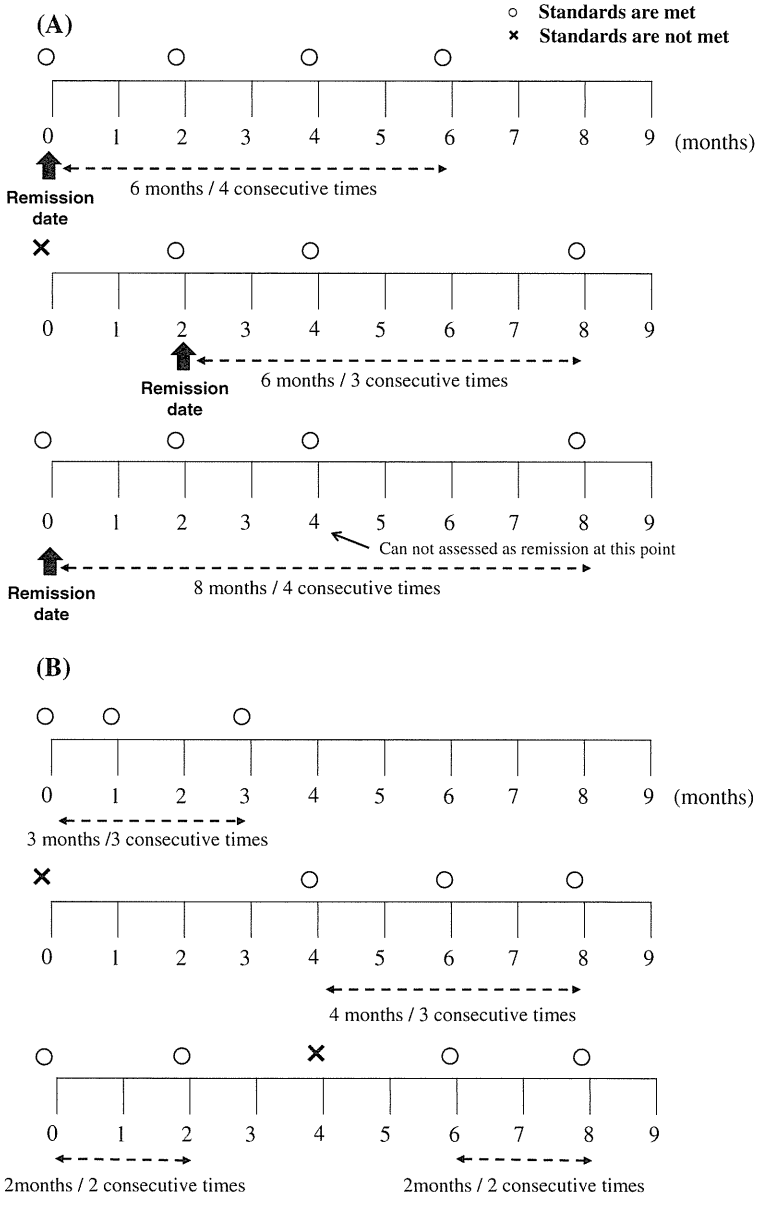

Fig. 1 **a** Examples that can be assessed as remission. All cases are those in which the criteria were met in tests over 6 months or more; from the first day, the criteria were met for 3 consecutive times or more. **b** Examples that cannot be assessed as remission. All cases are those in which the criteria were not met in over of 6 months or more; from the first day, the criteria were not met for 3 consecutive times or more

used in evaluation of diabetic nephropathy. However, these criteria may be not practical, at least in the early stages, for patients with long-term disease. Although the proposed criteria may not apply to all types of treatment response, such as in cases with improvement but no remission after treatment, it may allow nephrologists to make a uniform assessment of treatment outcome. Standardized and universally accepted explanations of patient condition after treatment will be useful for physicians in daily practice and for treatment of patients with IgA nephropathy.

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EXPERT OPINION

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Paradigm shift in activity assessment of IgA nephropathy – optimizing the next generation of diagnostic and therapeutic maneuvers via glycan targeting

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Introduction: IgA nephropathy (IgAN) is the most common glomerular disease and has a poor prognosis. Appropriate therapeutic strategies are not currently available due to the lack of information regarding IgAN pathogenesis and the absence of appropriate tools to assess disease activity in IgAN, a long-term chronic disease. However, recent evidence revealed that aberrantly glycosylated serum IgA1, mostly galactose-deficient IgA1 (Gd-IgA1) and immune complexes (ICs) with autoantibodies against glycan-containing epitopes on Gd-IgA1 are essential effector molecules.

Areas covered: Assessing disease activity by urinalysis/renal biopsy has some limitations, resulting in conflicts regarding the efficacy of possible IgAN-specific therapies. We summarize the characteristics and molecular basis of Gd-IgA1 and related ICs, their clinical application for activity assessment and early diagnosis, and discuss glycan as a potent target of therapeutic agents based on glycan engineering in IgAN.

Expert opinion: Recently, Gd-IgA1 and related ICs have shown clinical value for disease activity assessment and IgAN diagnosis. This suggests a paradigm shift in IgAN treatment thus allowing development of appropriate clinical trials of patients with IgAN stages and objective evaluation of the efficacy of future treatments. Early screening and diagnosis may increase therapeutic options, including quantitative regulation of nephritogenic Gd-IgA1 using therapeutic antibodies and selective depletion of Gd-IgA1-producing cells via glycan engineering.

Keywords: anti-glycan antibody, biomarker, disease activity assessment, early diagnosis, galactose-deficient IgA1, glycan engineering, IgA immune complex, IgA nephropathy

Expert Opin. Biol. Ther. [Early Online]

1. Introduction

Immunohistological analysis is the gold standard for diagnosing IgA nephropathy (IgAN), in which dominant or co-dominant mesangial deposition of IgA is essential. Elevated levels of circulating IgA immune complexes (ICs) have been reported in a significant number of patients with IgAN [1-3]. Thus, primary IgAN can be considered an IC-mediated glomerulonephritis that is immunohistologically defined by the presence of glomerular IgA deposits that instigate glomerular damage via unknown mechanisms [4]. The primary abnormal manifestation of IgAN is recurring bouts of hematuria with or without proteinuria. However, the disease spectrum

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Article highlights.

- IgAN remains a disease with a poor prognosis, which may be partly due to the limitations of existing methods for diagnosing the disease and assessing its activity. Additionally, the unclear elucidation of pathogenesis and absence of a conclusive therapeutic strategy hampers prognosis.
- Present disease activity assessment by urinalysis/renal biopsy has some limitations, resulting in conflicts regarding the efficacy of possible IgAN-specific therapies.
- Gd-IgA1 and related ICs are essential effector molecules in IgAN, and thus can be used as novel biomarkers.
- Specific measurement of circulating Gd-IgA1 and related ICs has clinical value for disease activity assessment and IgAN diagnosis.
- Noninvasive and real-time assessments with these biomarkers in combination with present biochemical and histological methods will provide a paradigm shift in IgAN clinical practice, including early diagnosis and objective evaluation of the efficacy of future treatments.
- In addition, further biochemical and molecular understanding of these biomarkers will optimize the next generation of diagnostic and therapeutic manoeuvres through glycan engineering.

This box summarizes key points contained in the article.

of IgAN is composed of many common manifestations. In clinical practice, ~ 30 – 40% of patients with IgAN progress to end-stage kidney disease (ESKD) within 20 years [4,5], whereas 10 – 20% of patients show spontaneous clinical remission [4-8]. However, there is currently no definitive method to predict these different outcomes. Thus, the highly variable clinical course and unpredictable progression of IgAN partly hinder the development of a treatment strategy.

In Japan, where urine analysis is conducted annually from childhood, there are many cases in which an early diagnosis and treatment are possible. However, intervention for many patients is delayed, resulting in frequent relapses, treatment resistance and decreased renal function. Therefore, even in Japan, IgAN remains a disease with a poor prognosis, which may be partly due to the limitations of existing methods for diagnosis and activity assessment of this disease, in addition to the lack of clear elucidation of the pathogenesis and a conclusive therapeutic strategy.

2. Paradigm shift in IgAN activity assessment is required for next-generation therapies

2.1 Limitations of proteinuria in IgAN activity assessment

A recent report based on the Japan Renal Biopsy Registry (2009 – 2010) revealed that 55% of patients with IgAN were diagnosed before the age of 40 years and ~ 70% of patients with IgAN are diagnosed with stage 1 or 2 chronic kidney disease (CKD) [9]. Therefore, in Japan, early diagnosis can be achieved via annual urinalysis. Indeed, around 75% of Japanese patients with IgAN are initially diagnosed through chance hematuria in urinalysis. This scenario also indicates that hematuria may be an initial manifestation of IgAN.

Although hematuria is a risk factor for the development of IgAN [10], the degree of proteinuria presents a greater risk for disease. This observation makes sense because glomerular injury events leading to hematuria may precede those leading to persistent proteinuria. In fact, previous epidemiological studies assessing risk factors for CKD [11,12] further support the idea that hematuria is a risk factor for proteinuria.

At present, the degree of proteinuria is considered one of the most important prognostic factors not only for IgAN, but for all renal diseases [12,13]. There have been a substantial number of clinical research studies on renal disease [14,15] in which both a decrease in kidney function and proteinuria have been evaluated as end points. Therefore, many clinical guidelines recommend therapeutic modalities based on the degree of proteinuria [16,17].

However, there are many cases in which both hematuria and proteinuria occur in the acute phase of disease and, with time, hematuria resolves and only proteinuria is observed. The possibility cannot be excluded that proteinuria in these patients is not an inflammatory reaction triggered by the IgA deposition in the glomeruli, which is a defining feature of true IgAN but is dependent on the so-called 'common pathway' accompanying glomerulosclerosis and nephron reduction. In general, IgAN may have a 20-year disease course with a mixture of acute inflammatory lesions with IgA-dependent specific activity (**Figure 1A**, blue triangle) and the activation of the common pathway results in chronic fibrotic lesions (**Figure 1A**, red triangle) [4]. Therefore, proteinuria may be derived from both types of lesions, which makes it difficult to qualitatively discriminate proteinuria at points A and B by present urinalysis (**Figure 1B**), suggesting the limitation of proteinuria-based disease activity assessment.

2.2 Necessity of noninvasive and real-time assessing the IgAN activity

Pathological analysis of renal biopsy tissue is the gold standard for diagnosing IgAN, as well as for assessing disease activity and renal prognosis. However, pathological findings may differ according to the timing of renal biopsy during the 20-year course of IgAN (**Figure 1A**, arrows A–C) [4,5]. In many countries, abnormalities identified during urinalysis may be overlooked or purposely not followed-up by further examinations until impairment of renal function becomes evident [18]. This raises a controversial issue among nephrologists of whether to perform renal biopsy in circumstances without renal function impairment or nephritic range proteinuria because of the perception that specific treatment is not yet available. Commonly, renal biopsy is not recommended for patients presenting with isolated hematuria or mild proteinuria in the UK, Canada or the US, as renal biopsy is reserved

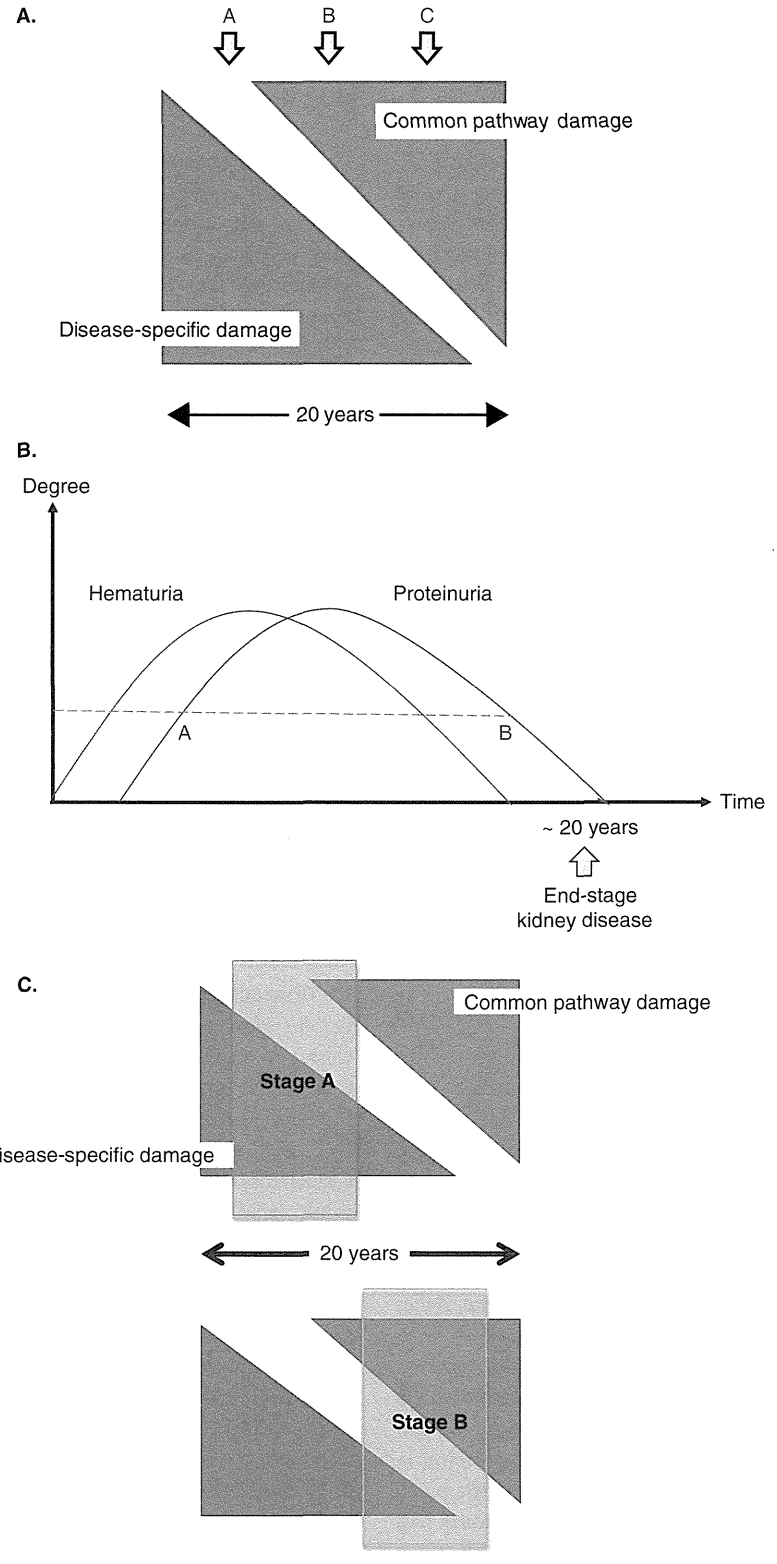

Figure 1. Image of clinical course of IgA nephropathy.

for those with increasing proteinuria or worsening renal function [18]. On the other hand, in Japan, symptom-free individuals with microscopic hematuria with or without mild proteinuria are more likely to undergo renal biopsy, leading to an increase in the diagnosis of IgAN.

Variations in intervention periods may yield different findings on renal biopsy. For example, in studies using a renal biopsy database from the southern US, IgAN was commonly identified in renal biopsy tissue specimens from patients aged < 40 years, whereas focal segmental glomerulosclerosis (FSGS) was reportedly the primary finding in patients aged ≥ 40 years [19]. The authors pointed out that although primary FSGS was most common in non-white individuals aged ≥ 40 years, secondary FSGS was more common in Caucasians because of 'burned-out' IgAN. Moreover, renal biopsy is not frequently performed because of procedural risks and/or limited insurance coverage. These observations emphasize that in some cases, renal biopsy provides only a snapshot of the disease status and thus has limitations in assessing disease activity, although a refined glomerular histologic grading system provides important activity information and stands as an independent morphological predictor of renal outcome in IgAN [20]. Furthermore, even if biopsy is performed early in the course of disease, findings may be inconclusive and prognosis may be difficult to predict. Even mild IgAN, presenting as hematuria or mild proteinuria with mild histological lesions at the time of renal biopsy, progresses to renal failure in 30% of cases [6-8]. To determine disease stage, a noninvasive, real-time activity assessment method in combination with present assessment by renal biopsy is desirable.

2.3 Need of reasonable activity assessment for future curative treatment of IgAN

Such limitations to present disease activity assessment techniques such as urinalysis and renal biopsy demonstrate practical problems that impede the development of IgAN-specific therapy. In fact, there are currently discussions underway between western countries and Japan to resolve the conflict regarding the benefits of tonsillectomy [21], although general treatments such as steroid and renin–angiotensin blockades are similarly accepted. This conflict is based on discrepancies between two European retrospective studies [22,23] and several Asian studies [24-26]. However, the clinical stages of patients with IgAN appeared to differ. A German clinical study included patients with relatively advanced stages of the disease; 55% of the participants had hypertension, 35% had elevated serum creatinine (> 150 mmol/l), 62% had severe proteinuria (> 1.5 g/day) and most surprisingly, 25% of the participants progressed to ESKD within 2.3 years after tonsillectomy [22]. A recent Italian study included only patients with stage 1 and 2 CKD who underwent tonsillectomy to analyze the efficacy of tonsillectomy in patient with and without IgAN [23]. A Japanese study of 118 patients with moderate CKD (38.1% had proteinuria of > 0.5 g/day and the mean serum creatinine level was 1.07 mg/dl) [24] found that patients who underwent tonsillectomy achieved better outcomes at long-term follow-ups. Moreover, a recent Japanese study that included patients with relatively early-stage IgAN confirmed the efficacy of tonsillectomy after adjustment for known risk factors, including blood pressure, proteinuria and histological findings [26]. Such discrepancies in the efficacy of tonsillectomy between European and Asian studies may be partly due to the clinical stage of IgAN at the time of intervention. Thus, it is reasonable that the efficacy of disease-specific treatments differs between stages A and B (**Figure 1C**). Although the justification for tonsillectomy should be verified by more clinical and experimental studies, future curative treatments based on the pathogenesis of IgAN should be evaluated among patients who have been adjusted for disease activity. To determine the stage of disease, a paradigm shift for activity assessment with a novel noninvasive real-time method in combination with simple urinalysis and renal biopsy is greatly desirable for IgAN.

Emerging evidence from clinical and experimental studies has revealed that galactose (Gal)-deficient IgA1 (Gd-IgA1) and Gd-IgA1 ICs with endogenous anti-glycan antibodies are essential effector molecules in IgAN [2].

In the next chapters, we introduce current perspectives on noninvasive testing methods with aberrantly glycosylated IgA (Gd-IgA1)-related molecules and its contribution to the paradigm, and discuss a strategy incorporating glycan engineering as a future curative therapy.

3. Noninvasive testing for activity assessment of IgAN with aberrantly Gd-IgA1

3.1 Characteristics of aberrantly Gd-IgA1 in IgAN

IgA in glomerular deposits is exclusively of the IgA1 subclass [27]. IgA1 contains a hinge region in its heavy chain that is the site of attachment of 3 – 6 O-glycans [28-32]. Patients with IgAN have elevated levels of circulating IgA1 with some *O*-glycans consisting of Gal-deficient *N*-acetylgalactosamine (GalNAc) with or without *N*-acetylneuraminic acid (NeuAc) [33-36]. In contrast, normal serum IgA1 is believed to contain relatively few Gal-deficient *O*-glycans (**Figure 2**) [28].

Immortalized IgA1-secreting cells derived from the circulation of patients with IgAN and healthy controls have provided a new insight that Gal-deficiency of IgA1 is related to decreased expression and activity of core 1 β1,3-galactosyltransferase (C1GalT1) [1], which adds Gal to GalNAc, and elevated expression and activity of α-*N*-acetylgalactosaminide α-2,6-sialyltransferase 2 (ST6GalNAc-II) that adds NeuAc to GalNAc [37].

A relatively high Gd-IgA1 serum level is a hereditable trait, suggesting the involvement of genetic co-determination factors in the pathogenesis of IgAN [38]. Meanwhile, mucosal infections in patients with IgAN, such as tonsillitis and upper respiratory infections associated with macro hematuria, may alter production of multiple cytokines, which is notable as

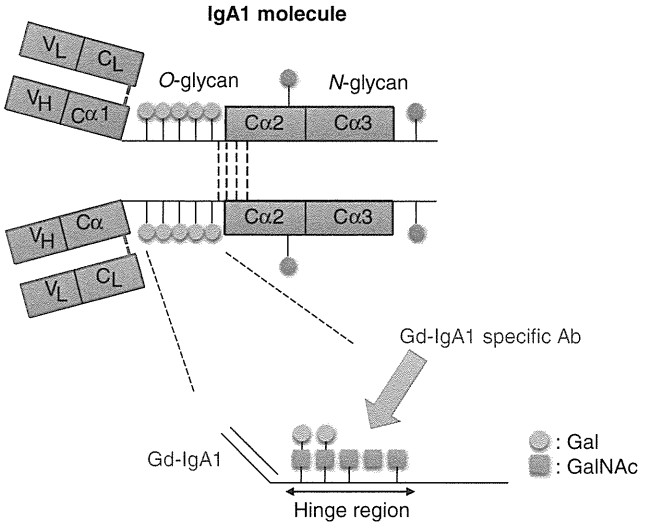

Figure 2. Possible structure of hinge-region of galactose-deficient IgA1.

IL-6 and IL-4 reportedly accentuate Gal-deficiency of IgA1 via coordinated modulation of C1GalT1 and ST6GalNAc-II [39].

3.2 Pathogenic role of Gd-IgA1- and Gd-IgA1-specific antibodies

There is increasing evidence that Gd-IgA1 plays a pivotal role in the pathogenesis of IgAN [2,40], as Gd-IgA1 serum levels are higher in patients with IgAN than in healthy controls or patients with other kidney diseases [36,41]. Multiple observations support this concept, including a study that found that glomerular IgA eluted from tissue specimens from patients with IgAN is exclusively of the IgA1 subclass, predominantly in the polymeric form and aberrantly glycosylated [42,43]. However, Gd-IgA1 containing IC, but not Gd-IgA1 alone, induced *in vitro* proliferation of mesangial cells [44]. Moreover, analysis of familial cases indicated that most people with elevated Gd-IgA1 levels do not exhibit clinical signs of renal injury. Thus, these findings indicate that additional pathogenic hits are necessary in the pathogenesis of IgAN.

Gd-IgA1 in the serum of patients with IgAN is found nearly exclusively within IC bound to IgG or IgA1 antibodies. We recently reported that these IgG antibodies recognize GalNAc-containing epitopes bound to Gd-IgA1 [45]. Glomerular IgA1 arises from deposition of IC from the circulation and/or *in situ* binding of anti-glycan antibodies against deposited Gd-IgA1 (**Figure 3**) [2,35,40,46-48]. In the mesangium, these IgA1-containing ICs activate resident mesangial cells, and thereby stimulate their proliferation and overproduction of extracellular matrix, leading to glomerular injury [44,49-53].

3.3 GdIgA1 and related ICs as biomarkers for activity assessment and diagnosis of IgAN

A noninvasive and real-time method to assess disease activity is desirable in order to determine the disease stage of IgAN. Recent studies have revealed that increased circulating Gd-IgA1 levels were associated with worsening proteinuria and a greater risk for progression of renal dysfunction in IgAN [54]. In addition, the combination of high serum Gd-IgA1 levels and circulating levels of advanced oxidation protein products were correlated with a more rapid decline in estimated glomerular filtration rate, suggesting that oxidative stress linked to Gd-IgA1 may be involved in the pathogenesis of IgAN [48]. Although these studies did not analyze serum levels of IgA-containing ICs, the serum levels of Gd-IgA1-specific IgG autoantibodies were correlated with disease severity, as assessed by the magnitude of proteinuria [45]. Furthermore, Berthoux *et al.* [55] reported that serum levels of IgG and IgA antibodies against Gd-IgA1 at the time of renal biopsy were significantly associated with clinical progression of IgAN toward dialysis or death.

To assess the efficacy of therapy, we evaluated changes in serum Gd-IgA1 levels before and 4 weeks after tonsillectomy. This study found that patients with IgAN who demonstrated a significant decrease in serum Gd-IgA1 levels after tonsillectomy achieved significantly better improvement in hematuria [56]. Another study recently examined serum levels of Gd-IgA1 and IgA–IgG IC in combination with urinary abnormalities in 50 patients with IgAN, who showed complete or partial clinical remission following tonsillectomy with steroid pulse therapy, before and 3 – 5 years after treatment [57]. Cross-sectional analysis revealed that the degree of hematuria and proteinuria were significantly associated with serum levels of Gd-IgA1 and levels of IgA–IgG ICs. Disease activity of IgAN, as assessed by the degree of hematuria and proteinuria, was correlated with serum levels of and changes to Gd-IgA1 and IgA–IgG IC during the course of therapy [57].

These findings further support the multi-hit hypothesis for the disease mechanism of IgAN (**Figure 3**) [2] and indicate the

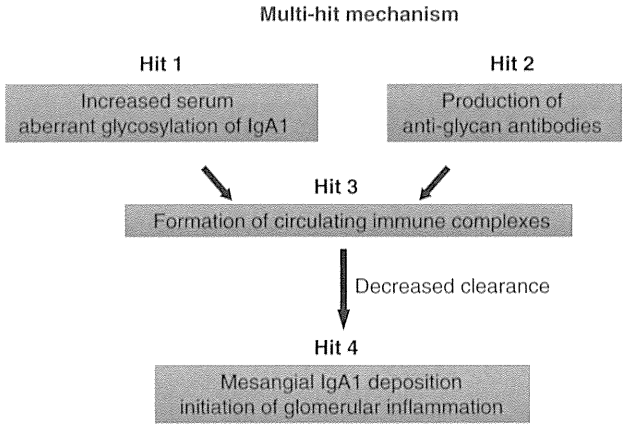

Figure 3. Proposed mechanism of pathogenesis of IgA nephropathy.

possibility that evaluation of not only serum levels of Gd-IgA1, but also those of Gd-IgA1-specific autoantibodies [45,46] are sufficient disease markers of IgAN. These new noninvasive markers of disease activity may be useful to formulate an activity scoring system and to guide therapeutic approaches. These serum biomarkers have potential as diagnostic indicators of IgAN, even though renal biopsy is the gold standard for diagnosis and prognosis of IgAN.

Moldoveanu *et al.* [36] investigated the value of Gd-IgA1 serum levels for diagnostic testing. By receiver operating characteristic (ROC) curve analysis, the Gd-IgA1 serum level that provided a sensitivity of 0.77 had a specificity of 0.90 in the differentiation of patients with IgAN from healthy controls, whereas a level with a specificity of 1 had a sensitivity of 0.44 [36]. However, elevated Gd-IgA1 serum levels have been detected in healthy relatives of individuals with IgAN, suggesting that such levels alone are insufficient to cause disease [38].

IgG specific for Gd-IgA1 represents another potential biomarker, as serum levels of this antibody are significantly elevated in patients with IgAN and correlated with proteinuria. ROC curve analysis indicated that when the specificity of the level of serum IgG antibody directed against Gd-IgA1 reached 0.95, the corresponding sensitivity was 0.88 [45].

A recent study by Yanagawa *et al.* [41] reported that serum levels of IgA, Gd-IgA1, Gd-IgA1-specific IgG and Gd-IgA1-specific IgA were elevated in patients with IgAN compared with those in healthy controls and those with other renal disease, suggesting that these parameters may be useful for the diagnosis of IgAN. It is important to note that a substantial overlap in serum levels of individual biomarkers between healthy controls and patients with IgAN or other renal diseases was observed. Consequently, no single biomarker was sufficiently specific for IgAN. These findings suggest that a panel of serum biomarkers may be more helpful to differentiate IgAN from other glomerular diseases.

3.4 Present limitations of those biomarkers

A recent study revealed that serum levels of Gd-IgA1, Gd-IgA1-specific IgG and Gd-IgA1-specific IgA may be useful for the diagnosis of IgAN [41], although several important limitations to these examinations have arisen. First, lower Gd-IgA1 serum levels have been found in patients with IgAN as compared to healthy controls, suggesting that such levels alone are insufficient to cause disease. Second, elevated levels of Gd-IgA1-specific autoantibodies are frequently observed in IgAN patients with normal serum Gd-IgA1 levels. These findings suggest that even with normal circulating levels of Gd-IgA1, there may be a sufficient number of Gal-deficient residues on IgA1 to form pathogenic ICs with Gd-IgA1-specific antibodies. Furthermore, a lectin-based assay using *Helix aspersa* lectin may be insufficient to discriminate Gal-deficient sites specific to IgAN. Therefore, some pathogenic glycosylation defects may exist below the detection level of a *H. aspersa* agglutinin (HAA) lectin-based method. Although, Gal-deficient sites randomly occur on the hinge region of IgA1, a new method to better identify Gd-IgA1 specific to IgAN is greatly desired.

4. Glycan as a potent target of therapeutic agents

4.1 Glycans and disease

Glycosylation is one of the most fundamental and complex posttranscriptional modifications of various biomolecules, such as glycoproteins, lipids and proteoglycans. Abnormal *in vivo* glycan profiles have been associated with various diseases.

In cancer cells, the structures of glycans on cellular membranes undergo drastic changes during malignant transformation. For instance, hypoxic conditions induce expression of some glycosylase genes in progressive cancer [58]. Another example is the involvement of sialic acid in diabetes. A study

of neuraminidase 3 (NEU3) transgenic mice revealed that NEU3 participated in the onset of diabetes [59].

In some diseases, the glycans of the antibody itself can undergo alterations, thereby inducing changes to the characteristics of the molecule. Aberrations of *N*-link glycans on the constant region of IgG and *O*-link glycans on the hinged region of IgA1 are possibly related to chronic rheumatoid arthritis and IgAN, respectively [60,61].

Anti-glycan antibodies produced by exogenous pathogens with various surface glycans sometimes recognize endogenous molecules with similar structures to the original antigens, which in turn can cause autoimmune diseases and allergy [62].

4.2 Glycan-focused pharmaceutical approaches

Innovative molecularly targeted drugs and novel diagnostic agents featuring glycans are desirable. There are several attractive glycan-focused cancer therapies; one of the advantages of focusing on glycans is the ability to specifically target cancer cells. In many cases, molecules known to be involved in cancer progression are tissue-unspecific and expressed in normal cells as well as cancer cells. To increase specificity for cancer cells, a method to select antibody clones that can distinguish differences between glycan types and patterns among cancer cells versus normal cells is an important pharmaceutical strategy [63].

Pancreatic cancer is associated with a poor prognosis and is difficult to diagnose at early stages of progression; thus, it is a good candidate for glycan-based diagnosis. The β chain of fucosylated haptoglobin has been identified as a disease-specific circulating molecule and may be a useful serum diagnostic marker of pancreatic cancer [64]. The development of an ELISA for fucosylated haptoglobin using fucose-specific lectin is under investigation [65].

In general, lectins possess the unique characteristic of glycan recognition and have therefore been applied in the diagnosis of various diseases [35,66].

The heterogeneity of glycosylation renders glycan analysis (e.g., mass spectrometry) and consequent pharmaceutical approaches challenging [30,67]. From the point of antibody engineering, because glycans themselves are self-antigens, it is difficult to acquire anti-glycan antibodies with high affinity to specific glycan types and patterns. Combining the phage display method and artificial glycolipids is a recent example of approaches to efficiently obtain anti-glycan antibodies for therapeutic use [68,69].

4.3 Anti-glycan antibody recognizing Gd-IgA1 in patients with IgAN

Based on the hypothesis of glycan abnormalities in IgAN, specific measurements of circulating Gd-IgA1 serum levels in patients with IgAN has become important to arrive at a definitive diagnosis and disease activity assessment. Currently, the HAA lectin-based assay for Gd-IgA1 is a common method to assess Gd-IgA1 serum levels; however, unstable reactivity to Gd-IgA1 has recently been recognized, which is a critical problem [36,70]. A Gd-IgA1-specific monoclonal antibody was established by simple immunizations of rats with synthesized GalNAc-conjugated peptides and consequently a desired monoclonal antibody against Gd-IgA1 was obtained through several selections of hybridoma clones (in preparation). This antibody has notable potential for applications for an ELISA to specifically detect serum Gd-IgA1 [71]. The ELISA consists of anti-Gd-IgA1 monoclonal antibody that can be stably acquired from hybridoma cells, whereas HAA lectin can only be isolated from a natural source and is unstable. Therefore, this glycan-specific antibody suggests the importance and usefulness of an anti-glycan antibody to better elucidate pathophysiological mechanisms and presents a potential diagnostic method of chronic intractable disease.

The next step will be the development of a medication for IgAN by lowering the pathological effect of Gd-IgA1 in patients. Here again, molecularly targeted drugs featuring glycans may also be indispensable for: i) specific neutralization of Gd-IgA1 itself; ii) inhibition of abnormal enzymatic glycosylation of IgA1; and iii) specific depletion of source cells that produce Gd-IgA1 or auto-antibody, which are probably considerable based on glycan aberrance of IgA. Particularly, regarding the depletion of source cells, each population of immune cells, including IgA-producing B cells, are known to possess characteristic structures or patterns of surface glycans, which suggests the possibility of targeting specific populations of immune cells with glycan-targeted drugs. Therapeutic drugs to decrease serum levels of Gd-IgA1 may result in reduction of glomerular pathogenic ICs containing Gd-IgA1 and consequent inhibition of disease progression in patients with IgAN, according to multi-hit hypothesis (**Figure 3**) [2].

Thus, diagnostic and therapeutic agents for IgAN, along with anticancer agents, are expected as beneficial outcomes of glycan targeting.

5. Conclusion

Although a small number of candidate molecules for IgAN-specific therapy have been identified, optimization of glycan engineering will facilitate the development of the next generation of glycan-targeted therapies for IgAN. However, indications by activity assessment and early diagnosis with aberrantly Gd-IgA1 and related ICs may be important for the development of IgAN-specific therapies.

6. Expert opinion

IgAN is the most common form of glomerular disease worldwide and is associated with a poor prognosis. Thus, the development of a curative treatment and strategies for early intervention are urgently needed. However, there is no specific therapeutic agent for the treatment of IgAN recommended in major clinical guidelines. Although recent clinical and experimental studies have suggested tonsillectomy

in combination with steroid pulse therapy for IgAN, there is no consensus on treatment. As discussed in this review, this conflict may be partly due to inappropriate comparisons of the results of clinical studies because patients had different stages of IgAN. Emerging evidence indicates that Gd-IgA1 and related ICs are essential effector molecules in IgAN. Therefore, a therapeutic strategy targeting such aberrantly glycosylated molecules is now desirable. However, even if curative treatment targeting such molecules is developed, the same conflict will be undoubtedly continued without further clinical trials with appropriate indications.

Patients with IgAN have a long-term disease course. Most cases will develop ESKD within 20 years from onset. We encounter patients with stage A and B disease in daily clinical practice (**Figure 1C**), but treatments for these different stages should be appropriately selected. Treatment selection should be ideally based on proper staging to objectively and timely evaluate disease-specific activity of IgAN. However, urinalysis and renal biopsy have limitations, as discussed here. Because serum Gd-IgA1 and related ICs are correlated with disease activity in IgAN, staging methods with these serum molecules present a promising candidate for activity assessment to discriminate stage A from B. In addition, urinary levels of these molecules may further improve the accuracy of assessment. On the other hand, our recently established ELISA, which uses monoclonal antibodies against Gd-IgA1, can provide a stable and massive scale measurement of Gd-IgA1 that is unachievable by existing HAA-lectin ELISAs. This assessment may also be valuable for the evaluation of therapeutic efficacy in diseases with long-term courses; thus, reasonable clinical trials of specific IgAN treatments are warranted.

Another important factor in the development of therapeutic strategies may be an approach for early diagnosis. At present, the diagnosis of IgAN is restricted to renal biopsy. Therefore, the diagnosis of IgAN is primarily limited to the determination of stage and is dependent on insurance coverage, which is subject to socioeconomic circumstances, to cover the expense of general checkups and dictates the frequency of urinalysis. If the proposed diagnostic approaches with these biomarkers, as introduced in this review, can be improved by future large clinical studies through the addition of markers, such as urinary levels of Gd-IgA1 or IC, low-cost and convenient approaches will be implemented for the early diagnosis and intervention of IgAN. Furthermore, early intervention may dramatically increase the availability of treatment options, including glycan-targeting methods. For example, if serum levels of nephritogenic Gd-IgA1 or related ICs in patients with early-stage IgAN with only hematuria or hematuria and mild proteinuria can be quantitatively regulated by short-term glycan-targeting therapy with therapeutic agents, associated medical expenses may be lower than those for chronic management of more advanced IgAN cases. In addition, staging with these biomarkers may be applicable to second screenings of patients with hematuria during general checkups. When we consider that hematuria generally precedes proteinuria in IgAN, new screening approaches may dramatically change the importance of hematuria screening.

Therapeutic glycan targeting for IgAN may include not only neutralizing of nephritogenic GdIgA1 by biologics including the antibodies, but also depletion of specific B cells that produce Gd-IgA1 or endogenous anti-glycan antibodies. Such targeting may avoid the necessity of invasive treatments such as tonsillectomy or immune treatments that cause serious side effects. The selective targeting of specific B cells may also require glycan engineering, such as augmentation in activity of antibody-dependent cellular cytotoxicity, as observed in cancer therapy. Furthermore, if immune tolerance can be controlled in targeting (Tn-positive) specific B cells, anti-IgAN vaccination will become a more attractive option. On the other hand, recent analysis of GalNAc-containing epitopes in IgA1 from IgAN indicates that there are many variations in aberrant glycosylation, further suggesting that IgAN is a heterogeneous disease. Therefore, future analysis to identify key variations of aberrant glycosylation, including truncated *O*-glycans and sialylated versions of this disease, is required in succession to fully understand IgAN pathogenesis. This information may also provide important clues not only for new therapeutic strategies that employ glycan targeting, but also new classifications of this heterogeneous disease. A paradigm shift in activity assessment of IgAN with pathogenic products based on aberrant glycosylation patterns and further understanding of the biochemical and molecular mechanisms will optimize the next generation of diagnostic and therapeutic maneuvers through glycan engineering.

Declaration of interest

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Change of Chylous Ascites During Low-Density Lipoprotein Apheresis in a Patient With Idiopathic Nephrotic Syndrome

Dear Editor

Chylous ascites is a well-known complication of severe nephrotic syndrome (1). However, the pathogenesis of chylous ascites in nephrotic syndrome remains to be elucidated. We investigated the factors associated with chylous ascites in a patient with severe nephrotic syndrome.

A 32-year-old Japanese man presenting with rapid onset of generalized edema was diagnosed with minimal change nephrotic syndrome (MCNS) and was treated with intravenous methylprednisolone at a dose of 500 mg daily for 3 days followed by oral prednisolone (50 mg/day) in 2002. However, the clinical findings indicating steroid-resistance and high proteinuria selectivity index (0.29), led us to suspect focal segmental glomerulonephrosis (FSGS). Therefore, we also applied cyclosporin A (CyA) and therapeutic apheresis (low-density lipoprotein [LDL] apheresis: LDL absorption [LDL-A] using a dextran sulfate cellulose column, double filtration plasmapheresis [DFPP], as described previously) (2). After the initiation of CyA (100 mg/day) and LDL apheresis, the renal function and severe edema improved with no obvious improvement of the urinary protein excretion (UPE) or hypoalbuminemia. When the prednisolone was gradually tapered to 5 mg/day, generalized edema and renal failure developed rapidly. Upon admission, physical examination revealed massive ascites and severe generalized edema. Biochemical analysis of the paracentesis fluid was comparable with the chylous fluid (i.e., the maximum triglyceride level and the triglyceride to cholesterol ratio in the fluid were 221 mg/dL and 20.4, respectively). Chylomicron was positive on electrophoresis. Examination of the chylous fluid revealed neither inflammation nor malignancy. After administration of high dose oral prednisolone, CyA and LDL apheresis, the renal function (Fig. 1), massive ascites and severe edema improved immediately, and the proteinuria improved gradually.

Therapeutic paracentesis was performed regularly, because the patient wanted to achieve symptomatic relief of the abdominal distension. We measured the ascites triglyceride levels (aTG) in all of the paracentesis fluid samples, and plotted the serum albumin, cholesterol and triglyceride levels, and UPE

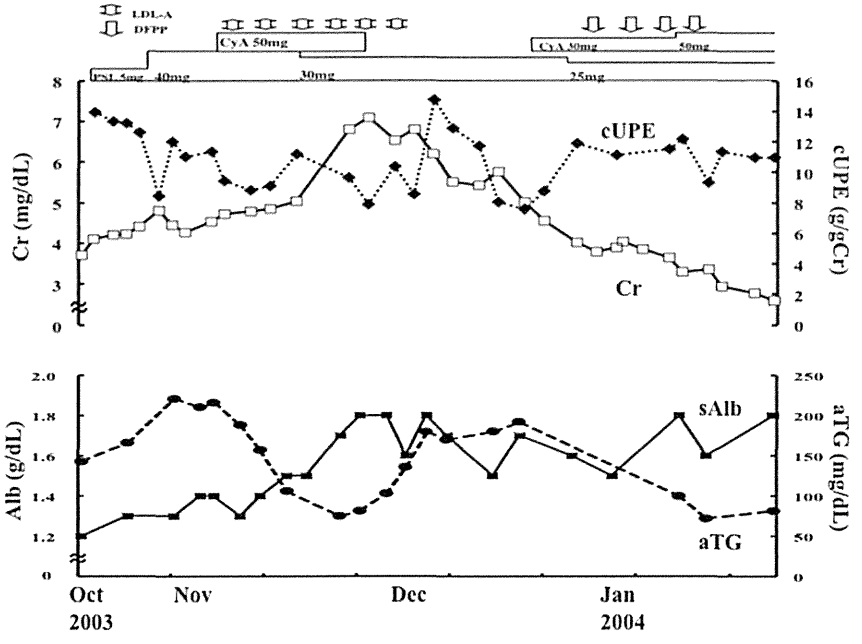

FIG. 1. Longitudinal changes in serum creatinine (Cr), corrected urinary protein excretion (cUPE), ascites triglyceride (aTG) and serum albumin levels (sAlb). aTG was reduced by low density lipoprotein absorption (LDL-A) and double filtration plasmapheresis (DFPP), and increased after suspension of LDL-A and DFPP. Because renal function worsened during the treatment with LDL-A, and we previously reported that DFPP was potentially effective for nephrotic syndrome with acute renal failure (2), we decided to switch from LDL-A to DFPP.

against the aTG during hospitalization. All parameters correlated significantly with aTG ($P < 0.005$, Wilcoxon signed-rank test). However, multiple linear regressions showed that only serum albumin level was an independent factor influencing the aTG among all parameters.

To our knowledge, this is the first report to show serial changes of chylous ascites with treatment. While it has been reported that hypoalbuminemia may be related to chylous ascites (1,3), we demonstrated for the first time that there was an association between hypoalbuminemia and chylous ascites. Lindenbaum and Scheidt suggested that hypoalbuminemia-induced bowel edema might predispose to changes in the permeability of the mucosal or serosal lymphatics, which could result in the leakage of chylomicrons into the peritoneal cavity and chylous ascites (3). In addition, we showed that aTG was reduced by therapeutic apheresis and elevated after suspension of the therapeutic apheresis (Fig. 1). LDL apheresis has been reported to be effective by removing the permeability factors in MCNS and FSGS patients (4). Thus, alternative permeability factors that aggravate the permeability of the lymphatics may contribute to the pathogenesis of chylous ascites, and the waste fluid of therapeutic apheresis should be used to identify novel permeability factors on a large scale.

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ORIGINAL ARTICLE

Validation of the Japanese histologic classification 2013 of immunoglobulin A nephropathy for prediction of long-term prognosis in a Japanese single-center cohort

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Abstract
Background A new Japanese histologic classification (JHC) of immunoglobulin A nephropathy (IgAN) for prediction of long-term prognosis was proposed in 2013. The goal of this study was to validate the JHC system in a Japanese single-center cohort.
Methods A retrospective study was conducted in 198 Japanese adult patients with IgAN. Clinical findings including blood pressure, urinary protein, estimated glomerular filtration rate (eGFR), and outcomes were evaluated in these patients. The glomerular lesion percentage score (GLPS) [number of glomeruli with cellular crescents, fibrocellular crescents, global sclerosis, segmental sclerosis, or fibrous crescents/number of total obtained glomeruli × 100 (%)] was assessed in each patient and categorized into histologic grades (HGs) of HG1 (<25 %), HG2 (25–49 %), and HG3/4 (≥50 %). Associations of GLPS (HG) with disease progression (50 % eGFR decline or end-stage renal disease requiring dialysis) within 10 years after biopsy and the rate of annual eGFR decline were examined.
Results During a median follow-up period of 12.0 years after biopsy, disease progression occurred in 12.8 % (12/94) of HG1 patients, 32.3 % (21/65) of HG2 patients, and 46.2 % (18/39) of HG3/4 patients. The risk of disease progression was significantly higher in the HG2 and HG3/4 groups than in the HG1 group (odds ratios: 3.3 and 5.9 vs. 1). A higher GLPS was significantly associated with a higher risk of disease progression and a greater annual eGFR decline.
Conclusion The newly proposed JHC system 2013 based on GLPS (HG) was well correlated with long-term prognosis in our cohort of Japanese adult patients with IgAN.

Keywords Japanese histologic classification · IgA nephropathy · Long-term prognosis

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Introduction

Immunoglobulin A nephropathy (IgAN) was first described by Berger and Hinglais in 1968 [1] and is the most common primary glomerular disease worldwide [2]. The prevalence of IgAN is approximately 10–40 % of all biopsied cases or all cases of primary glomerulonephritis [3–5]. The current frequency of IgAN in renal pathological diagnoses is 31.0 %, as classified by pathogenesis in the Japan Renal Biopsy Registry 2009 and 2010 [6]. The diagnostic hallmark of IgAN is the predominance of IgA deposits in the glomerular mesangium. The exact pathogenesis is unknown, but recent studies suggest the importance of circulating galactose-deficient IgA1-containing immune complexes [7].

IgAN is not a benign disease because approximately 20–40 % of cases progress to end-stage renal disease (ESRD) within 10–20 years from onset [8, 9]. Clinical and histologic prognostic factors have been evaluated, including clinical parameters such as blood pressure, daily urinary protein (UP) excretion, and estimated glomerular filtration rate (eGFR), and histologic features of renal biopsy specimens [8, 9]. Such information permits prediction of clinical courses and planning of therapeutic strategies in patients with IgAN.

Several histologic prognostic classifications of IgAN have been developed, including the Oxford classification [10, 11]. In 2013, a new Japanese histologic classification (JHC) of IgAN for prediction of long-term prognosis was proposed by the IgAN Study Group of the Progressive Renal Disease Study Committee organized by the Ministry of Health, Labor, and Welfare of Japan [12]. In this study, multicenter retrospective analyses of 287 patients with IgAN were performed to identify glomerular histologic lesions at initial biopsy associated with long-term progression to ESRD [12]. Multivariate logistic regression analysis revealed that cellular crescents (CC), fibrocellular crescents (FCC), global sclerosis (GS), segmental sclerosis (SS), and fibrous crescent (FC) were significant histologic prognostic variables. In contrast, the Oxford classification, published as an international IgAN classification in 2009, does not include crescent formations as prognostic variables, but includes tubular atrophy/interstitial fibrosis (TA/IF) [10, 11]. TA/IF was not directly included in the JHC 2013 because it was shown that GS could be substituted for TA/IF [12].

Histologic grades (HGs) in the JHC 2013 system are based on only glomerular lesions in renal biopsy specimens of patients with IgAN: the HG 1, HG2, HG3, and HG4 categories reflect <25, 25–49, 50–74, and ≥75 of glomeruli exhibiting CC, FCC, GS, SS, or FC, and the odds ratios for development of ESRD increase significantly in higher HG groups [12]. The JHC 2013 system has started to be used in Japan for simple and straightforward assessment of histologic lesions in IgAN and is likely to be useful for predicting long-term prognosis. The present long-term retrospective cohort study was performed to validate the JHC 2013 system in a single-center cohort of 198 Japanese patients with IgAN.

Patients and methods

Patients

The study was based on renal histologic records (January 1980 to April 2001) of 4,275 patients (excluding transplant patients) treated at Akita University Hospital and its affiliated hospitals. Renal biopsies were performed in all patients after written informed consent was obtained. Out of the 4,275 patients, 1,111 were diagnosed with primary IgAN, giving a prevalence rate of IgAN of 26.0 % among all renal biopsies.

Pathologic analyses of renal biopsy specimens were performed at Akita University Hospital by at least three nephrologists. The glomerular lesions percentage score (GLPS) was defined as follows: [number of glomeruli with CC, FCC, GS, SG, or FC/number of total obtained glomeruli × 100 (%)]. The reproducibility of categorizing HGs in the JHG 2013 system among three nephrologists (R.S., A.K., and H.O.) was assessed using an intraclass correlation coefficient (ICC).

For light microscopy examination, specimens were fixed in 10 % phosphate-buffered formalin (pH 7.2), embedded in paraffin, and sliced into 2- to 3-μm sections. These specimens were stained with hematoxylin and eosin, periodic acid Schiff reagent, Mallory-Azan reagent, and periodic acid silver methenamine. For histologic examinations using light microscopy, we used a standardized form developed in the Oxford study [10, 11]. For immunofluorescence studies, cryostat sections were stained with fluorescein isothiocyanate-conjugated rabbit anti-human γ heavy chain (HC), α HC, μ HC, κ light chain (LC), λ LC, C3, and C1q (DakoCytomation, Glostrup, Denmark). Mesangial IgA deposition as the predominant or codominant immunoglobulin was shown in all patients diagnosed with IgAN.

Clinical data were collected from medical records for age, sex, blood pressure, urinalysis, serum creatinine, and eGFR [13] at the time of renal biopsy. To determine the outcome in each patient after biopsy, treatment information and follow-up laboratory data were collected via a questionnaire. Chronic kidney disease (CKD) classification was based on GFR categories [14].

Among the 1,111 patients with IgAN, those meeting any of the following criteria were excluded from the study: (1)

insufficient number of glomeruli (<10) in biopsy specimens for light microscopy; (2) age <18 years; (3) insufficient clinical data for blood pressure, daily UP, hematuria, eGFR at the time of renal biopsy, and details of immunosuppressive therapy or treatment with renin–angiotensin system (RAS) blockade; (4) follow-up period <10 years (including patients who developed ESRD requiring dialysis within 10 years, but excluding those who were lost to follow-up). Based on these criteria, 198 Japanese patients with primary IgAN were retrospectively enrolled in the study (Fig. 1). The study protocol was approved by the Ethics Committee of Akita University Hospital (Approval number 1,026).

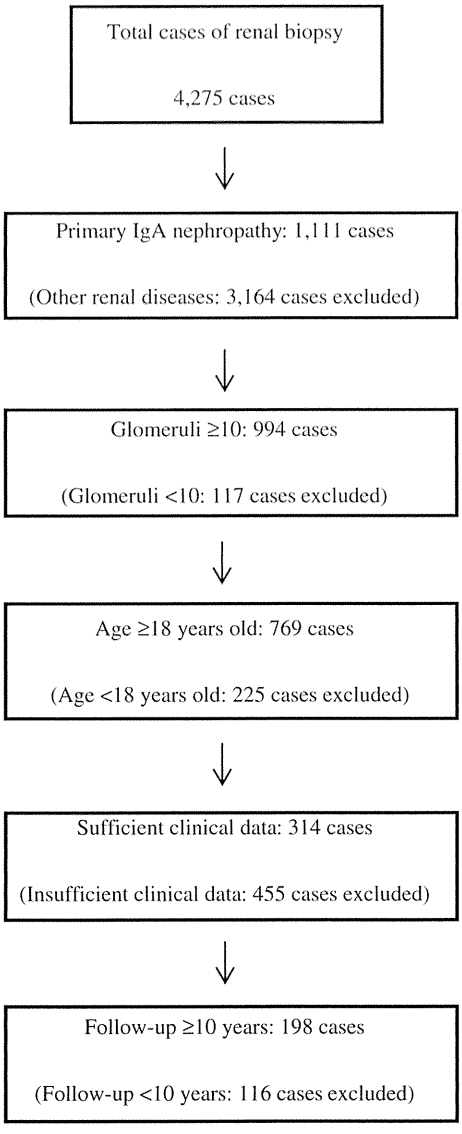

Fig. 1 Cohort selection based on the inclusion and exclusion criteria used in the study

Statistical analysis

Data were analyzed using IBM SPSS Statistics Ver. 20 (Chicago, IL, USA) and are presented as the mean ± SD, median with range, or counts and percentages. GLPS was categorized into HG1 (<25 %), HG2 (25–49 %), and HG3/4 (≥50 %) in this study because only 9 patients had a GLPS ≥75 % (HG 4 in the JHC 2013). The number of HG4 patients was too small to compare the long-term prognosis of HG4 with that of the other three HGs statistically, therefore this study combined HG3 (50–74 %) and HG4 (≥75 %) into HG3/4 (≥50 %). Differences of means, medians and rates of clinical variables among the three HG groups were analyzed by one-way analysis of variance, Kruskal–Wallis test, and Cochran–Armitage trend test, respectively.

Primary renal outcome was defined as disease progression of IgAN (50 % eGFR decline or ESRD requiring dialysis) at the time of 10 years after biopsy. 50 % decline in eGFR was defined as primary renal outcome in Oxford study [10], therefore this study followed the definition of Oxford study. ESRD requiring dialysis (Introduction of dialysis) was added as primary renal outcome because one patient was introduced to dialysis therapy before 50 % decline in eGFR. The patients who were reached to 50 % decline in eGFR at the time of more than 10 years after biopsy were not included in the patients who were reached to primary renal outcome. GLPS, initial mean arterial blood pressure (MAP), initial daily UP, initial eGFR, immunosuppressive therapy, and RAS blockade were defined as covariates in logistic regression analysis. The correlation between renal outcome and each covariate was examined by univariate logistic regression analysis. Associations between renal outcome and covariates were examined by multivariate logistic regression analyses. To analyze whether each covariate was an independent predictor of renal outcome, multivariate logistic regression analyses were performed with adjustment for GLPS, initial MAP, UP, and eGFR in Model 1; and for GLPS, initial MAP, UP, eGFR, immunosuppressive therapy, and RAS blockade in Model 2. A Hosmer–Lemeshow test was used to evaluate the suitability of the two models.

Linear regression analyses were performed with the annual eGFR decline as the dependent variable and GLPS, initial MAP, UP, eGFR, immunosuppressive therapy, and RAS blockade as independent variables. The correlation between the annual eGFR decline and each independent variable was examined by univariate linear regression analysis. Associations between the annual eGFR decline and independent variables were examined by multivariate linear regression analyses. To analyze whether each independent variable was an independent factor influencing the annual eGFR decline, two multivariate linear regression

analyses were performed with adjustment for GLPS, initial MAP, UP, and eGFR in Model 1; and for GLPS, initial MAP, UP, eGFR, immunosuppressive therapy, and RAS blockade in Model 2.

The risk of disease progression (50 % eGFR decline or ESRD requiring dialysis) and the annual eGFR decline among the three HG groups were compared by univariate logistic regression analysis and univariate linear regression analysis, respectively.

P values <0.05 were considered significant in all analyses. Confidence intervals included 95 % of the predicted values. By conversion, an ICC <0.40 indicates poor inter-rater reliability, 0.40–0.59 moderate, 0.60–0.79 very good, and ≥0.80 outstanding [11].

Results

Clinical features and outcomes in 198 patients with IgAN

Baseline characteristics at the time of renal biopsy and treatments and outcomes during follow-up in all 198 cases of IgAN are shown in Table 1. The median number of glomeruli in biopsy specimens was 20 (range 10–100). Based on the GLPS, 94 patients (47.5 %) were categorized into the HG1 group, 65 (32.8 %) into the HG2 group, and 39 (19.7 %) into the HG3/4 group. The reproducibility of this categorization among three nephrologists was very good (almost outstanding) based on the ICC score of 0.78 (confidence interval: 0.71–0.84, $P < 0.0001$).

Table 1 Clinical characteristics

	HG 1	HG 2	HG3/4	P_{trend}
Number of patients	94	65	39	
Baseline characteristics				
Age (years)	42 (18–72)	43 (21–73)	49 (21−70)	0.43*
Male [*n* (%)]	39 (40.9 %)	36 (56.7 %)	20 (51.3 %)	0.13**
MAP (mmHg)	96 (61.3–136.7)	98 (70–138)	98 (75.3–143.3)	0.23*
Proteinuria (g/day)	0.88 (± 0.78)	1.85 (±2.68)	2.58 (±2.16)	<0.0001***
<0.5 g/day (n (%))	39 (41.5 %)	12 (18.5 %)	2 (5.1 %)	<0.0001**
0.5–1.0 g/day [*n* (%)]	17 (18.1 %)	14 (21.5 %)	5 (12.8 %)	0.72**
≥1.0 g/day [*n* (%)]	38 (40.4 %)	39 (60.0 %)	32 (82.1 %)	<0.0001**
Hematuria [*n* (%)]	79 (84.0 %)	60 (92.3 %)	35 (89.7 %)	0.18**
eGFR (mL/min/1.73 m^2)	79.7 (±22.1)	69.2 (±26.5)	55.1 (±25.2)	<0.0001***
CKD stage [*n* (%)]				
G1	25 (26.6 %)	8 (12.3 %)	3 (7.7 %)	0.003**
G2	54 (57.4 %)	29 (44.6 %)	13 (33.3 %)	0.008**
G3a	11 (11.7 %)	18 (27.7 %)	7 (17.9 %)	0.09**
G3b	3 (3.3 %)	10 (15.4 %)	9 (23.1 %)	0.0003**
G4	1 (1.1 %)	0 (0 %)	6 (15.4 %)	0.002**
G5	0 (0 %)	0 (0 %)	1 (2.6 %)	0.13**
Treatments and outcomes during follow-up period				
Observational period (months)	172 ± 52 (79–327)	150 ± 50 (30–307)	140 ± 55 (5–279)	0.002*
RAS blockade therapy [*n* (%)]	57 (60.6 %)	42 (64.6 %)	21 (53.8 %)	0.17**
Immunosuppressive therapy [*n* (%)]	39 (41.5 %)	48 (73.8 %)	30 (76.9 %)	<0.0001**
PSL or mPSL pulse therapy (*n* (%)]	39 (41.5 %)	48 (73.8 %)	30 (76.9 %)	<0.0001**
Cyclosporin A or Cyclophosphamide [*n* (%)]	1 (1.1 %)	7 (10.8 %)	7 (17.9 %)	0.0004**
Tonsillectomy [*n* (%)]	0 (0 %)	1 (1.5 %)	1 (2.6 %)	0.15**
eGFR decline (mL/min/1.73 m^2/year)	−1.50 (±2.05)	−2.75 (± 3.53)	−3.40 (±3.51)	0.001***
50 % eGFR decline [*n* (%)]	8 (8.5 %)	13 (20 %)	9 (23.1 %)	0.01**
ESRD requiring dialysis [*n* (%)]	4 (4.3 %)	8 (12.3 %)	9 (23.1 %)	0.001**
Death [*n* (%)]	0 (0 %)	0 (0 %)	0 (0 %)	

Continuous data are presented as the mean ± SD or median (range), and categorical data as the number of patients (%)

P for a significant trend in the three analyses

CKD chronic kidney disease, *eGFR* estimated glomerular filtration rate, *ESRD* end-stage renal disease, *MAP* mean arterial pressure, *mPSL* methylprednisolone, *n*, number, *PSL* prednisolone, *RAS* renin–angiotensin system, *SD* standard deviation

* Kruskal–Wallis test, ** Cochran–Armitage trend test, *** one-way analysis of variance

The mean age at the time of biopsy was 42 (range 18–73) years in all 198 patients and median ages did not differ significantly among the three HG groups. There were 95 males and 103 females. The mean MAP in all 198 patients was 98.3 ± 14.7 mmHg and 42.9 % of the patients had MAP ≥100 mmHg (approximately equivalent to 130/85 mmHg). The median MAP did not differ significantly among the three HG groups. The median daily UP in all 198 cases was 1.0 (range 0.01–20) g/day. The rates of patients with daily UP <0.5 g/day were significantly lower and the rates of daily UP ≥1 g/day were significantly higher in the HG2 and HG3/4 groups, compared to those in the HG1 group. Almost 90 % of the patients had hematuria and the rates of hematuria did not differ significantly among the three HG groups. The mean eGFR in all 198 patients was 71.3 ± 25.9 mL/min/1.73 m^2, and the mean eGFRs in the HG2 and HG3/4 groups were significantly lower than that in the HG1 group. The rates of CKD stages G1 and G2 were significantly lower and those of CKD stage G3b were significantly higher in the HG2 and HG3/4 groups, compared to the respective rates in the HG1 group (all *P* values are shown in Table 1).

The median observational period was 12.0 years (range 5–327 months) and 89.9 % of patients were followed for >10 years. The mean observational periods differed significantly among the three HG groups. Antihypertensive therapy and RAS blockade were administered in 73.2 and 60.6 % of the patients, respectively. The rates of RAS blockade were not significantly different among the three HG groups. In immunosuppressive therapy, 59.1 % of patients received variable doses of corticosteroids with additional agents (cyclosporine A or cyclophosphamide in 7.6 % of cases). Tonsillectomy was performed in 1.0 % of patients. The rates of immunosuppressive therapy were significantly higher in the HG2 and HG3/4 groups, compared to the HG1 group. The median annual eGFR decline in all 198 patients was −1.5 (range −16.6 to 3.9) mL/min/1.73 m^2, and the means of the annual eGFR decline differed significantly among the three HG groups. A 50 % eGFR decline occurred in 30 patients (15.2 %) and hemodialysis or continuous ambulatory peritoneal dialysis had been started in 21 patients (10.6 %) at 10 years after biopsy. There were no deaths during the follow-up period. Disease progression (50 % eGFR decline or ESRD requiring dialysis) was significantly more common in the HG2 and HG3/4 groups than in the HG1 group (all *P* values are shown in Table 1).

Validation of the JHC 2013 system in 198 patients with IgAN

We analyzed predictors for disease progression of IgAN (50 % eGFR decline or ESRD requiring dialysis). In univariate logistic regression analysis (Table 2), the risk of disease progression was significantly associated with higher GLPS (per 10 %), higher initial MAP (per 10 mmHg), and lower initial eGFR (per 10 mL/min/1.73 m^2), but not with initial daily UP (per 1 g/day) or treatments. In multivariate logistic regression analysis (Table 2), disease progression was significantly associated with higher GLPS and higher initial MAP in Models 1 and 2. A Hosmer–Lemeshow test showed good suitability of the two models (Table 2).

We next analyzed predictors of the annual eGFR decline. In univariate linear regression analysis (Table 3), a greater annual eGFR decline was significantly associated with higher GLPS and higher initial MAP, but not with higher initial daily UP, lower initial eGFR, or treatments. In multivariate linear regression analysis (Table 3), a greater annual eGFR decline was significantly associated with higher GLPS, higher initial MAP, and lower initial eGFR in Models 1 and 2. Thus, the results for eGFR differed between the univariate and multivariate analyses.

We also analyzed the risk of disease progression of IgAN (50 % eGFR decline or ESRD requiring dialysis) and a greater annual eGFR decline in each HG group (Table 4). The rate of disease progression within 10 years after biopsy increased with an increased HG. In univariate logistic regression analysis, the risk of disease progression was significantly higher in the HG2 and HG3/4 groups compared to the HG1 group (odds ratios: 3.3 and 5.9 vs. 1). In univariate linear regression analysis, the annual eGFR decline was significantly greater in the HG2 and HG3/4 groups than in the HG1 group.

Discussion

In this study, 198 adult cases of IgAN diagnosed at a single Japanese center from 1980 to 2001 were analyzed to validate the JHC 2013 system based on the GLPS (HG) for prediction of long-term prognosis [12]. In our cohort, the mean daily UP levels at the time of biopsy were significantly higher in the HG2 and HG3/4 groups and the mean initial eGFRs at the time of biopsy were significantly lower in these groups, compared to the HG1 group. These results are similar to those in the JHC 2013 study [12]. The mean annual eGFR declines were also significantly greater in the HG2 and HG3/4 groups, as also found in the JHC 2013 study. The risk of disease progression (50 % eGFR decline or ESRD requiring dialysis) was significantly higher in the HG2 and HG3/4 groups (odds ratios: 3.3 and 5.9 vs. 1) and the annual eGFR decline was significantly greater in these groups, compared to the HG1 group. These results suggest that the JHC 2013 system for IgAN is useful for assessment of the disease state at the time of biopsy and for predicting the clinical outcome.