Noninvasive biomarkers in IgA nephropathy

IgA-nephropathy (IgAN) is the most common primary glomerulonephritis. It is considered to be an autoimmune disorder due to the pathogenesis underlying the disease. The formation of immune complexes consisting of galactose-deficient IgA1 (Gd-IgA1) and autoantibodies IgG/IgA1 against Gd-IgA1 is crucial for the development of the disease. These complexes form deposits in kidneys leading to mesangial cell activation, releasing inflammatory mediators, complement activation, and glomerular injury. Receptors: Fc alpha receptor (FcαRI/CDS9), transferrin receptor (TF, CD71), enzyme transglutaminase-2 (TG2), and interleukins are involved in this process. The gold standard for disease diagnosis is kidney biopsy. However, due to invasiveness and limitations of this procedure, there is an urgent need for new noninvasive biomarkers for monitoring course and progression of the disease and its response to treatment. This article focuses on noninvasive biomarkers that so far have been reported in the diagnosis of IgAN.

Introduction

IgA-nephropathy (IgAN) is the most common primary glomerulonephritis and is one of the leading causes of end-stage renal disease [1,2]. It presents with a wide spectrum of clinical and histological changes.

IgAN is diagnosed all over the world. It frequently appears in Asian and Caucasian populations while it is rare in African people [3]. There is also a large geographical diversity regarding gender: in Asia, it occurs with the same frequency in both sexes while in North America male-to-female ratio is 2-3:1 [4]. It usually affects people between 20-40 years of age [5].

Clinical symptoms of the disease are microscopic hematuria, proteinuria, hypertension and kidney disease in different stages. The onset of the disease is often accompanied by an infection of upper respiratory tract and episodes of macroscopic hematuria [4,6]. However, more than 50% of IgAN cases are asymptomatic and are recognized after the occurrence of proteinuria, hypertension or lower GFR which are all bad prognostic factors [7]. Biopsy samples of IgAN patients present with chronic glomerular inflammation, segmental glomerulosclerosis, mesangial hypercellularity and tubular-interstitial fibrosis [8].

Diagnosis of IgAN is established by biopsy samples with a presence of characteristic IgA-dominant glomerular immune deposits, frequently with complement C3-co-deposits, and presence of IgG or/and IgM. In 2009, an international group of nephrologists and renal pathologists published the Oxford classification that identified four specific pathological features considered prognostic values in disease progression regardless of clinical data: mesangial supercellularity, segmental glomerulosclerosis, excessive endocapillary cellularity and tubular atrophy / interstitial fibrosis. According to this classification, the pathologist is allowed to score each of these characteristics, which correlates with the clinical outcome [8,9].

Due to the invasiveness of kidney biopsy and its limitations, there is a need for noninvasive tests that enables to diagnose the subclinical cases of the disease, to monitor the course of IgAN and response...
to treatment and help identify patients with risk of progression to end-stage renal disease 

**Pathophysiology**

Galactose-deficient IgA1 (Gd-IgA1) molecule is considered to be the key factor in IgAN pathogenesis, however further studies are necessary. Human IgA has two subclasses: IgA1 and IgA2, which can exist in the form of polymer and monomer [6,10]. In healthy individuals, the hinge region of the IgA1 molecule is comprised of short O-linked oligosaccharide chains consisting of N-acetylgalactosamine conjugated with β1,3-linked galactose and sialic acid. There is strong evidence that another mechanism involved in the pathogenesis of IgAN is the release and activation via the mannose-binding lectin pathway. Component involvement in the disease would explain glomerular complement deposition in IgA nephropathy [16,17].

The mucosal immunity is also considered important in the pathogenesis of IgAN. One hypothesis assumes there is a connection on bowels and kidneys in the IgAN pathogenesis. Microbiota in guts may vary depending on different factors such as diet or environmental factors and may influence MALT activity. They control organisation of gut lymphoid tissue and take part in inflammatory bowel disease as well as autoimmune diseases.

Abnormal response to microbiota in guts, caused by incorrect immune tolerance, with changes in the intestinal barrier and activation of MALT can lead to subclinical intestinal inflammation. It may cause the abnormal reaction to food antigens and intestinal microbiota causing the production of Gd-IgA1, which after entering the circulation forms IgA mesangial deposits [6,18,19]. Mucosal immunity involvement in the pathogenesis of IgAN may be a new treatment target. Therapy with enteric budesonide, targeting the ileocolic region, provided some promising results in IgAN, avoiding systemic side effects of steroids [20].

**Biomarkers of IgA nephropathy**

**Galactose deficient IgA1 and IgA or IgG autoantibodies against gd-IgA1**

Galactose deficient immunoglobulin Gd-IgA1 was established to be an important biomarker in a disease with a specificity of 90% and sensitivity of 75% [21]. However, it should be remembered that high levels of Gd-IgA1 were found in patients with sporadic IgAN and healthy relatives, suggesting the involvement of other additional factors in the development of IgAN [22]. Recent studies have determined that elevated serum level of Gd-IgA1 is associated with worsening proteinuria and may be useful in predicting renal function impairment in IgAN patients [21,23,24]. A common method, used for years, allowing to measure a serum level of Gd-IgA1 is using a snail helix aspersa agglutinin (HAA) lectin-based assay. Several studies measuring serum level of Gd-IgA1 were based on this method [25,26]. However, the HAA-lectin-based assay has its limitations mainly concerning its stability and bioactivity, which depends on the source of lectin. Recently, a new lectin-independent method using monoclonal antibody KM55 against Gd-IgA1 was presented. Results from KM55 ELISA assay correlated positively with HAA lectin-based assay at the same time eliminating its disadvantages. In addition, KM55 may be used for detection of IgA glomerular deposits in IgAN patients [27].

Formation of IgG and IgA autoantibodies against Gd-IgA1 – second hit of pathogenesis – has also been the subject of interest over the last few years. A study of Berthoux et al. demonstrated that serum levels of aberrantly glycosylated IgA1 and autoimmune antibodies IgG and IgA against Gd-IgA1 at the time of biopsy were higher in IgAN patients. The mean follow-up was 13.8 years. Furthermore, increasing serum levels of autoantibodies correlated with poor clinical outcome and were strongly related to the progression of the disease toward dialysis or death [25]. Suzuki et al. presented a study that compared serum levels of Gd-IgA1, IgG and IgA autoantibodies as a healthy controls and patients suffering from chronic kidney disease. Serum level of each biomarker was significantly elevated in IgAN patients in comparison to CKD or healthy controls. In addition, higher serum levels of IgG autoantibodies against Gd-IgA1 correlated with higher proteinuria [28,29]. Placzek et al. have recently carried out research looking for the association between serum levels of Gd-IgA1 autoantigen and specific IgG and IgA autoantibodies against Gd-IgA1. Serum samples obtained from 136 patients with biopsy-proven IgAN, 76 patients with other renal disease and 106 healthy controls were analyzed for Gd-IgA1 and IgGl/IgA autoantibodies. Results revealed that only patients with IgAN presented association between serum levels of Gd-IgA1 and serum levels of Gd-IgA1 specific IgA autoantibodies. In CKD patients and healthy controls, no such correlation was found. Moreover, in IgAN patients, the association was observed only between serum levels of Gd-IgA1 autoantigen and IgG-IgA1 specific IgG autoantibodies. No association was found in Gd-IgA1 specific IgA autoantibodies, which suggest that Gd-IgA1 specific IgG autoantibody is predominantly exhibited isotype in IgAN patients [30].

There is also a correlation between the pre-transplantation serum levels of Gd-IgA1 and Gd-IgA1 specific IgG autoantibodies and the recurrence of IgA nephropathy after transplantation. Hence, they may be considered valuable biomarkers in predicting disease relapse after transplantation [31].

**Advanced oxidation protein products**

Oxidative stress pathways are activated in IgA nephropathy. Advanced oxidation protein products are markers of oxidative stress as well as effector molecules and mediator of inflammation process [32–34]. In one study serum samples from 292 IgAN patients and 69 healthy controls were collected with a mean follow up of 4.46 years. Patients with IgAN had higher serum AOPPs levels, compared to healthy controls. In addition, the study revealed that serum levels of AOPPs are correlated with proteinuria and decline in renal function, which makes them a valuable indicator of clinical disease activity. The correlation is strengthened by Gd-IgA1. For this reason, using AOPPs with the association of Gd-IgA1 as a combined disease progression biomarker is suggested [24]. Another study revealed that high renal AOPPs expression independently has a prognostic value in CKD and renal fibrosis progression in patients with early stage of IgAN [35].
Immune complexes

In IgAN patients the presence of circular immune complexes (CIC): IgA1-C3, IgA1-IGL, IgA1-IGM was reported [36]. Undergalactosylated IgA1 is recognized by naturally occurring anti-IgG antibodies - predominantly IgG isotype or much less often IgA1, which are specific for N-acetylgalactosamine of a hinge region of Gd-IgA1 [26,37]. Some of IgA1-CIC that escape hepatic catabolism deposit in kidneys and result in complement and mesangial cells activation, release of cytokines (e.g. TNF-alpha, TGF-beta) and trigger renal injury [38]. Several studies have been carried out and revealed that Gd-IgA1 – immune complexes from IgAN patients binds to mesangial cells with higher affinity than healthy controls or uncomplexed IgA1 [39,40]. Cultured human mesangial cells were used as a model in IgA1-IC bioactivity assessment. Researchers collected sera from IgAN patients and healthy controls using size-exclusion chromatography and determined that large-molecular-mass serum fractions containing Gd-IgA1 stimulated proliferation of mesangial cells in contrast to immune complexes, which were inhibitory. In addition, IgA1-depleted fractions were devoid of MC activation properties [40]. In native sera of IgAN patients, immune complexes stimulated cell proliferation more than similar weight complexes collected from a healthy control group, however, without statistical significance. Furthermore, greater proliferation was stimulated by serum complexes obtained during an episode of macroscopic hematuria than CIC collected in non-hematuric phase [40].

Study conducted by Matousovic et al. analyzed urine samples collected from 29 biopsy-proven IgAN patients (Group I), 27 non-IgAN proteinuric patients and 28 healthy subjects. The IgG and IgA levels were significantly higher in groups I and II in comparison to group III. The urinary levels of immune complexes were significantly higher in IgAN patients than in non-IgAN patients and healthy volunteers. Size-exclusion chromatography revealed IC with large-molecular-mass fractions (650-850kDa) Levels of IC in urine was closely associated with proteinuria [41].

Another study evaluated serum samples of 50 IgAN patients with clinical remission after steroid therapy with tolnaftate. Their clinical data and serum samples were followed up for 3-5 years. Cross-sectional analysis demonstrated that serum levels of Gd-IgA1, as well as IgA1 immune complexes, were significantly correlated with the degree of proteinuria and hematuria. In addition, the disappearance of hematuria was associated with the decrease of Gd-IgA1 and IgA1 serum levels. Therefore, the use of immune complexes as noninvasive disease activity biomarker is suggested [42].

Receptors in IgA nephropathy

Formation and deposition of immune complexes in kidneys are accompanied by several molecules: myeloid IgA Fc alpha receptor (FcR/CD89), transglutaminase-2 (TG2) and transferrin receptor (TIR/CD71) [14].

FcR/CD89 is type I receptor transmembrane glycoprotein specific for IgA, which binds both IgA1 and IgA2 subclasses with similar affinity. CD89 is expressed on human myeloid cells: monocytes/macrophages, neutrophils, Kupffer cells, dendritic cells and eosinophils and participates in the catabolism of immune complexes. After binding IgA and IgA-IC, CD89 activates macrophages and eliminates IgA from the circulation [14,43,44]. FcR/CD89 is not expressed on human mesangial cells and does not participate in binding IgA1-CIC to MC in kidneys [39]. Gd-IgA1 causes release of soluble CD89 (sCD89) as well as generation and amplification of immune complexes in circulation [12,44]. Launay et al. reported for the first time that in sera of IgAN patients sCD89 is bound with IgA in circulating soluble FcR/CD89-IgA complexes. Studies in mice revealed that deposition of IgA-CD89 in mesangium stimulates macrophage infiltration, matrix expansion and inflammatory reaction in glomeruli and interstitium, which leads to hematuria and proteinuria [45].

Vuong et al. demonstrated that there is a significant correlation between sCD89-IgA complex in sera of IgAN patients and disease severity. They measured levels of IgA-CD89 at the time of first diagnosis (ELISA). The significant association between level of complexes in IgAN patients with and without progression was revealed. In control group of patients with another glomerulonephritis also suffering from proteinuria no such correlation was found. Furthermore, during a follow-up of 5-15 years, an IgAN group of patients with disease progression serum levels of sCD89 remained stable and low while in the non-progression group they were continuously high [46]. The study also demonstrated that a significant correlation was found between one of the five CD89 genetic variants and levels of sCD89 complexes. The single-nucleotide polymorphism (SNP) rs11084377 probably regulates production and expression of sCD89. Nevertheless, no correlation between gene polymorphism and susceptibility to IgAN was revealed [46].

Berthelot et al. investigated cooperation between IgAN receptors and found that IgA1-CD89 may stimulate overexpression of transferrin receptor 1 and transglutaminase-2 and activate mesangial cells in an alternative way leading to the development of IgAN [15]. Another multi-center study enrolled 160 biopsy-proven Caucasian IgAN/HSPN patients and revealed lower levels of urinary CD89 and TG-2 in patients with active IgAN/HSPN in comparison to patients in complete remission. IgA/CDAg, IgA/TG2, and IgA/CD89xTG2 ratios were significantly higher in active IgAN than in complete or partial remission. CD89xTG2 were, on the other hand, lower in patients with active IgAN than in complete or partial remission [44]. IgA-CD89 complexes could also have a prognostic value in predicting IgAN recurrence after kidney transplantation [31].

Transferin receptor (TIR, CD71) is a protein localized on the surface of mesangial cells binds polymeric IgA1 [47]. CD71 binds IgA1 to a mesangial cell, and its expression is enhanced on proliferating mesangial cells in IgAN patients [13]. One study assessed kidney biopsies from patients with HSPN/Berger disease and compared to a control group of patients with another glomerulonephritis (lupus erythematosus, mesangial proliferative glomerulonephritis, steroid-sensitive minimal change nephrotic syndrome and others), several of which also had IgA deposits, and to a healthy control group. In normal kidneys, CD71 was not expressed on mesangial cells but only on the tubular epithelium. In contrast, in a group of IgAN patients 105/107 glomeruli had strong CD71 expression. In patients with another glomerulonephritis CD71 expression was associated with IgA deposits. The study demonstrated that CD71 expression is not specific for IgA nephropathy. However, CD71 is the primary cell surface receptor for IgA1 in mesangium [48]. Delanghe et al. developed a new sensitive latex-enhanced immunoconcentrometric assay and measured level of soluble transferrin receptor (sTIR) in urine samples collected from IgAN/HSPN patients. The study revealed higher levels of sTIR in patients with primary glomerulopathy than in healthy controls, which makes it a valuable biomarker for diagnosing and monitoring of the disease. In addition, urine concentrations of sTIR were higher in IgAN/HSPN comparing to other morphological types of glomerulonephritis [49].

TIR binds sCD89, and thus CD89-IgA1, which stimulates transglutaminase-2 induction and translocation to MC membrane. TG-2 in exchange induce expression of TIR on the surface of MC. Therefore transglutaminase-2 causes amplification loop increasing IgA1-CD89 deposits leading to cell activation and production of cytokines by these complexes, which triggers renal injury [15]. Urinary levels of FcR/CD89 and transglutaminase-2 (TG-2) in IgAN/HSPN patients were investigated. Concentrations of CD89 and TG-2 in urine were lower in IgAN/HSPN patients with active disease in comparison to patients with complete remission. The study also demonstrated a significant association between urinary CD89 and TG-2 levels and proteinuria. Therefore, urinary CD89 and TG-2 may be useful as a biomarker for identification of patients with active IgAN/HSPN [44].

Complement

Complement activation participates in the pathogenesis of IgA nephropathy (50). C3 complement deposits are present in more than 90% of IgAN kidney biopsies [5]. IgA is capable of activating complement through alternative or not-identified manana-binding lectin pathway [16,51]. Each pathway ends with activation of C3 convertase, which in the cooperation of other complement components results in assembly of the membrane attack com-
plex (C5b-9), called terminal pathway complete complex. Factor H (FH) is inhibitory for the alternative pathway [50]. Patients with IgAN have significantly higher urine levels of FH, MAC, and properdin (P) than healthy subjects. Furthermore, positive association between MAC and FH levels and serum creatinine, urinary b2 microglobulin, urinary N-acetyl-b-D-glucosaminidase, urinary protein, interstitial fibrosis and the percentage of glomerular sclerosis was observed [50]. One study with 162 biopsy-proven IgAN patients in nal injury in IgAN patients. Properdin correlated positively only with proteinuria [52]. One study evaluated urine samples of 162 biopsy-proven IgAN patients in comparison to 50 healthy volunteers and found that urinary levels of mannose-binding lectin (MBL) were significantly associated with renal function and proteinuria. IgAN patients had significantly higher urine MBL levels than healthy controls. There was also a correlation between MBL levels and histopathological parameters: mesangial hypercellularity, segmental glomerulosclerosis, endocapillary hypercellularity and tubular atrophy/interstitial fibrosis. In addition, at the end of the follow-up, non-remission patients had higher urine MBL level than patients in remission. For this reason, MBL is suggested to be a valuable biomarker for the prediction of disease progression and estimation of IgAN severity [53]. Onda et al. demonstrated that serum levels of complement components: CH50, C4, factor B, P, factor I, and factor H were significantly higher in IgAN patients than in healthy controls. However, the only C4 component was associated with poor renal outcome and can be a valuable biomarker in the prediction of disease progression [54].

Interleukins
Interleukins are also considered to be a useful biomarker of IgA nephropathy [55,56]. Interleukin-6 is produced in human kidneys by mesangial and tubular cells [56]. Urinary levels of IgAN patients were compared to healthy subjects in a study with a mean follow-up of 8.07 ± 1.72 years. Patients were divided into two groups (progressors and non-progressors) based on the renal function after follow-up time. It appeared that on enrollment progressors had significantly higher urinary IL-6 levels than non-progressors. Urinary IL-6 levels greater than 2.5 ng/day at diagnosis was associated with a 7.8-fold higher risk in comparison to urinary IL-6 levels <1.0 ng/day which makes it a promising predictor of long-term renal outcome [57]. It was also reported that high urinary IL-6 levels are correlated with histological progression in IgAN patients [58]. Also, a significant positive correlation between urinary IL-6 levels and chronic histological lesions was observed [59]. One study analyzed biopsies of IgAN patients by PCR and revealed an increased expression of IL-6 mRNA in the total renal cortex in comparison to healthy controls. In addition, renal IL-6 expression was strictly associated with the degree of tubulointerstitial damage [58].

Interleukin-2 is produced by activated T cells and has a crucial meaning in their proliferation. Activated T cells express receptors for IL-2 (IL-2R) and releases a soluble form of IL-2R (sIL-2R). For this reason, sIL-2 is considered to be a marker of continuous T cells activation [60]. Lundberg et al. measured serum levels of sIL-2R and analyzed its correlation with disease progression in IgAN patients in comparison to healthy controls in a mean follow-up of 5 years. It appeared that plasma sIL-2R levels were significantly higher in IgAN patients comparing to healthy subjects and it was positively associated with the serum creatinine values and yearly GFR decline. Furthermore, biopsy samples of patients with high serum sIL-2R levels presented advanced tubulointerstitial fibrosis. sIL-2R had a prognostic value in prediction of severe renal outcome and disease progression [60].

IL-10 [61] and IL-17 [62] are also considered to play a role in the pathogenesis of IgAN. Chemokines involved in tubulointerstitial fibrosis were also considered to be a valuable biomarker for diagnosis and monitoring treatment effects [59].

Urinary podocalyxin
Podocalyxin (PCX) is a sialomucin expressed, among others, by kidney podocytes. It covers the apical cell surface of podocyte and regulates its adhesion and morphology. PCX is also crucial for proper kidney development [63]. In injured podocytes, a sialomucin is released into the urine. Therefore, in one study urine samples from 51 IgAN patients were collected on the day of biopsy and analyzed the correlation between u-PCX and histological assessment. It appeared that levels of u-PCX and the number of urinary podocytes were significantly associated with glomerular abnormalities in biopsy specimens. Moreover, u-PCX correlated with the activity of IgAN in the acute phase [64]. A study carried out on Japanese children reported that u-PCX might be a valuable indicator of glomerular injury in IgAN [65]. Also, urinary podocytes are suggested to be a reliable marker of IgAN severity assessment [66].

Other
There are some other molecules considered to be a valuable biomarkers for diagnosis and monitoring of IgA nephropathy.

Monocyte chemoattractant protein-1 (MCP-1)
Stimulated monocytes release MCP-1 and MCP-2. MCP-1 can also be secreted by renal resident cells: mesangial cells and tubular epithelial cells. It is one of the best-known chemokines involved in renal the injury, implicated with the crescentic and fibrogenic process [67,68]. Stangou et al. carried out a prospective study on IgAN patients and found that their urine MCP-1 levels were significantly higher than in healthy subjects. In addition, a significant positive association between u-MCP-1 levels and degree of histological damage and proteinuria was reported [59]. Researchers from Spain also observed a positive correlation between u-MCP-1 and degree of interstitial lesions [69].

Epidermal growth factor (EGF)
EGF is a peptide produced in Henle’s loop and the distal tubule [56], which participates in the regeneration process after acute tubular injury and accelerates renal function recovery [70]. In IgAN patients a negative correlation between EGF renal expression as well as its urinary levels and progression of renal damage was reported [56]. A prospective study also revealed a significant association between EGF excretion and creatinine clearance and suggested it to be a reliable biomarker in predicting disease outcome as well as in monitoring treatment effects [59].

B-cell activating factor (BAFF)
BAFF belonging to tumor necrosis factor family is responsible for B-cell development and homeostasis. Serum BAFF levels were significantly higher in patients with IgAN than healthy controls and correlated with IgA deposition in glomeruli [71]. BAFF was also associated with serum creatinine level and the severity of tubular atrophy/interstitial fibrosis [72].

N-acetyl-b-D-glucosaminidase
N-acetyl-b-D-glucosaminidase (NAG) originates from proximal tubular cells is suggested to be a biomarker of tubular damage. Machiguchi et al. observed significantly higher urine NAG levels in IgAN patients than in healthy subjects. In addition, an inverse correlation between serum Epo levels and u-NAG was reported. U-NAG was a good indicator of progression in both clinical and histological findings and reflected severity of IgAN [73].

MicroRNAs
MicroRNAs are a short noncoding RNA which play a role in the regulation of gene expression at the post-transcriptional level. In IgAN a dysregulation of mRNAs was observed [74]. Considering that mRNA is stable and easily accessible in body fluids [75], its potential as a new biomarker of IgA nephropathy was investigated [74-79]. Wang et al. revealed that intrarenal and urinary levels of miR-146a and miR-155 are significantly elevated in IgAN patients in comparison to healthy controls. In addition, an inverse correlation between intrarenal levels of miR-146a and miR-155 and estimated glomerular filtration rate (eGFR) was reported. Both intrarenal and urinary levels of miR-146a and miR-155 positively correlated with proteinuria. Intrarenal level of miR-155 was also significantly associated with tubulointerstitial scarring [76]. IgAN patients had significantly lower urinary miR-29b and miR-29c, and higher miR-93 levels than healthy subjects. A correlation between miR-29b, miR-29c and proteinuria, as well as the renal function, was ob-


