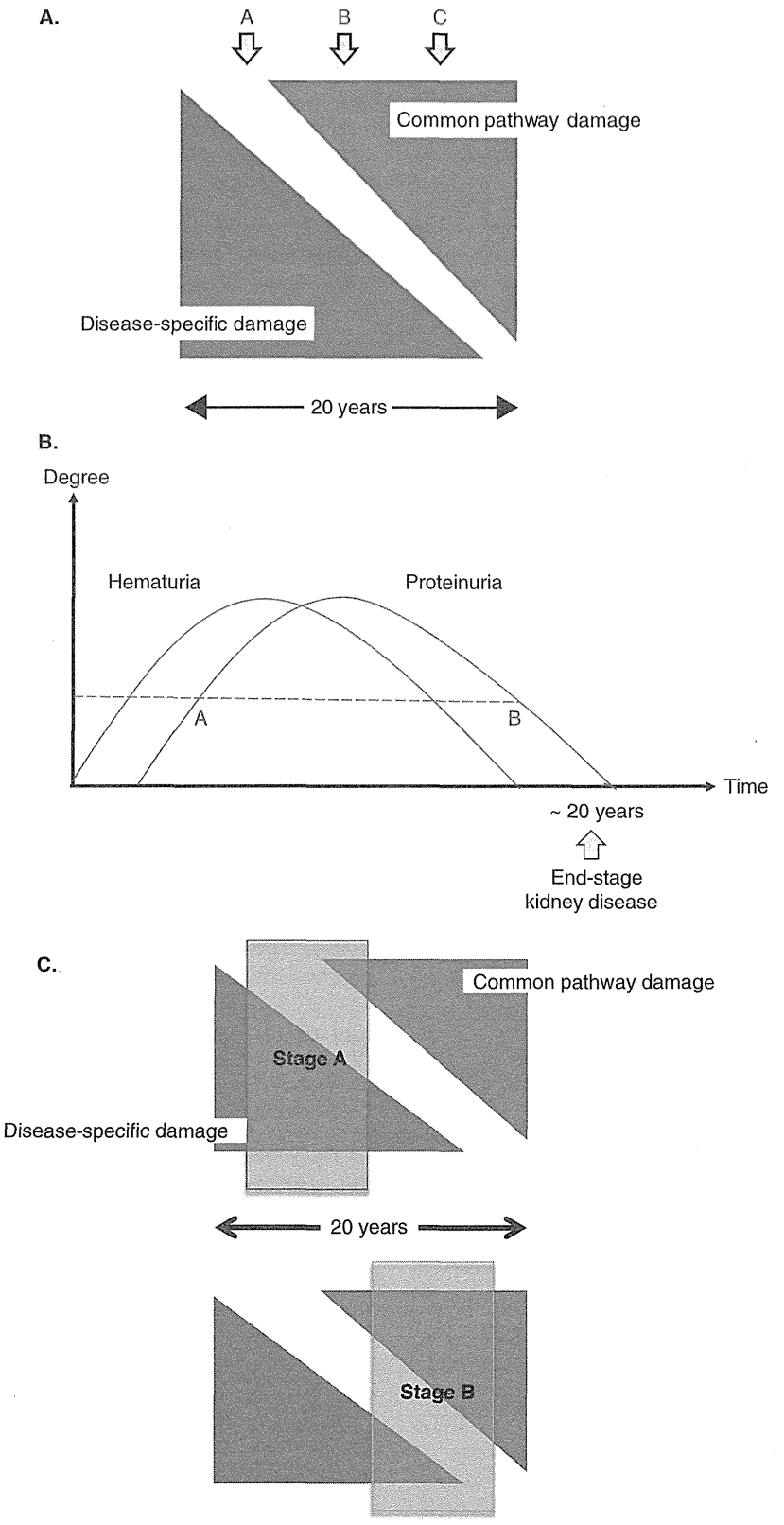

Figure 1. Image of clinical course of IgA nephropathy.

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

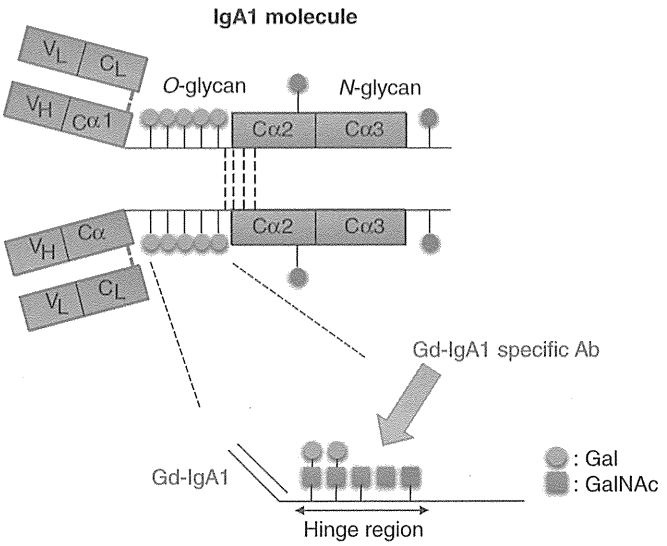

Figure 2. Possible structure of hinge-region of galactose-deficient IgA1.

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

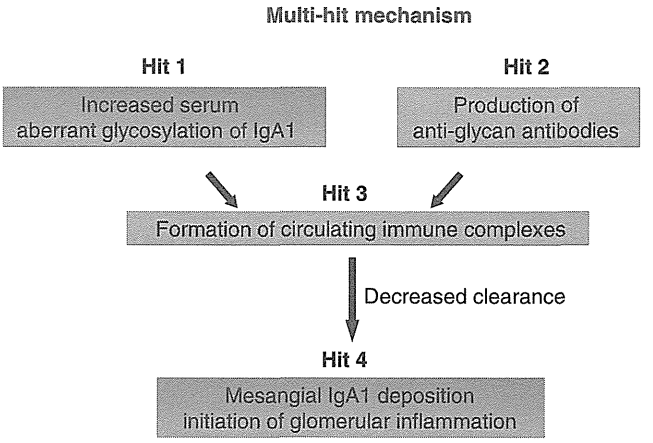

Figure 3. Proposed mechanism of pathogenesis of IgA nephropathy.

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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Open Access Full Text Article REVIEW

Diagnosis and activity assessment of immunoglobulin A nephropathy: current perspectives on noninvasive testing with aberrantly glycosylated immunoglobulin A-related biomarkers

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Abstract: Immunoglobulin (Ig) A nephropathy (IgAN) is the most common form of glomerular disease worldwide and is associated with a poor prognosis. Thus, development of a curative treatment and strategies for early diagnosis and treatment are urgently needed. Pathological analysis of renal biopsy is the gold standard for the diagnosis and assessment of disease activity; however, immediate and frequent assessment based on biopsy specimens is difficult. Therefore, a simple and safe alternative is desirable. On the other hand, it is now widely accepted that multi-hit steps, including production of aberrantly glycosylated serum IgA1 (first hit), and IgG or IgA autoantibodies that recognize glycan containing epitopes on glycosylated serum IgA1 (second hit) and their subsequent immune complex formation (third hit) and glomerular deposition (fourth hit), are required for continued progression of IgAN. Although the prognostic and predictive values of several markers have been discussed elsewhere, we recently developed a highly sensitive and specific diagnostic method by measuring serum levels of glycosylated serum IgA1 and related IgA immune complex. In addition, we confirmed a significant correlation between serum levels of these essential effector molecules and disease activity after treatment, suggesting that each can be considered as a practical surrogate marker of therapeutic effects in this slowly progressive disease. Such a noninvasive diagnostic and activity assessment method using these disease-oriented specific biomarkers may be useful in the early diagnosis of and intervention in IgAN, with appropriate indication for treatment, and thus aid in the future development and dissemination of specific and curative treatments.

Keywords: galactose-deficient immunoglobulin A1, anti-glycan antibody, immune complex, N-acetylgalactosamine, surrogate marker

Limitations of renal biopsy and urinalysis in assessment of disease activity

Immunoglobulin A nephropathy (IgAN) is the most common form of primary glomerulonephritis worldwide, and is associated with a poor prognosis, resulting in end-stage kidney disease in approximately 40% of cases.[1–3] Because the poor prognosis of IgAN is partly a result of delayed diagnosis, strategies for early diagnosis leading to early and effective medical intervention are urgently needed.

Pathological analysis of renal biopsy tissue is the gold standard for diagnosis of IgAN as well as assessment of disease activity and renal prognosis. However, the findings may differ according to the timing of renal biopsy during the 20-year course of IgAN.[1,2] Different intervention periods may yield different findings on renal biopsy.

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For example, in studies using a renal biopsy database from the southern USA, IgAN was commonly identified in renal biopsy tissue specimens from patients aged <40 years, whereas focal glomerulosclerosis was reportedly the primary finding in patients aged ≥40 years.[4] The authors pointed out that although primary focal glomerulosclerosis was most common in non-white individuals aged ≥40 years, secondary focal glomerulosclerosis was more likely in Caucasians because of "burned-out" IgAN. Moreover, renal biopsy is not frequently performed because of procedural risks and/or limitation of insurance coverage. These observations emphasize that renal biopsy provides only a snap-shot of the disease status and thus also has limitations in the assessment of disease activity. 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.[3,5,6] To determine disease stage, a noninvasive, real-time activity assessment method separate from renal biopsy is desirable.

Urinalysis is often used to assess disease activity, although this method also has limitations and restrictions. However, to date, there is no clear method to distinguish proteinuria from acute glomerular inflammatory lesions such as cellular crescents or burned-out sclerotic glomerulus in IgAN. Therefore, the rationale for treatment indication based on urinary protein levels in many clinical guidelines must be rigorously limited.

Taken together, activity assessment methods other than renal biopsy and urinalysis are needed. In addition to an objective method to assess disease activity, a simple and safe method of early diagnosis using valid biomarkers based on the pathogenesis of IgAN should be established.

Aberrantly glycosylated IgA and related IgA immune complexes as effector molecules in IgAN

Levels of the polymeric form of IgA are elevated in the serum of IgAN patients, whereas glomerular IgA in this disease is mainly of the IgA1 subtype.[7,8] IgA1 has a longer hinge region, which can be glycosylated with various *O*-linked-glycans with N-acetylgalactosamine (GalNAc), galactose, and sialic acid, depending on the activity of specific glycosyltransferases.[9] Previous reports have indicated that glomerular IgA1 in IgAN is either under-galactosylated.[10,11] Therefore, aberrantly glycosylated IgA1 has long been considered as a possible cause of IgAN.[12] In recent years, an increase in serum galactose-deficient IgA1 levels in patients with IgAN was quantitatively confirmed for the first time by Moldoveanu et al by use of an enzyme-linked immunosorbent assay using helix aspersa agglutinin lectin, which recognizes GalNAc residues.[13] Moreover, Suzuki et al established immortalized B-cell lines that produce IgA1 from peripheral blood B cells obtained from IgAN patients and analyzed the characteristics of GdIgA1.[14] IgA1 from IgAN was polymeric-dominant and galactose-deficient or over-sialylated on terminal GalNAc.[9,13] In addition, enzyme analysis revealed abnormal enzymatic activity, such as a decrease in β1,3-galactosyltransferase and an increase in α2,6-sialyltransferase activities, which are critical for incorporation of galactose and sialic acid into GalNAc, respectively.[14] Recent studies have shown that these enzymatic activities are possibly regulated by genetic mechanisms and dysregulation of mucosal immunity.[15–17]

A question arises regarding where such GdIgA1 is generated. Some clinical evidence, such as exacerbation of IgAN after upper respiratory tract infection, suggests mucosal contribution in the pathogenesis of IgAN. Indeed, some Japanese groups demonstrated aberrant glycosylation in IgA from palatine tonsils of IgAN patients,[18,19] whereas some Chinese groups reported abnormal cytokine profiles in the tonsils leading to an imbalance in β1,3-galactosyltransferase and an increase in α2,6-sialyltransferase,[20,21] indicating that palatine tonsils may be the primary site of GdIgA1.

Our recent studies further confirmed this notion. Most IgAN patients have decreased serum IgA levels after tonsillectomy. In our analysis, we found an average decrease of nearly 10% at 4 weeks after tonsillectomy. Moreover, patients demonstrating a relatively large decrease in serum IgA levels (>10%) achieved better clinical outcomes after tonsillectomy,[22] suggesting that nephritogenic IgA may play a role in the decrease in IgA after tonsillectomy. Indeed, high serum IgA levels (>315 mg/dL) and serum IgA/C3 ratio (>3.01) in association with hematuria (more than five red blood cells per high power field) and proteinuria (>0.3 g/day) have a diagnostic value for IgAN.[23] To further confirm this idea, we directly evaluated changes in serum GdIgA1 levels by helix aspersa agglutinin lectin enzyme-linked immunosorbent assay before and 4 weeks after tonsillectomy.[24] IgAN patients who demonstrated a significant decrease in serum GdIgA1 levels after tonsillectomy achieved significantly better improvement in hematuria, strongly indicating that at least some factors associated with nephritogenic GdIgA1 are delivered from the palatine tonsils.[24]

To determine whether GdIgA1 is the sole effector molecule of IgAN, Gharavi et al noted increased serum helix aspersa agglutinin-reactive GdIgA1 levels in patients with IgAN compared with those in their relatives; however, serum GdIgA1 levels in the relatives were still elevated compared with those in normal individuals who were not blood relatives, regardless of the absence of nephropathy.[25] Thus, IgAN pathogenesis cannot be explained on the basis of elevated GdIgA1 levels alone, because other pathological factors may be involved in the continued progression of IgAN.

In addition, the concentration of immune complexes containing IgA, specifically GdIgA1, is increased in the blood and urine of IgAN patients.[8,9,12,26] Serum levels of GdIgA1-IgA and GdIgA1-IgG in immune complexes are increased in IgAN.[27] Suzuki et al have recently reported that the IgG autoantibodies that recognize glycan-containing epitopes on GdIgA1 exhibit unique features in the complementarity-determining region 3 of the variable region of their heavy chains.[28] Furthermore, serum levels of IgG autoantibodies specific for GdIgA1 correlated with disease severity, as assessed by magnitude of proteinuria.[28] The onset and progression of IgAN is believed to require GdIgA1 (first hit), as well as endogenous anti-glycan antibodies (second hit) and subsequent immune complex formation (third hit) and glomerular depositions of both (fourth hit).[29]

These multi-hit steps in disease progression were also reported in murine models of IgAN. IgAN-prone mice have elevated serum levels of mucosally regulated polymeric forms of IgA with aberrant oligosaccharide contents at the IgA hinge region[30,31] and disease severity is not correlated with serum levels of IgA but rather with IgA immune complexes.[32] Indeed, complement activity, including the lectin pathway, was strongly induced in the murine IgAN model.[33]

Assessment of disease diagnosis and activity using serum biomarkers based on etiology

The above-described clinical and experimental findings suggest that GdIgA1 and its related immune complexes with anti-glycan autoantibodies are essential effector molecules in IgAN pathogenesis; thus, serum levels of these molecules have shown potential as diagnostic markers for the clinical severity of IgAN.[13,25,27,28]

Recent studies demonstrated that increased GdIgA1 levels were associated with worsening proteinuria and a greater risk of deterioration of renal function in IgAN.[34,35] In addition, the combination of high serum GdIgA1 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 GdIgA1 may be involved in the pathogenesis of IgAN.[35] Although these reports did not analyze serum IgA immune complex levels, Berthoux et al reported that serum levels of IgG and IgA autoantibodies (specific for GdIgA1) at the time of diagnostic biopsy were significantly associated with clinical progression of IgAN towards dialysis/death.[36] The findings of previous reports further confirmed the multi-hit hypothesis for the disease mechanism of IgAN,[29] and indicate the possibility that evaluation of not only serum levels of the autoantigen (GdIgA1) but also those of autoantibodies to GdIgA1 or immune complexes with such antibodies[27,28] should be required as disease markers for IgAN.

Our recent study further demonstrated the value of these biomarkers for the assessment of disease activity.[37] We evaluated changes in serum levels of GdIgA1 and immune complexes with autoantibodies and demonstrated correlations with changes in urinary abnormalities in 50 IgAN patients, who showed complete or partial clinical remission following tonsillectomy with steroid pulse therapy before and 3–5 years after treatment. Cross-sectional analysis revealed that the degree of hematuria and proteinuria was significantly associated with serum levels of GdIgA1 and IgA immune complexes. In addition, longitudinal analyses showed that improvement of urinary abnormalities was well correlated with decreased serum levels of GdIgA1 and IgA immune complex from baseline values before treatment. Because the disease course of IgAN is slowly progressive, clinical studies to investigate the efficacy of therapy with hard end-point evaluations as observed in diabetic nephropathy, will take a long time to complete. However, these findings emphasize the potential value of these effector molecules as practical surrogate markers for evaluation of the efficacy of therapies for IgAN.

Higher serum levels of GdIgA1 and related immune complexes indicate their diagnostic value for IgAN.[13,25,27,28] However, previous studies have also shown elevated serum levels of GdIgA1 and immune complexes in some healthy individuals as well as other chronic kidney disease patients.[13,14,25,27,28] In our analysis, 41% of IgAN patients had elevated serum GdIgA1 levels and 91% of these patients exhibited GdIgA1-specific IgG levels above the 90th percentile of healthy controls,[38] consistent with a prior report.[39] Although up to 25% of controls with chronic kidney disease, particularly those with immune-mediated glomerular diseases including lupus nephritis, also had elevated serum levels of GdIgA1-specific IgG, most IgAN patients had

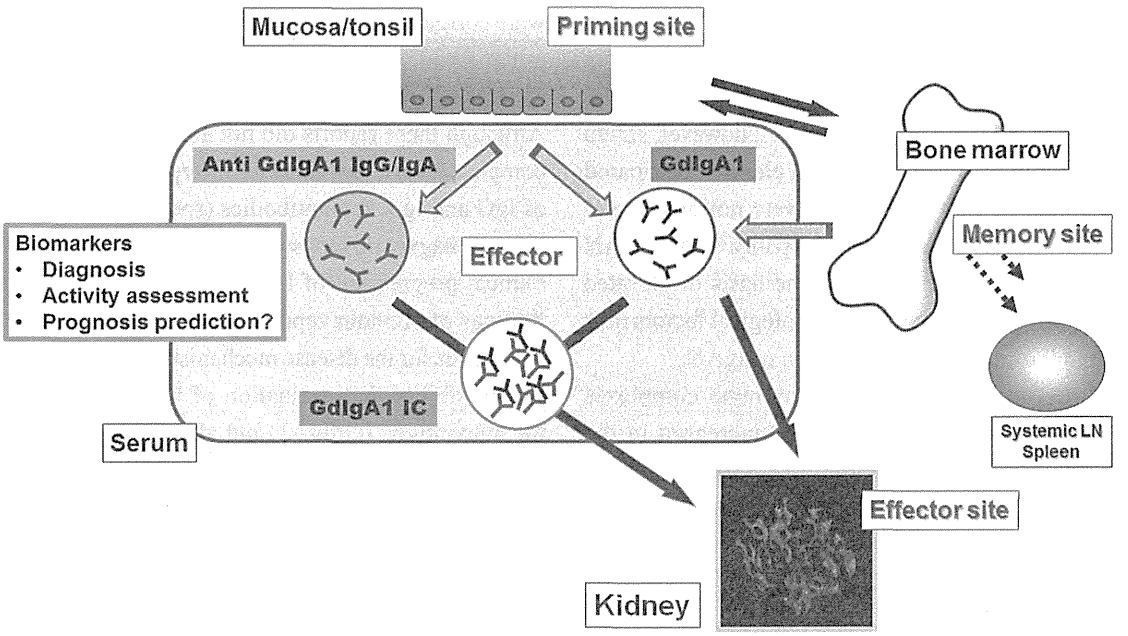

Figure 1 GdIgA1 and its related immune complex with anti-glycan autoantibodies are prospective disease-specific surrogate markers in IgA nephropathy. Recent clinical and experimental studies show that GdIgA1 and auto anti-glycan antibodies may be derived from the mucosa bone marrow axis.
Abbreviations: GdIgA1, galactose-deficient IgA1; IC, immune complex; Ig, immunoglobulin; LN, lymph nodes.

elevated levels of Gd-IgA1-specific antibody of both isotypes. Such a substantial overlap in serum levels of individual biomarkers between patients with IgAN, controls with chronic kidney disease, and healthy controls suggests that no single biomarker was sufficiently specific for IgAN.[38]

Serum levels of GdIgA1-specific IgG were significantly higher in IgAN patients with elevated GdIgA1 levels ($P<0.001$). However, approximately 91% of IgAN patients with normal serum GdIgA1 levels also exhibited elevated levels of GdIgA1-specific IgG,[38] suggesting that auto-antibody production may occur independently of GdIgA1. Although serum levels of GdIgA1-specific autoantibodies have excellent predictive properties to discriminate IgAN patients from healthy controls and patients with non-immune-mediated kidney disease, the specificity is lower for discriminating IgAN patients from those with other types of immune-mediated kidney disease.[38] Indeed, the assessment of GdIgA1-specific IgG showed the best performance for diagnosis of IgAN, with a sensitivity of 89% and specificity of 92%. However, these findings also suggest that a panel of such serum biomarkers may be helpful to differentiate IgAN from other glomerular diseases. In addition, other related markers, such as urinary GdIgA1 and immune complexes, should be assessed for addition to a panel in a larger cohort. Indeed, a multicenter trial of the diagnostic use of the panel with these biomarkers has already been initiated in Japan. Although their capability as prognostic markers to separate progressors and nonprogressors to end-stage kidney disease must be of keen interest, it would take more time because the prognostic value should be evaluated by more follow-up data about IgAN activity assessment with these biomarkers.

Conclusion

Specific curative treatment is required for IgAN, which is the most common form of glomerulonephritis and is associated with poor prognosis. Future research is needed to establish specific treatment regimens. However, such specific treatments may require early diagnosis of IgAN through specific targets before progression of commonly induced pathways that result in further kidney damage, such as sclerotic lesions, and an appropriate clinical trial with practical and disease-specific surrogate markers. As explained in this report, recent clinical and experimental studies emphasize that one of prospective diagnostic and disease assessment markers for IgAN are GdIgA1 and anti-glycan autoantibodies derived from the mucosa-bone marrow axis,[40] and subsequent immune complexes (Figure 1). Therefore, noninvasive and real-time testing with such reasonable biomarkers on the basis of pathogenesis is critical to accomplish this goal.

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Disclosure

The authors report no conflicts of interest in this work.

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ORIGINAL ARTICLE

Proposal of remission criteria for IgA nephropathy

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Abstract
Background The remission criteria of immunoglobulin A (IgA) nephropathy have varied depending on the clinical study. Therefore, nephrologists cannot make a uniform assessment of treatment outcomes and the standardization of explanations of the condition is difficult in patients with IgA nephropathy. This study aims to propose clinical remission criteria for IgA nephropathy based on a nationwide opinion survey in Japan regarding IgA nephropathy remission/relapse.

Method This nationwide survey was sent to 312 teaching facilities of the Japanese Society of Nephrology by Progressive Renal Disease Research, Research on Intractable Disease, from the Ministry of Health, Labour and Welfare of Japan.

Results Valid answers were obtained from 193 facilities (61.9 %) (136 internal medicine facilities and 57 pediatric facilities), of which 134 (69.4 %) thought that both hematuria and proteinuria should be used in the remission standards. Approximately half of the survey respondents shared the opinion on standards of negative results for hematuria and proteinuria and the duration and frequency of these conditions.

Conclusion In this paper, we propose a standardized set of criteria for defining IgA nephropathy remission: three consecutive negative results over a 6-month period in urinary occult blood tests; urinary sediment red blood cell count of <5/high-power field (hematuria remission); and urinary protein of <0.3 g/day (g/g Cr; proteinuria remission). Clinical remission is defined as cases with both hematuria and proteinuria remission. These consensus-based remission criteria should be verified in future studies. In the meantime, they may be useful in predicting therapeutic outcome in cases of IgA nephropathy.

Keywords Remission criteria · IgA nephropathy · Hematuria · Proteinuria

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Introduction

Immunoglobulin A (IgA) nephropathy is the most common form of chronic glomerulonephritis in Japan, and approximately 40 % of patients progress to renal failure within 20 years without therapeutic intervention [1]. In the past,

one of the most popular treatments was the administration of antiplatelet agents or renin–angiotensin system (RAS) inhibitors. However, since steroid pulse therapy (TSP) was shown to be effective by Pozzi et al. [2] in 1999 and tonsillectomy combined with TSP was shown to be effective by Hotta et al. [3] in 2001, these have evolved as the standard treatments for adult, but not pediatric, patients in Japan [4]. In addition, in Japan, where annual tests for urine analysis are well developed, there are many cases in which an early diagnosis and early treatment, followed by subsequent clinical remission, are possible. However, the pathogenesis of this disease remains unclear, and thus there are many patients who show frequent relapses, or are treatment-resistant, with decreasing renal function. IgA nephropathy therefore remains a disease with a poor prognosis.

There have been several studies [5–7] reporting on the remission of IgA nephropathy; however, the degree, time period, and frequency of abnormal urinary findings vary depending on each nephrologist's definition of remission, rendering the state of the disease ambiguous. The fact that the standards for remission are ambiguous makes a uniform assessment of the treatment outcome and the standardization of the explanation of the condition difficult in patients with IgA nephropathy. A set of standard criteria for remission will therefore be useful to both patients and physicians.

The extreme endpoint for patients with kidney disease is end-stage kidney disease (ESKD), and to truly evaluate the therapeutic outcome a reduction in renal mortality rates should be the primary endpoint. However, IgA nephropathy is often diagnosed in its early stages, especially in Japan, and the progression of this disease is often slow. Therefore, observation of the endpoint (ESKD) within the period of observation in the same hospital can actually be very difficult. These facts indicate that it is necessary to define remission as a practical and clinically useful alternative outcome and use it as an effective assessment standard.

Accordingly, based on a consensus obtained through a survey of domestic nephrologists called "Opinion Survey Regarding IgA Nephropathy Remission/Relapse" by the Special Study Group (IgA Nephropathy) on Progressive Renal Diseases Research, Research on Intractable Disease, from the Ministry of Health, Labour and Welfare of Japan and related references, we propose the following IgA nephropathy remission criteria.

Subjects and methods

The survey was sent to 312 facilities (226 internal medicine facilities and 86 pediatric facilities) which are teaching hospitals in the Japanese Society of Nephrology and which also answered the "Nationwide survey on current treatments for IgA nephropathy in Japan" conducted in 2008 by the Special Study Group on IgA Nephropathy [4]. The content of the survey was determined after validation of the question and answer methods by a pilot study in members of this special study group.

Results

Valid answers were obtained from 193 facilities (61.9 %) (136 internal medicine facilities and 57 pediatric facilities). 95 facilities (50.2 %) had remission criteria of their own definition. Both hematuria and proteinuria were considered in the criteria in 81 of these facilities (87.0 %). Of the facilities without remission criteria, 53 facilities (53.5 %) were of the opinion that both hematuria and proteinuria should be emphasized, whereas 33 facilities (37.4 %) and 8 facilities (9.1 %) thought that only proteinuria or hematuria, respectively, should be emphasized.

Approximately half of the survey respondents shared the opinion that 3 consecutive negative results over a 6-month period of urine occult blood, or a urinary sediment red blood cell count of less than 5/high-power fields (HPF) for hematuria and protein ranging from (−) to (±) or less than 0.2 g/day or g/g Cr for proteinuria, should be the criteria for the remission.

Discussion and proposal

Items of remission criteria

The degree of proteinuria is important as a prognostic factor not only in IgA nephropathy but also in all renal diseases [8, 9], and there have been a substantial number of clinical research studies on renal disease [10, 11] in which both a decrease in kidney function and proteinuria have been considered as an endpoint.

However, in Japan, when IgA nephropathy is diagnosed, "chance hematuria" is observed during physical checkup in more than 70 % of cases [12]. In other words, the main initial symptom is hematuria. In Japan, where tests for urine analysis have been well developed and renal biopsies are more actively utilized than in Western countries, there are many opportunities to manage the disease from a very early stage. In addition, although both hematuria and proteinuria do not generally occur simultaneously from the early stages, both often occur together as the disease progresses and after the hematuria period has passed.

On the other hand, there are also cases in which both hematuria and proteinuria occur at the beginning of the

disease, and as time passes hematuria disappears and only proteinuria is observed. The possibility cannot be excluded that proteinuria in these patients is not an inflammatory reaction triggered by the deposition of IgA in the glomeruli, which is a defining feature of true IgA nephropathy, but depends on the so-called "common pathway" accompanying glomerulosclerosis and nephron reduction.

In the opinion survey we conducted, in the 87.0 % of facilities that had their own remission criterion, "disappearance of urinary findings" was the standard used, and 53.5 % of facilities without their own remission criteria suggested both hematuria and proteinuria as "items that should be focused on during remission." In the prognostic scores in Japan [13], both urinary abnormalities were considered as prognostic factors for IgA nephropathy. In addition, there were several studies [14, 15] demonstrating that 7–20 % of IgA nephropathy patients with hematuria alone or associated with mild proteinuria at renal biopsy showed a decrease in renal function over long-term observation. Accordingly, the persistence of hematuria is important, and remission of IgA nephropathy assessed by proteinuria alone is considered to lack validity in the light of the disease state. Based on the above observations, we have included both hematuria and proteinuria as assessment items in the present criteria.

Hematuria cutoff criteria

In the opinion survey, nearly all facilities responded that a change to negativity in a urine dipstick and less than 5 red blood cells of urinary sediment per HPF was used as a remission criteria of hematuria.

The Japanese Committee for Clinical Laboratory Standard studies is unifying the test strips' (1+) in over-the-counter urine occult blood reaction test strips as hemoglobin density of 0.06 mg/dL and red blood cells of 20/μL in the flow cytometry (FCM) technique. Since 2006, the detection sensitivity of the test paper has been mostly standardized among Japanese manufacturers [16]. If a red blood cell count of 20/μL in the FCM technique is converted to the microscopy cutoff value, it is generally 5/HPF or more (magnified 400×, 1 field of vision) [17]. From the above standards, the disappearance of hematuria is set and standardized at a urine occult blood reaction ranging from (−) to (±) and/or urinary sediment red blood cells of less than 5/HPF.

Urinary sediment microscopic examination method

The cutoff value of urinary sediment microscopy is defined as red blood cells of 4/HPF, according to the above observations; however, the lower limit of sediment red blood count (such as 1–4/HPF or less and 1–5/HPF or less) is believed to be different. Therefore, in facilities where the lower limit is 1–5/HPF or less, it is necessary to consider and assess the dipstick method for urinary blood and the FCM technique results.

False positive/negative urine occult blood reaction

When the urine occult blood reaction is measured using test paper, false positives with regard to hemoglobinuria/myoglobinuria and false negatives with regard to reducing substances, such as ascorbic acid, may occasionally occur [18]. For this reason, in the event of substantial differences in sediment red blood cell counts in the occult blood reaction in the test paper method and urinary sediment microscopy method, sediment red blood cell count takes precedence.

Proteinuria cutoff criteria

In the opinion survey, among those facilities that used a proteinuria criteria, 0.2 g/day (g/g Cr) or less was the most common cutoff in 142 facilities (73.6 %), whereas 32 facilities (16.6 %) used 0.3 g/day (g/g Cr) or less. In a past report regarding proteinuria remission, Reich et al. [5] reported that when proteinuria was controlled at less than 0.3 g/day in IgA nephropathy patients, the 15-year renal survival rate was 96 %. Hwang et al. [6] also showed that the long-term renal survival was favorable in a group in which the proteinuria level was maintained at less than 0.3 g/day through treatments.

In nephrotic syndrome, proteinuria of less than 0.3 g/day is defined as "complete remission" by the treatment policies proposed by the Special Study Group (Nephrotic Syndrome) on Progressive Renal Diseases Research, Research on Intractable Disease, from the Ministry of Health, Labour and Welfare of Japan [19]. However, in clinical trials conducted in other countries, the complete remission criteria differ, with proteinuria levels of 0.2 g/day or less as well as less than 0.3 g/day (albumin 200 mg/day) being used. Furthermore, in the Clinical Practice Guidebook for Diagnosis and Treatment of Chronic Kidney Disease 2012 by the Japanese Society of Nephrology [18], proteinuria is defined as urinary protein excretion of more than 0.15 g/day.

Considering the above observations, a consensus "proteinuria negativity criteria" in Japan has not been established. We thus compared the parameters of proteinuria with those of other diseases and defined the proteinuria cutoff value as less than 0.3 g/day. However, in the future, it will be necessary to verify the cutoff value using large-scale cohort research studies.

Duration and frequency in remission assessment

In these criteria, we considered the frequency of hospital visits by patients with IgA nephropathy for everyday medical care and the remission survey results (approximately half of the facilities provided 3 consecutive results over a 6-month period), and determined that at least 3 consecutive results over a six-month period were necessary for assessment.

There is room for debate regarding the continuity of the findings, and it can be hypothesized that there are cases in which urinalysis was conducted, but not consequently assessed as remission (did not achieve the 3 consecutive results standard). However, considering the IgA nephropathy disease state, continued negative findings for urine abnormalities are considered important during remission, and thus we defined remission as "cases in which the criteria are fulfilled 3 consecutive times".

Proposal of IgA nephropathy remission criteria

Based on the above discussion, we propose the following criteria (Table 1). In the case where the criteria are met for 3 consecutive times or more over at least 6 months, patients are classified as being in "hematuria remission" or "proteinuria remission," and both hematuria and proteinuria remission is defined as "clinical remission." Hematuria 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. Specific examples of cases in which remission can (a) or cannot (b) be assessed are demonstrated in Fig. 1.

Limitations of the opinion survey

Our survey has several limitations. First, targeted facilities primarily included mid- to large-scale hospitals, with a special emphasis on facilities that could conduct early, specific treatments within the facility, such as renal biopsy and TSP. Hence, it is possible that deviations may occur in items included in the remission criteria (hematuria and proteinuria) and assessment periods and frequencies (3 consecutive times over a 6-month period). Second, because we conducted one survey per facility, in the event of differences in opinion between nephrologists within a facility, it is possible that only the opinion of the answering individual was reflected. Third, in Japan, IgA nephropathy is often diagnosed in its early stages. Therefore, present opinion may be partly based on the clinical practice of IgA nephropathy patients in early stages. In addition, the consensus-based remission criteria presented here must be verified in future studies.

Table 1 Remission criteria for IgA nephropathy

Hematuria remission
Urine occult blood reaction: (−) to (±) or
Urinary sediment red blood cells: less than 5/HPF[a]
Proteinuria remission
Proteinuria qualitative reaction: (−) to (±) or
Proteinuria amount less than 0.3 g/day (g/g Cr)
In cases where the standards are met for 2 subsequent times or more (for a total of 3 times) during at least 6 months, patients are "*hematuria remission*" or "*proteinuria remission*," and hematuria and proteinuria remission together are defined as "*clinical remission*"

[a] In the event that nonglomerular hematuria or complication with thin basement membrane disease is suspected, this possibility should be assessed

Medical care after the remission

Because the underlying mechanisms for onset and progression of IgA nephropathy are still unclear, there is currently no specific treatment for this disease. There are cases in which relapses occur in various situations, even after remission. Nephrologists should therefore carefully and periodically follow up on the urinary findings of these patients after remission under the scope of continuous medical examinations.

Practitioners providing medical care after remission need to know the criteria by which they can recognize relapse and recurrence of IgA nephropathy. "Relapse" and "recurrence" may be defined as a return of symptoms of IgA nephropathy during clinical remission and as a deterioration of symptoms without clinical remission or during partial remission, respectively. Therefore, verification and permanent establishment of the remission criteria are required; these definitions may be helpful in the process.

Proposal for long-term cohort research

This proposal is based on an opinion survey, and not on results from long-term cohort studies. Therefore, the clinical impact of the remission proposed in this report on the renal prognosis is unclear. In the future, these remission criteria should be verified using data from long-term cohort research using the ongoing Japan Kidney Disease Registry (J-KDR) and Japan IgA Nephropathy Cohort Study (J-IGACS).

For IgA nephropathy developing over the long term (e.g., 20 years), evaluation of therapeutic efficacy should be conducted according to different criteria from that for patients with shorter-term disease. Hard endpoints for the latter patients may include end-stage kidney disease or doubling of serum creatinine levels, similar to the criteria

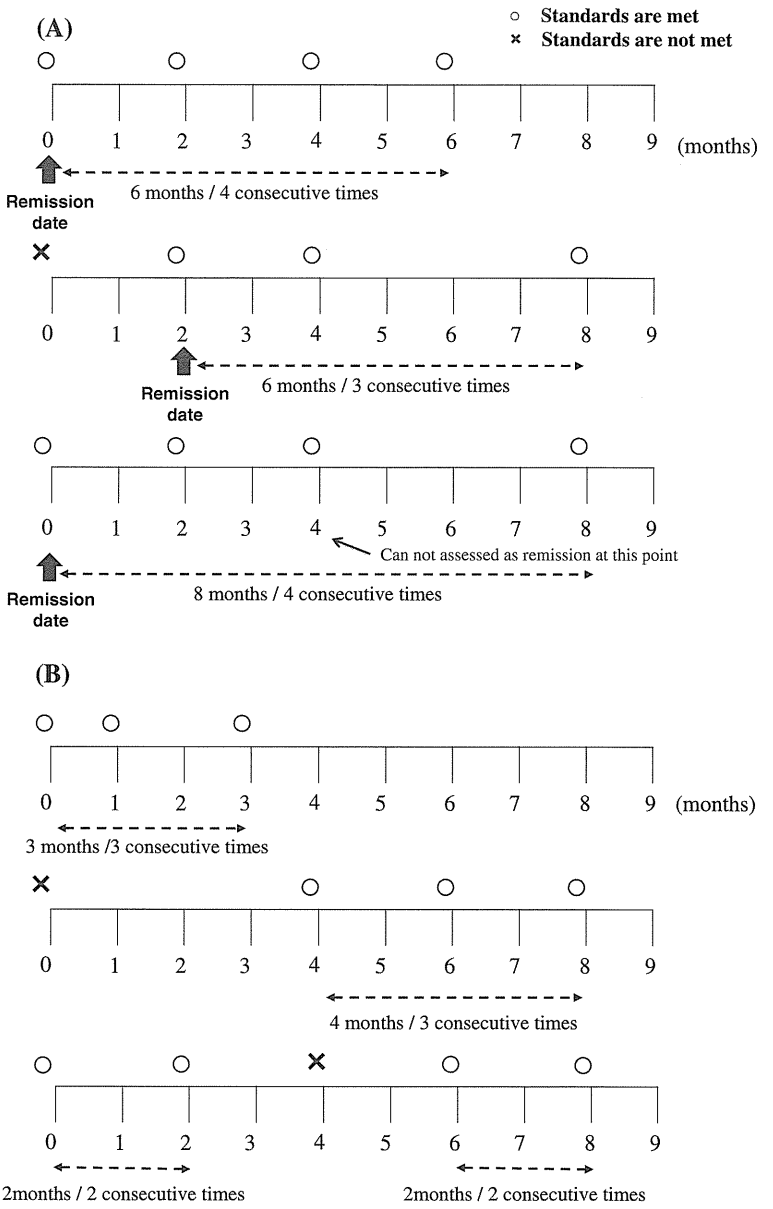

Fig. 1 **a** Examples that can be assessed as remission. All cases are those in which the criteria were met in tests over 6 months or more; from the first day, the criteria were met for 3 consecutive times or more. **b** Examples that cannot be assessed as remission. All cases are those in which the 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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Conflict of interest The authors have declared that no conflict of interest exists.

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