga. Kõrgem arterioskleroosi skoor ja MEST skoor leiti nendel patsientidel, kellel FU lõpus oli kõrgem proteinuuria ja madalam eGFR. Ravimata patsientide rühmas esines ainult üks statistiliselt oluline korrelatsioon patomorfoloogiliste ja kliiniliste riskifaktorite vahel: korrelatsioon arteriolo-skleroosi ja mikrohematuuria vahel (rs = -0,4, p = 0,04).

IgA progresseerumise riskitegurid

Neerufunktsiooni langus oli leitud kõikides uuritud patsientide rühmades (Artikkel III, Joonis 1A). eGFR muutused esinesid alljärgmiselt: eGFR alanemine registreeriti 54,8% patsientidest, 12% patsientidest jäi eGFR samale tasemele ja 30,2% patsientidest eGFR kergelt suurenes. ANOVA uuringu järgi leiti eGFR langus üldiselt kõikides uuringurühmades vaatamata sellele, kas nad said ravi või ei ja kas nendel esinesid kliinilised või morfoloogilised riski-faktorid. (Artikkel III, Joonis 1B). eGFR languse trend leiti nendes patsientide subgruppides, kus esinesid kliinilised, morfoloogilised või mõlemad riskitegurid, kuid mis statistiliselt jäi mitteusutavaks. Kasutades Mann-Whitney võrdlustesti analüüsisime IgAN ravi saanute rühmi eraldi. Analüüsi tulemusena leidsime, et patsientidel oli madalam neerufunktsioon (eGFR <60 ml/min/1.73 m²), kõrgem MAP ($p < 0,01$) ja proteinuuria vaatamata sellele kas patsiendid said ($p< 0,01$) või ei saanud RASb ravi ($p< 0,01$). FU proteinuuria taseme erinevusi ei olnud. Lõpuks moodustasime täiendavalt uuritavate rühmad vastavalt ainult kliinilistele riskiteguritele (ülekaalulisus/tüsedus, hüpertensioon, suitsetamine ja proteinuuria) ja leidsime järgneva tulemuse: ravita ja kliiniliste riskiteguritega patsientidel oli eGFR alanemine 13% algsest ning nendel patsientidel, kellel esinesid kliinilised riskitegurid ja kes said RASb ravi, eGFR langus oli 10,5%, siiski sellel erinevusel ei olnud statistilist usutavust. (Artikkel III, Joonis 1c).

Järeldused

1. Käesoleva uurimustöö raames läbi viidud neerubiopsia materjali analüüsil leiti, et primaarsete glomerulopaatiate rühmas esinesid kõige sagedamini glomerulonefriidid ehk põletikulised glomerulopaatiad, mille hulka kuulub ka IgAN. Võrreldes uuringu andmeid 1991–1994 läbiviidud uuringuga täheldasime muutust mittepõletikuliste glomerulopaatiate esinemise suurenemise suunas. Erinevate glomerulopaatiate levimus sarnaneb teiste Euroopa maade neerubiopsia uuringute tulemustega. IgA nefropaatia oli kõige sagedasem glomerulonefriit meie populatsioonis ja selle levimus ei ole muutunud aja jooksul. (Artikkel I).
2. Asümptomaatiline mikrohematuuria and asümptomaatiline mikrohematuuria proteinuuriaga olid peamised kliinilised sündroomid IgAN haigetel moodustades vastavalt 48% ja 39% kõikidest juhtudest. Nefrootiline sündroom ja makroskoopiline hematuuria, mis olid seotud peamiselt ägeda respiratoorse

infektsiooniga, esines harva biopteerimise ajal (vastavalt 7% ja 4%,). Tüüpiline leid päsmakestes oli mesangiaalse maatriksi rohkenemine ja mesangiaalsete rakkude proliferatsioon (73% juhtudest). Segmentaarne glomerulоskleroos/adhesioon ja tuubulite atroofia/interstitsiaalne fibroos esines vastavalt 52% ja 10% neerubiopsiatest. (Artikkel II).

3. Mesangiaalne hüpertsellulaarsus, endokapillaaarne hüpertsellulaarsus, segmentaarne skleroos/adhesioon ja tuubulite atroofia/interstitsiaalne fibroos (MEST) korreleerus kliiniliste andmetega ja MEST osade kõrgemad väärtused korreleerusid eGFR. Me leidsime statistiliselt usutava korrelatsiooni M1 ja eGFR vahel meestel, kuid mitte naistel. Teised MEST osad – S1 – korreleerus proteinuuria ja eGFR neerubiopsia ajal nii naistel kui meestel, T1 korreleerus meestel eGFR ja proteinuuriaga, E1 puhul ei leitud naistel korrelatsioone eGFR, kuid statistiliselt usutavad korrelatsioonid olid proteinuuriaga (Artikkel II).
4. Suurem eGFR langus jälgimisperioodi lõpus olid patsientidel, kellel esinesid nii kliinilised kui morfoloogilised riskitegurid (hüpertensioon, proteinuuria, ülekaal/rasvumus, segmentaarne glomerulоskleroos, tuubulite atroofia/ interstitsiaalne fibroos). Kliinilistel riskiteguritel oli oluline mõju kaugtulemustele. Kiirem IgA nefropaatia progresseerumine täheldati meestel, kellel esines suurem eGFR taseme langus. (Artikkel III).
5. IgA nefropaatia progresseerumist ei olnud patsientidel ilma riskifaktoriteta ja ravita ning ilma riskifaktoriteta ja RASb raviga rühmades (Paper III).

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