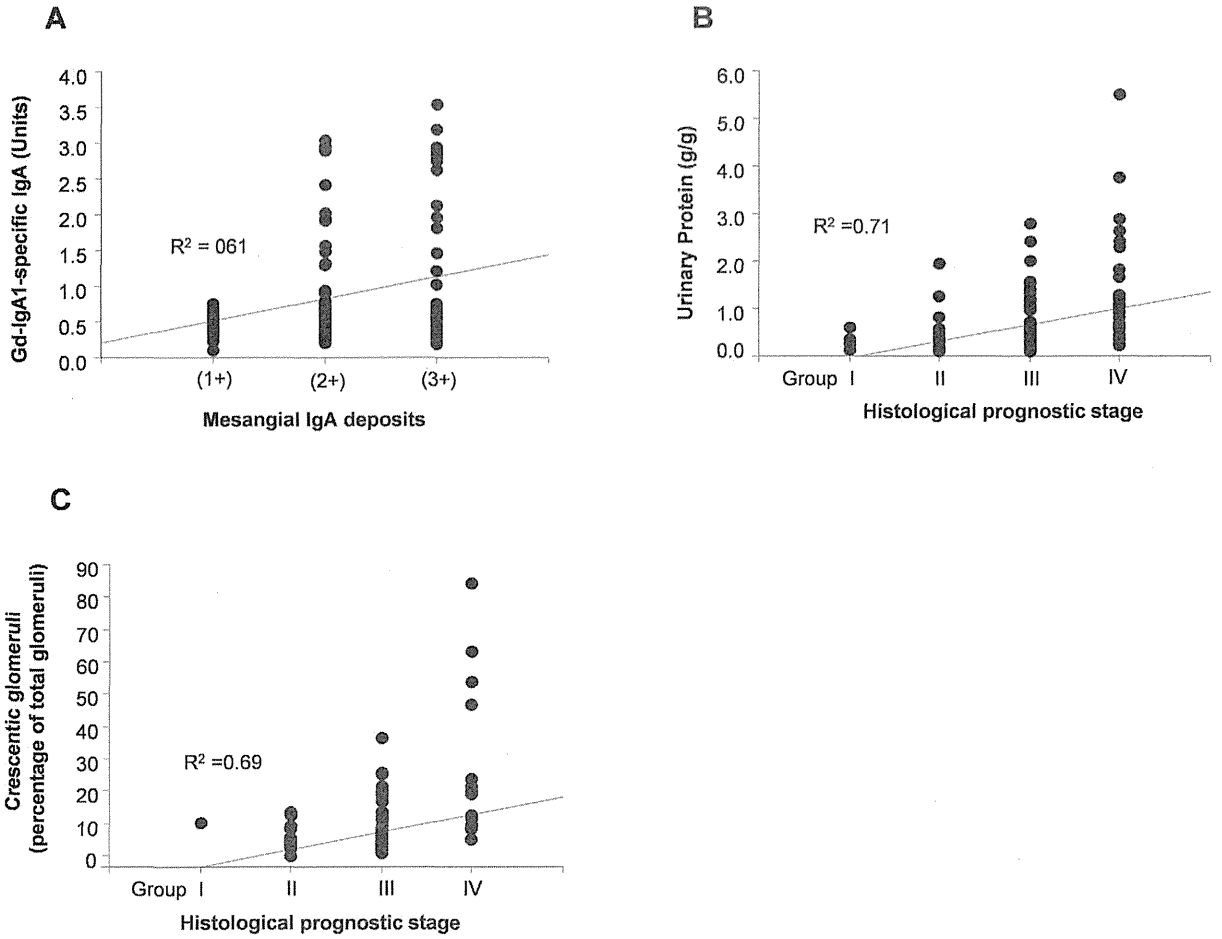

Figure 3. Correlation between biomarkers, histological findings and clinical findings. The strength of correlation between biomarkers, histological findings and clinical findings was measured by the Spearman's correlation coefficient. The serum level of Gd-IgA1-specific IgA correlated with the amount of mesangial IgA deposits (A). Histological prognostic stage (Clinical Guidelines for IgA Nephropathy in Japan, second version) [17] correlated with the urinary protein/creatinine ratio (B), and percentage of glomeruli with a crescent (C).
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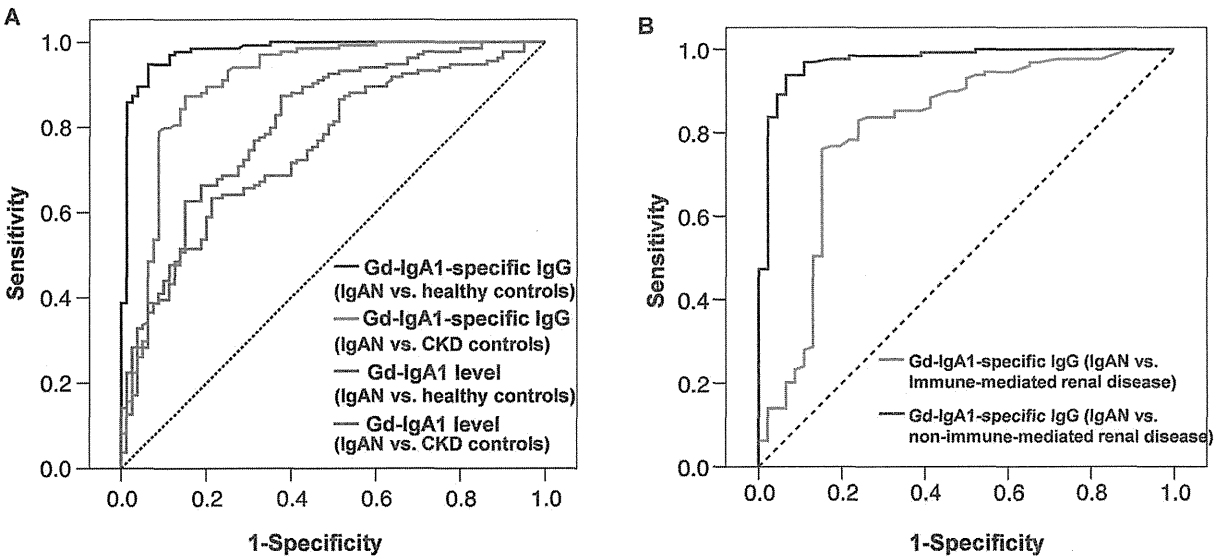

Figure 4. Receiver operating characteristic (ROC) curves. (A) Discrimination between IgAN versus healthy and CKD controls for serum Gd-IgA1 and Gd-IgA1-specific IgG levels; (B) Discrimination between IgAN versus CKD controls with immune-mediated renal disease and non-immune-mediated renal disease for serum Gd-IgA1-specific IgG levels.
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Table 4. Statistics summarized data; Discrimination between IgAN versus healthy and CKD controls for serum Gd-IgA1 and Gd-IgA1-specific IgG levels.

	Area under the ROC curve (C-statistic)	Asymptotic 95% Confidence Interval for the C-statistic		Asymptotic Significance (P-value)	AIC***
		Lower Bound	Upper Bound		
Gd-IgA1-specific IgG (IgAN vs. healthy controls)	0.965	0.943	0.987	1.7E-35	57.4
Gd-IgA1-specific IgG (IgAN vs. CKD controls)	0.906	0.858	0.953	3.2E-23	188.5
Gd-IgA1-specific IgG (IgAN vs. CKD non-immune-mediated renal disease*)	0.973	0.948	0.999	1.7E-21	75.3
Gd-IgA1-specific IgG (IgAN vs. CKD immune-mediated renal disease**)	0.813	0.730	0.895	3.2E-10	145.2
Gd-IgA1 (IgAN vs. healthy controls)	0.800	0.745	0.855	9.9E-16	264.6
Gd-IgA1 (IgAN vs. CKD controls)	0.749	0.683	0.815	1.2E-09	246.3
Gd-IgA1-specific IgA (IgAN vs. healthy controls)	0.722	0.659	0.786	2.7E-09	295.6
Gd-IgA1-specific IgA (IgAN vs. CKD controls)	0.690	0.619	0.761	3.5E-06	266.1

* CKD non-immune-mediated renal disease includes diabetic nephropathy, nephrosclerosis, interstitial nephritis and Fabry's disease.
** CKD immune-mediated renal disease includes lupus nephritis, membranous nephropathy, minimal change disease, membranoproliferative glomerulonephritis, other types of non-IgAN glomerulonephritis.
***AIC: Akaike's Information Criterion.
doi:10.1371/journal.pone.0098081.t004

cohorts of IgAN cases, and some variability may also be expected when the diagnosis (the time of renal biopsy and serum sampling) occurs at different stages of the disease process.

Second, in this study, we frequently observed elevated levels of Gd-IgA1-specific autoantibodies in IgAN patients with normal serum Gd-IgA1 levels. One potential explanation of these data is that even when the circulating level of Gd-IgA1 is normal, there may be a sufficient number of galactose-deficient residues on these molecules to induce formation of pathogenic immune complexes in the setting of elevated Gd-IgA1-specific antibodies. Furthermore, because the *O*-glycosylation defects may occur preferentially at specific amino acid positions, [32] and the lectin-based assay is not able to discriminate which sites are involved, some pathogenic glycosylation defects may exist below the detection level of our lectin-based method.

We also found that serum levels of Gd-IgA1-specific IgA correlated with the intensity of mesangial IgA1 deposits, implying that Gd-IgA1-specific IgA plays a role in glomerular deposition of IgA1-containing immune complexes. Lastly, serum levels of Gd-IgA1-specific IgG associated with histological grading, and we detected a trend for higher serum levels of Gd-IgA1-specific IgG in IgAN patients with at least moderate proteinuria ($\geq$1.0 g/g), when compared to patients with less proteinuria. These data suggest that Gd-IgA1-specific IgG may represent a marker of disease severity, but its prognostic utility will require validation in independent prospective cohorts.

We noted a substantial overlap in serum levels of individual biomarkers between patients with IgAN, CKD controls, and healthy controls. Consequently, no single biomarker was sufficiently specific for IgAN. The levels of Gd-IgA1-specific IgG and IgA were particularly elevated in patients with non-IgAN immune-mediated kidney disease, such as lupus nephritis in which increased propensity to auto-antibody production is well established. Notably, some of these autoantibodies may be polyreactive, *i.e.*, binding to several autoantigens [33,34], thus complicating the assay for IgAN. Our data raise a question as to whether the anti-Gd-IgA1 autoantibodies in patients with lupus nephritis are detected as an artifact of polyreactivity or constitute pathogenic mediators of kidney injury in non-IgAN kidney disease. Additional studies are necessary to examine this aspect of non-IgAN glomerular diseases.

Another limitation of our study was that the CKD control group did not contain sufficient numbers of patients with glomerular disorders that are clinically difficult to differentiate from IgAN, such as Alport syndrome, thin basement membrane disease, post-infectious glomerulonephritis or membranoproliferative glomeru-

Table 5. Statistics summarized data; Discrimination between IgAN versus immune-mediated CKD and non-immune-mediated CKD controls for serum Gd-IgA1-specific IgG levels.

	Sensitivity	Specificity	PPV	NPV
Gd-IgA1-specific IgG (IgAN vs. healthy controls)	89%	92%	92%	89%
Gd-IgA1-specific IgG (IgAN vs. all CKD controls)	89%	81%	82%	88%
Gd-IgA1-specific IgG (IgAN vs. non-immune-mediated CKD)	89%	96%	96%	90%
Gd-IgA1-specific IgG (IgAN vs. immune-mediated CKD)	85%	67%	72%	82%
Gd-IgA1 (IgAN vs. healthy controls)	41%	91%	82%	61%
Gd-IgA1-specific IgA (IgAN vs. healthy controls)	34%	92%	81%	58%

PPV, Positive Predictive Value; NPV, Negative Predictive Value.
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lonephritis. Because of the relative rarity of these conditions, large multicenter studies will be required to validate our results in these settings.

Conclusions

Serum levels of Gd-IgA1-specific antibodies are elevated in most IgAN patients, even in the setting of normal serum level of Gd-IgA1. Our study suggests that a panel of serum biomarkers may be helpful in differentiating IgAN from other glomerular diseases.

References

1. D'Amico G (1987) The commonest glomerulonephritis in the world: IgA nephropathy. Q J Med 64: 709–727.
2. Julian BA, Waldo FB, Rifai A, Mestecky J (1988) IgA nephropathy, the most common glomerulonephritis worldwide. A neglected disease in the United States? Am J Med 84: 129–132.
3. D'Amico G (2000) Natural history of idiopathic IgA nephropathy: role of clinical and histological prognostic factors. Am J Kidney Dis 36: 227–237.
4. Russell MW, Mestecky J, Julian BA, Galla JH (1986) IgA-associated renal diseases: antibodies to environmental antigens in sera and deposition of immunoglobulins and antigens in glomeruli. J Clin Immunol 6: 74–86.
5. Mestecky J, Tomana M, Crowley-Nowick PA, Moldoveanu Z, Julian BA, et al. (1993) Defective galactosylation and clearance of IgA1 molecules as a possible etiopathogenic factor in IgA nephropathy. Contrib Nephrol 104: 172–182.
6. Allen AC, Harper SJ, Feehally J (1995) Galactosylation of *N*- and *O*-linked carbohydrate moieties of IgA1 and IgG in IgA nephropathy. Clin Exp Immunol 100: 470–474.
7. Novak J, Julian BA, Tomana M, Mestecky J (2008) IgA glycosylation and IgA immune complexes in the pathogenesis of IgA nephropathy. Semin Nephrol 28: 78–87.
8. Hiki Y, Horii A, Iwase H, Tanaka A, Toda Y, et al. (1995) *O*-linked oligosaccharide on IgA1 hinge region in IgA nephropathy. Fundamental study for precise structure and possible role. Contrib Nephrol 111: 73–84.
9. Moldoveanu Z, Wyatt RJ, Lee JY, Tomana M, Julian BA, et al. (2007) Patients with IgA nephropathy have increased serum galactose-deficient IgA1 levels. Kidney Int 71: 1148–1154.
10. Hiki Y, Odani H, Takahashi M, Yasuda Y, Nishimoto A, et al. (2001) Mass spectrometry proves under-*O*-glycosylation of glomerular IgA1 in IgA nephropathy. Kidney Int 59: 1077–1085.
11. Allen AC, Bailey EM, Brenchley PE, Buck KS, Barratt J, et al. (2001) Mesangial IgA1 in IgA nephropathy exhibits aberrant *O*-glycosylation: observations in three patients. Kidney Int 60: 969–973.
12. Czerkinsky C, Koopman WJ, Jackson S, Collins JE, Crago SS, et al. (1986) Circulating immune complexes and immunoglobulin A rheumatoid factor in patients with mesangial immunoglobulin A nephropathies. J Clin Invest 77: 1931–1938.
13. Coppo R, Basolo B, Piccoli G, Mazzucco G, Bulzomi MR, et al. (1984) IgA1 and IgA2 immune complexes in primary IgA nephropathy and Henoch-Schönlein nephritis. Clin Exp Immunol 57: 583–590.
14. Tomana M, Matousovic K, Julian BA, Radl J, Konecny K, et al. (1997) Galactose-deficient IgA1 in sera of IgA nephropathy patients is present in complexes with IgG. Kidney Int 52: 509–516.
15. Tomana M, Novak J, Julian BA, Matousovic K, Konecny K, et al. (1999) Circulating immune complexes in IgA nephropathy consist of IgA1 with galactose-deficient hinge region and antiglycan antibodies. J Clin Invest 104: 73–81.
16. Suzuki H, Fan R, Zhang Z, Brown R, Hall S, et al. (2009) Aberrantly glycosylated IgA1 in IgA nephropathy patients is recognized by IgG antibodies with restricted heterogeneity. J Clin Invest 119: 1668–1677.
17. Tomino Y, Sakai H (2003) Special Study Group (IgA Nephropathy) on Progressive Glomerular Disease: Clinical guidelines for immunoglobulin A (IgA) nephropathy in Japan, second version. Clin Exp Nephrol 7: 93–97.
18. Moore JS, Kulhavy R, Tomana M, Moldoveanu Z, Suzuki H, et al. (2007) Reactivities of *N*-acetylgalactosamine-specific lectins with human IgA1 proteins. Mol Immunol 44: 2598–2604.
19. Suzuki H, Moldoveanu Z, Hall S, Brown R, Vu HL, et al. (2008) IgA1-secreting cell lines from patients with IgA nephropathy produce aberrantly glycosylated IgA1. J Clin Invest 118: 629–639.
20. Akaike H (1974) A new look at the statistical model identification. IEEE Trans Automat Contr 19: 716–723.
21. van der Boog PJ, van Kooten C, van Seggelen A, Mallat M, Klar-Mohamad N, et al. (2004) An increased polymeric IgA level is not a prognostic marker for progressive IgA nephropathy. Nephrol Dial Transplant 19: 2487–2493.
22. Suzuki H, Kiryluk K, Novak J, Moldoveanu Z, Herr AB, et al. (2011) The pathophysiology of IgA nephropathy. J Am Soc Nephrol 22: 1795–1803.
23. Coppo R, Amore A (2004) Aberrant glycosylation in IgA nephropathy (IgAN). Kidney Int 65: 1544–1547.
24. Barratt J, Feehally J (2005) IgA nephropathy. J Am Soc Nephrol 16: 2088–2097.
25. Julian BA, Novak J (2004) IgA nephropathy: an update. Curr Opin Nephrol Hypertens 13: 171–179.
26. Novak J, Julian BA, Tomana M, Mesteck J (2001) Progress in molecular and genetic studies of IgA nephropathy. J Clin Immunol 21: 310–327.
27. Novak J, Vu HL, Novak L, Julian BA, Mestecky J, et al. (2002) Interactions of human mesangial cells with IgA and IgA-containing immune complexes. Kidney Int 62: 465–475.
28. Novak J, Tomana M, Matousovic K, Brown R, Hall S, et al. (2005) IgA1-containing immune complexes in IgA nephropathy differentially affect proliferation of mesangial cells. Kidney Int 67: 504–513.
29. Lai KN, Leung JC, Chan LY, Saleem MA, Mathieson PW, et al. (2008) Activation of podocytes by mesangial-derived TNF-alpha: glomerulo-podocytic communication in IgA nephropathy. Am J Physiol Renal Physiol 294: F945–955.
30. Lai KN, Leung JC, Chan LY, Saleem MA, Mathieson PW, et al. (2009) Podocyte injury induced by mesangial-derived cytokines in IgA nephropathy. Nephrol Dial Transplant 24: 62–72.
31. Gharavi AG, Moldoveanu Z, Wyatt RJ, Barker CV, Woodford SY, et al. (2008) Aberrant IgA1 glycosylation is inherited in familial and sporadic IgA nephropathy. J Am Soc Nephrol 19: 1008–1014.
32. Takahashi K, Wall SB, Suzuki H, Smith AD 4th, Hall S, et al. (2010) Clustered O-glycans of IgA1: defining macro- and microheterogeneity by use of electron capture/transfer dissociation. Mol Cell Proteomics 9: 2545–2557.
33. Zhang J, Jacobi AM, Wang T, Berlin R, Volpe BT, et al. (2009) Polyreactive autoantibodies in systemic lupus erythematosus have pathogenic potential. J Autoimmun 33: 270–274.
34. Mietzner B, Tsuiji M, Scheid J, Velinzon K, Tiller T, et al. (2008) Autoreactive IgG memory antibodies in patients with systemic lupus erythematosus arise from nonreactive and polyreactive precursors. Proc Natl Acad Sci USA 105: 9727–9732.

Supporting Information

Table S1 Serum levels of biomarkers in high-proteinuria and low-proteinuria CKD control subgroups.
(PDF)

Author Contributions

Conceived and designed the experiments: HS YT. Performed the experiments: HY HS. Analyzed the data: KM KK AGG. Contributed reagents/materials/analysis tools: BAJ JN. Wrote the paper: HY HS BAJ JN. Collected serum samples: YS. Collected clinical data: YM.

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ORIGINAL ARTICLE

Serum levels of galactose-deficient immunoglobulin (Ig) A1 and related immune complex are associated with disease activity of IgA nephropathy

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Abstract
Background The primary abnormal manifestation in immunoglobulin A nephropathy (IgAN) is recurring bouts of hematuria with or without proteinuria. Although immunohistochemical analysis of renal biopsy tissue remains the gold standard not only for diagnosis but also for evaluating the activity of IgAN, new sensitive and reasonably specific noninvasive tests are emerging to guide therapeutic strategy applicable to all stages of IgAN. The present study examined serum levels of galactose-deficient IgA1 (Gd-IgA1) and its immune complex (IgA/IgG-IC) as noninvasive markers for the disease activity.
Methods We enrolled 50 IgAN patients (male 40 %, median age 37 years) showing complete or partial clinical remission after steroid pulse therapy with tonsillectomy (TSP) whose clinical data and serum could be followed up for 3–5 years.
Results Cross-sectional analysis revealed that the degree of hematuria and proteinuria were significantly associated with levels of Gd-IgA1 and levels of IgA/IgG-IC. Longitudinal analysis further showed that from the group of 44 patients with heavy hematuria before TSP, 31 patients showed complete disappearance of hematuria (group A), but the remaining patients did not (group B). Although the levels of Gd-IgA1 and IgA/IgG-IC in the two groups before TSP were similar, percentage decrease of Gd-IgA1 and IgA/IgG-IC levels in group A was significantly higher than in group B.
Conclusion Disease activity of IgAN assessed by hematuria and proteinuria correlated with serum levels and changes of Gd-IgA1 and IgA/IgG-IC. These new noninvasive disease activity markers can be useful for future activity scoring system and guiding therapeutic approaches.

Keywords IgA nephropathy · Disease activity · Underglycosylated IgA · Immune complex · Biomarker

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Introduction

The primary abnormal manifestation of immunoglobulin A nephropathy (IgAN) is recurring bouts of hematuria with or without proteinuria. However, IgAN has a disease spectrum with many common manifestations, where mesangial IgA immune deposits instigate glomerular damage via unknown mechanisms [1]. From clinical practice, it is known that approximately 30–40 % of IgAN patients progress to end-stage kidney disease within 20 years [1, 2], whereas 10–20 % of patients show spontaneous clinical remission [1–5]. However, there is no definitive method for discriminating patients with these different outcomes.

Thus, the highly variable clinical course and unpredictable progression of IgAN hinder its treatment strategy.

Urinary protein levels may provide acceptable indicators of prognosis [1, 6–10]. However, assessing IgAN activity based on proteinuria should be carefully considered because proteinuria may partly be due to secondary focal segmental glomerulosclerosis (FSGS), known as 'burned-out IgAN', depending on the timing of biopsy during the clinical course [9]. Hematuria is the most important indicator of IgAN activity [1, 6, 7], but clinical evaluation using hematuria can be problematic because there are limitations to its quantification because of false-positive/negative reactions in dipstick tests. The clinical detection of urinary casts and dysmorphic red blood cells accompanying either macroscopic or microscopic hematuria clearly indicate that urinary tract bleeding is glomerular in origin, but they do not accurately indicate disease activity.

Immunohistochemical analysis of renal biopsy specimens is the gold standard for diagnosing and evaluating IgAN activity. However, over the prolonged clinical course of IgAN (approximately 20 years) the histological phenotype is dependent on the timing of renal biopsy [11]. In many countries, abnormalities found during urinalysis may be overlooked or purposely not followed up by further examination until renal function impairment is evident [6]. 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 a perception that a specific treatment is not yet available. Routine screening for urinary abnormalities is performed for all school-aged children in Japan [5, 12, 13]. Furthermore, symptom-free individuals with microscopic hematuria are more likely to undergo renal biopsy, leading to increased diagnosis of IgAN in Japan. However, it is a common practice not to recommend renal biopsy for patients presenting with isolated hematuria or mild proteinuria in the UK, Canada, and the USA, where renal biopsy is reserved for those who develop increasing proteinuria or worsening renal function [6]. Differences in the pathological variables used for renal prognosis in the Japanese and Oxford classifications may partly account for the timing of renal biopsy [14, 15]. Renal biopsies cannot be performed frequently because of the risks involved with the procedure and for socioeconomic reasons. Therefore, renal biopsy is still a snapshot evaluation method and is not a practical method for determining disease activity.

New sensitive and adequately specific noninvasive tests are developing that may guide therapeutic strategies applicable to all IgAN stages. Multivariable pathophysiological processes may mediate IgAN initiation and progression, although IgAN is attributable to mesangial IgA or IgA immune complex (IC) deposition. The nephritogenic roles of galactose-deficient IgA1 (Gd-IgA1) and Gd-IgA1 bound with anti-glycan IgG in an IC (IgA/IgG-IC) have been discussed [16–20]. Berthoux et al. [21] recently reported that Gd-IgA1 and IgA/IgG-IC may have a predictive value for outcome of renal death in IgAN. We examined these biomarkers from a perspective that is different from their study. The present study examined whether serum levels of these noninvasive biomarkers can be a potential index for the disease activity of IgAN equivalent to urinalysis, in patients with complete or partial clinical remission after steroid pulse therapy in combination with tonsillectomy (TSP) whose clinical data and serum were obtained 3–5 years after TSP.

Materials and methods

Patients and treatment

IgAN diagnosis requires renal biopsy with IgA as the dominant or co-dominant Igs in a typical mesangial distribution in the absence of clinical and laboratory evidence of systemic disease. We enrolled IgAN patients showing complete/partial clinical remission after TSP from 1999–2001 in Sendai Shakaihoken Hospital and who could be followed up and whose serum could be obtained serially for 3–5 years after TSP. Clinical remission was defined as negative proteinuria and hematuria as assessed using a dipstick test and/or a urinary erythrocyte count of <5 cells per high-power field during 3 consecutive visits. We defined patients with complete remission as those who showed no further urinary abnormalities throughout the observation period after urinary abnormalities disappeared. Patients who exhibited a relapse of proteinuria and/or hematuria after remission were excluded from the complete remission group, but were included in a partial remission group.

The steroid pulse therapy included 0.5 g methylprednisolone per day for 3 consecutive days, 3 times a week, for at least 1 week after tonsillectomy. Furthermore, 0.5 mg/body weight (kg) prednisolone was administrated once every 2 days for 6–12 months with a gradual tapering of the dose within 1 year [22]. Patients who had received a kidney transplant or who required dialysis were excluded from this study. This study was approved by the ethics committee of the Sendai Shakaihoken Hospital at Miyagi, Japan, and all patients provided written informed consent.

Clinical, laboratory and pathological data

We collected the baseline clinical data immediately before TSP, while qualitative hematuria and proteinuria data and serum were collected at a minimum of three time points, i.e., immediately before, 1 year after, and 3–5 years after TSP. Baseline clinical data (age, sex, duration from onset

to tonsillectomy, systolic blood pressure, total protein, albumin, blood urea nitrogen, serum creatinine, creatine clearance rate [CCr], quantitative proteinuria, amount of proteinuria, and quantitative hematuria) and histological findings were collected from hospital medical records. CCr was calculated based on the mean 24-h urine collection and adjusted for body surface area. Hematuria was evaluated by both dipstick and microscopy in about 70 % of evaluation points, while dipstick evaluation was carried out in all points. Since there was clear correlation in hematuria between both methods, we used quantitative data by dipstick analysis for this study. The histological findings were evaluated based on the index of the glomerular lesion (IGL), as previously reported [23]. IGL is a histological score which is graded from 0–4 with a modification to evaluate sclerotic changes.

Measurement of serum Ig, Gd-IgA1 and IgA/IgG-IC by ELISA

We measured serum Ig, Gd-IgA1, and IgA/IgG-IC at the same time, with all stock serum samples taken immediately before, 1 year after, and 3–5 years after TSP.

Serum IgA and IgG levels were determined using capture ELISA [17, 24]. ELISA plates were coated with 1 μg/ml of the $F(ab')_2$ fragment of goat IgA specific for human IgA and IgG (Jackson Immuno Research Laboratories Inc., West Grove, PA, USA). The captured Igs were then detected using a biotin-labeled $F(ab')_2$ fragment of goat IgG anti-human IgA, or IgG antibody (BioSource). Avidin-conjugated horseradish peroxidase (ExtrAvidin; Sigma-Aldrich) and peroxidase chromogenic substrate *o*-phenylenediamine/H_2O_2 (Sigma-Aldrich) were then added. The color reaction was stopped with 1 M sulfuric acid, and the absorbance was measured at 490 nm using the EL312 BioKinetics Microplate Reader (BioTek). The results were calculated using DeltaSoft III software (BioMetallics).

High-adsorption polystyrene 96-microwell plates (Nalge Nunc International, Rochester, NY, USA) were coated overnight with 2.5 μg/ml $F(ab')_2$ fragments of goat IgG anti-human IgA (Jackson Immuno Research Laboratories) in phosphate-buffered saline (PBS). Coated plates were blocked with 2 % bovine serum albumin (BSA; Sigma-Aldrich) in PBS containing 0.05 % Tween-20 (PBST) and serial two-fold dilutions of duplicate samples and standards in blocking solution were incubated overnight at 4 °C. The captured IgA was subsequently desialylated by treatment for 3 h at 37 °C with 10 mU/ml neuraminidase (Roche) in 10 mM sodium acetate buffer (pH = 5). Samples were then incubated for 3 h at 37 °C with GalNAc-specific biotinylated HAA lectin (Sigma-Aldrich) diluted 1:500 in blocking buffer [16]. The bound lectin was detected with avidin-conjugated horseradish peroxidase and the reaction was developed as described above. HAA reactivity of IgA1 of each sample was calculated as the optical density (OD)/1 μg of IgA. Gd-IgA1 (Ale) purified from the plasma of a patient with IgA1 multiple myeloma was treated with neuraminidase and used as the standard [16, 18].

Serum IgA/IgG-IC was determined using cross-capture ELISA [25]. High-adsorption polystyrene 96-microwell plates were coated with 1 μg/ml $F(ab')_2$ fragments of goat anti-human IgG (Jackson Immuno Research Laboratories). After washing and blocking with 1 % BSA in PBST, samples were diluted 11-fold with the same buffer. The captured Ig was detected with a horseradish peroxidase (HRP)-labeled $F(ab')_2$ fragment of goat IgG anti-human IgA (BioSource) and the reaction was developed as described above.

Statistical analysis

Statistical analysis was performed using Stata version 11 (StataCorp, College Station, TX, USA). Normally distributed continuous variables were expressed as the mean ± SD and compared using the Student's *t* test. Non-normally distributed continuous variables were expressed as the median (interquartile range) and compared using the Mann–Whitney *U* test. Categorical variables were expressed as numbers (proportions) and analyzed using the chi-squared test or Fisher's exact test. The trend for each value was analyzed using the Jonckheere–Terpstra [26] test. All probability values were 2-tailed and all confidence intervals were computed at the 95 % level.

Results

Patient characteristics

In this study, we enrolled 50 IgAN patients with complete or partial clinical remission after TSP. The basic characteristics of the enrolled patients ($N = 50$) whose clinical parameters could be collected are summarized in Table 1. The study population included 40 % males with a median age of 37 years. The average CCr and urinary protein excretion levels were 98.2 ml/min and 0.54 g/day, respectively. A total of 52 % of the patients had complete clinical remission after TSP. Only the duration from onset to tonsillectomy was significantly different among patients with complete or partial remission after TSP (Table 2).

Cross-sectional analysis

We first performed cross-sectional analysis to evaluate potential correlation between severity of hematuria or proteinuria and serum levels of Gd-IgA1 or IgA/IgG-IC

Table 1 Clinical background of IgAN patients

	Number of patients ($N = 50$)
Age	37 (25–48)
Sex (male %)	20 (40.0 %)
Onset to tonsillectomy (years)	2.0 (1.0–4.0)
SBP (mmHg)	122.3 ± 20.5
TP (g/dl)	6.8 ± 0.57
Albumin (g/dl)	4.2 ± 0.41
BUN (mg/dl)	15 ± 5.8
S-Cre (mg/dl)	0.82 ± 0.34
CCr (ml/min)	98.2 ± 26.8
UP (dipstick)	3+; 13, 2+; 8, 1+; 19, ± or −: 10
UP (g/day)	0.54 (0.3–1.3)
U-OB (dipstick)	3+; 27, 2+; 17,1+; 4, ±; 2
IGL score	1.47 (1.3–1.99)
Gd-IgA1 (units/mg IgA)	117.3 ± 45.6
IgA/IgG-IC (OD)	0.81 ± 0.31

Continuous data are presented mean ± SD or median [IQR], and categorical data as number of patients (%)

SBP systolic blood pressure, *BUN* blood urea nitrogen, *S-Cre* serum creatinine, *CCr* creatinine clearance, *UP* urinary protein, *U-OB* urinary occult blood, *IGL* index of the glomerular lesion, *TP* total protein

(Fig. 1). Significant correlations were observed for serum Gd-IgA1 levels and severity of hematuria (P for trend = 0.002) and proteinuria (P for trend = 0.035). Furthermore, significant correlations were observed for IgA/IgG-IC levels and severity of urinary findings (hematuria; P for trend <0.001, proteinuria; P for trend <0.001).

Longitudinal analysis of patients with hematuria

We divided the 44 patients (91.7 %) with heavy hematuria of >2+ by dipstick before TSP into group A [31 patients (64.6 %) with complete remission of hematuria] and group B (remaining patients who retained hematuria during the 3–5-year follow-up period) (Fig. 2a). There was no significant difference in serum Gd-IgA1 and IgA/IgG-IC levels before TSP in both groups [group A vs B, Gd-IgA1 (U/mg IgA); 122.1 ± 48.0 vs 107.7 ± 43.0, $P = 0.36$, IgA/IgG-IC (OD); 0.77 ± 0.31 vs 0.85 ± 0.29, $P = 0.43$]. Group A patients had a significantly higher percentage decrease in Gd-IgA1 ($P = 0.021$) and IgA/IgG-IC ($P = 0.016$) serum levels after TSP than group B patients (Fig. 2b).

Longitudinal analysis of patients with proteinuria

We then divided the 38 patients (79.2 %) with proteinuria before TSP into groups C ($N = 25$) and D ($N = 13$), with or without proteinuria 3–5 years after TSP, respectively (Fig. 3a). There was a significant difference in serum Gd-IgA1 levels, but not in IgA/IgG-IC levels, before TSP in both groups [group C vs D, Gd-IgA1 (U/mg IgA); 102.2 ± 37.6 vs 133.3 ± 41.4, $P = 0.03$, IgA/IgG-IC (OD); 0.81 ± 0.30 vs 0.98 ± 0.33, $P = 0.11$). Cross-sectional analysis indicated significant correlations between proteinuria severity and serum Gd-IgA1 and IgA/IgG-IC levels. However, the percentage decreases in Gd-IgA1 ($P = 0.87$) and IgA/IgG-IC ($P = 0.52$) serum levels after TSP were not significantly different between the 2 groups (Fig. 3b).

The average percentage decrease in IgA/IgG-IC levels before and after 3–5 years was 20 ± 17 in all patients.

Table 2 Clinical background and course of complete and partial remission groups

	Complete remission ($N = 26$)	Partial remission ($N = 24$)	P
Age	32.0 (24–43)	40.5 (28.5–50)	0.13
Sex (male %)	13 (50 %)	7 (29.2 %)	0.13
Onset to tonsillectomy (years)	1.0 (1.0–3.0)	3.0 (2.0–4.0)	0.02
SBP (mmHg)	122.4 ± 20.2	123.5 ± 21.4	0.85
TP (g/dl)	6.8 ± 0.51	6.8 ± 0.64	0.7
Albumin (g/dl)	4.3 ± 0.36	4.1 ± 0.44	0.13
BUN (mg/dl)	13.8 ± 3.7	16.1 ± 7.4	0.18
CCr (ml/min)	103.3 ± 24.2	92.8 ± 28.8	0.06
UP (g/day)	0.45 (0.3–1.0)	0.75 (0.36–1.45)	0.19
IGL score	1.40 (1.29–1.79)	1.62 (1.35–2.2)	0.18
S-Cre (mg/dl)			
Baseline	0.77 ± 0.19	0.82 ± 0.41	0.87
1 year	0.78 ± 0.24	0.84 ± 0.43	0.56
3–5 year	0.77 ± 0.26	0.91 ± 0.70	0.34
UP (dipstick)			
Baseline	3+; 7, 2+; 2, 1+; 9, ±or −; 8	3+; 6, 2+; 6, 1+; 10, ± or −; 2	0.17
1 year	2+; 1, 1+; 6, ± or −; 19	2+; 6, 1+; 7, ± or −; 11	0.01
3–5 year	± or −; 26	3+; 1, 2+; 6, 1+; 7, ± or −; 10	<0.001
U-OB (dipstick)			
Baseline	3+; 11, 2+; 13, 1+; 1, ±or −; 1	3+; 16, 2+; 4, 1+; 3, ± or −; 1	0.23
1 year	3+; 1, 2+; 2, 1+; 2, ± or −; 21	3+; 3, 2+; 1, 1+; 9, ± or −; 11	0.01
3–5 year	± or −; 26	3+; 2, 2+; 4, 1+; 8, ± or −; 10	<0.001

Continuous data are presented mean ± SD or median [IQR], and categorical data as number of patients (%). P based on complete remission and partial remission comparison

SBP systolic blood pressure, *BUN* blood urea nitrogen, *S-Cre* serum creatinine, *CCr* creatinine clearance, *UP* urinary protein, *U-OB* urinary occult blood, *IGL* index of the glomerular lesion, *TP* total protein

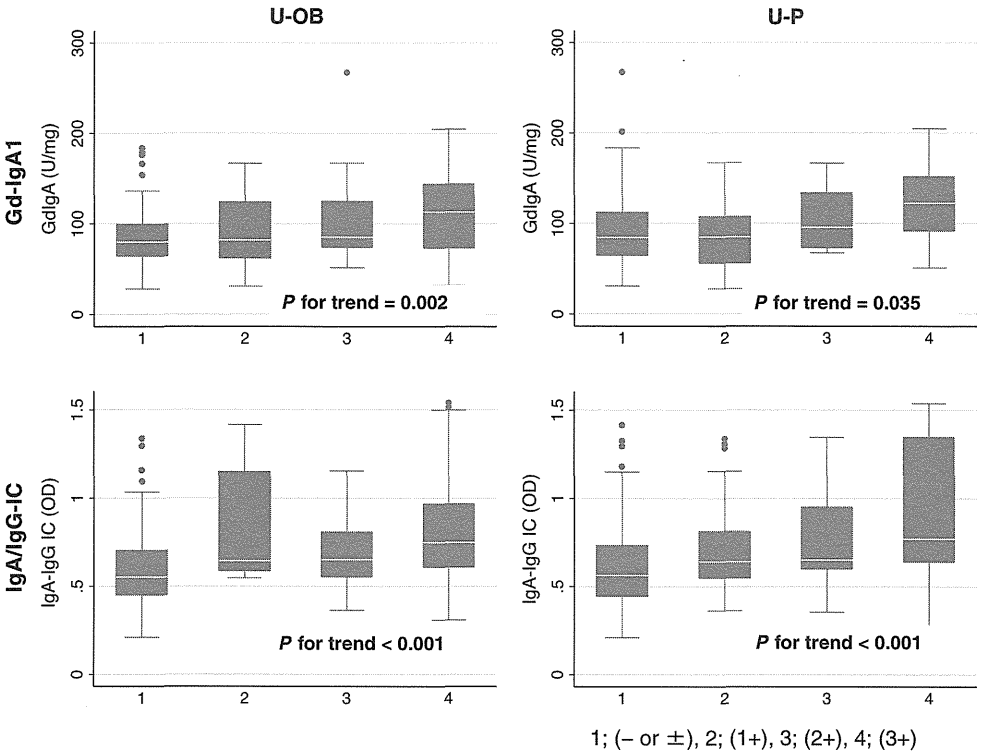

Fig. 1 Cross-sectional analysis of the correlation between severity of hematuria/proteinuria and serum Gd-IgA1 or IgA/IgG-IC levels. Significant correlations were found between serum Gd-IgA1 levels and hematuria (U-OB) and proteinuria (U-P), as determined by dipstick tests. Furthermore, significant correlations were also detected between serum IgA/IgG-IC levels and severity of urinary findings [1; (– or ±), 2; (1+), 3; (2+), 4; (3+) on *x* axis]

Next, we divided the patients according to the average percentage decrease in IgA/IgG-IC serum levels before TSP and 3–5 years after TSP into large delta IC (>20) and small delta IC (≤20) groups, and analyzed laboratory data for the patients in the large delta IC group. In this large delta IC group ($N = 25$; 50 %) of patients who had a greater than average percentage decrease (>20) in IgA/IgG-IC serum levels, proteinuria after 3–5 years was persistent only in 4 patients (16 %) who had severe sclerotic glomerular lesions before TSP (data not shown).

Discussion

This is the first report to demonstrate that assessment of IgAN activity based on urinary abnormality correlates with changes in serum levels of Gd-IgA1 and IgA/IgG-IC. This study indicates that Gd-IgA1 and IgA/IgG-IC could be extremely useful components for evaluation of IgAN activity in a noninvasive manner.

Annual routine screening for urinary abnormalities is conducted in school-aged children to adults in Japan [12, 13], and these screening procedures markedly increase the percentage of early stage IgAN patients presenting with microscopic hematuria and the overall IgAN prevalence. Indeed, chance microscopic hematuria is a leading event for renal biopsy in Japan [5, 7–10, 12, 13]. This observation suggests that hematuria is an initial manifestation of early stage IgAN and a primary manifestation of active IgAN. Recent studies revealed abnormalities of IgA1 glycosylation and formation of autoantibodies to these aberrantly glycosylated IgA1 molecules as key factors in the pathogenesis of IgAN [17–20]. Excessive production of IgA1 is an unlikely sole cause of glomerular IgA, because in IgA myeloma patients IgA rarely deposits in the kidney. Furthermore, only approximately one-third to a half of IgAN patients have increased IgA levels [1, 27, 28]. Thus, a structurally, immunologically, or physicochemically abnormal IgA1 molecule, such as Gd-IgA1, produced by IgAN patients, has been considered as a possible cause of glomerular IgA deposition. Indeed, serum Gd-IgA1 levels are elevated in IgAN patients where they are mainly regulated by genetic and environmental factors [16, 20, 29]. However, the clinical association between Gd-IgA1 levels and their clinical manifestation has not been completely evaluated. It is notable that serum Gd-IgA1 levels correlated with severity of hematuria. In addition, the disappearance or improvement of hematuria after TSP correlated with a decrease in serum Gd-IgA1 levels. These findings indicate that formation of Gd-IgA1

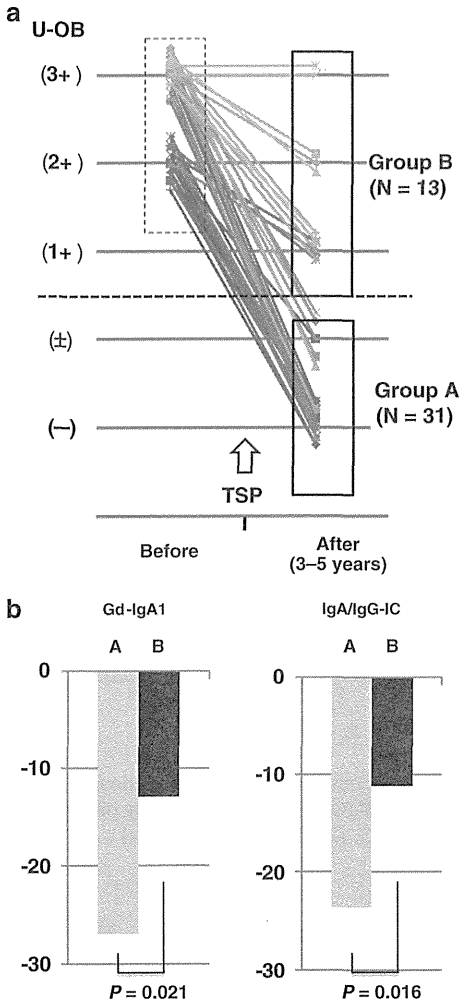

Fig. 2 Longitudinal analysis of patients with hematuria. Forty-four patients with heavy hematuria of >2+ in dipstick tests before TSP were divided into group A, which contained 31 patients with complete remission of hematuria, and group B, which contained the remaining patients who retained hematuria, during the 3–5-year follow-up period (**a**). Group A patients had a significantly higher percentage decrease in both serum Gd-IgA1 ($P = 0.021$) and IgA/IgG-IC ($P = 0.016$) levels than group B patients (**b**)

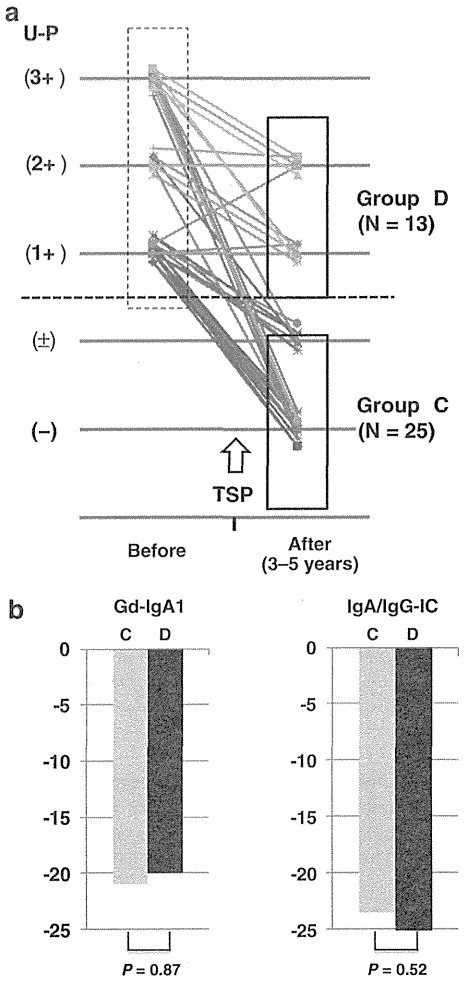

Fig. 3 Longitudinal analysis of patients with proteinuria. Thirty-eight patients with proteinuria before TSP were divided into groups C and D, with or without proteinuria 3–5 years after TSP (**a**). Cross-sectional analysis revealed significant correlations between severity of proteinuria and serum Gd-IgA1 and IgA/IgG-IC levels, but the percentage decrease in serum Gd-IgA1 and IgA/IgG-IC levels did not differ between the groups (**b**)

and Gd-IgA1-containing IC are key steps in the pathogenesis of IgAN, leading to glomerular deposition of these complexes and development of glomerular injury with subsequent hematuria [20]. However, specific serum Gd-IgA1 levels were still detected, even in patients who experienced complete remission after TSP. The absolute amounts of serum Gd-IgA1 were also independent of severity of hematuria before TSP. Therefore, threshold levels of Gd-IgA1 that induce hematuria may differ among individuals. Notably, elevated levels of Gd-IgA1 have been reported also in healthy relatives of IgAN patients [29], suggesting heterogeneity of Gd-IgA1 itself for the induction of glomerular damages.

The production site of nephritogenic Gd-IgA1 remains unclear, although there are some emerging clues. For example, we noted that hematuria in some IgAN patients improved after tonsillectomy alone and this improvement was associated with decreased serum Gd-IgA1 levels (Suzuki Y et al., unpublished data). We previously reported on an animal model of IgAN in which the mucosal activation of Toll-like receptor 9 (TLR9) was involved in IgAN pathogenesis [30, 31]. Furthermore, we reported that a single nucleotide polymorphism of TLR9 was linked with IgAN progression in humans [30]. Another recent study demonstrated that IgAN patients whose serum IgA levels decreased to more than average after tonsillectomy alone (large ΔIgA) showed a significantly higher mRNA expression of TLR9 in the tonsils than IgAN patients with a

smaller decrease (small ΔIgA) in these levels [32]. These findings suggest that nephritogenic Gd-IgA1 may be produced in the tonsils and that this production may involve TLR9 activation [33]. This conclusion is consistent with the observation that tonsillar TLR9 expression was elevated in IgAN patients whose serum Gd-IgA1 levels decreased significantly after tonsillectomy alone (Suzuki Y et al., unpublished data).

Increased IgA-IC levels were found in a large number of IgAN patients [27, 34]. A significant number of IgAN patients have an IC that contains both IgA1 and IgG [19, 35]. Mixed complexes with different Ig isotypes may emerge, at least in part, from specific Ig–anti-Ig interactions. Such an interaction could partly be the result of idiotype–anti-idiotype recognition, the presence of IgA rheumatoid factor (an IgA autoantibody specific to the Fc region of IgG), or IgG–anti-IgA as well as IgA1 anti-glycan antibodies [24]. Indeed, idiotype-positive antibody levels correlated with the clinical status of IgAN patients, as defined by their urinary abnormalities [36]. Recently, it was suggested that IgAN is characterized by a circulating IC composed of Gd-IgA1 and a glycan-specific IgG antibody. Suzuki et al. [18] reported that serum glycan-specific IgG antibody levels could differentiate between IgAN patients and healthy or diseased controls, with 88 % specificity and 95 % sensitivity. In addition, increased levels of this antibody in sera of IgAN patients correlated well with proteinuria. This study evaluated serum IgA/IgG-IC levels, and our findings regarding proteinuria and IgA/IgG-IC levels are consistent with previous studies [18, 35]. *O*-linked carbohydrates in the hinge region of IgA1 considerably affect IgA1 reactivity with such glycan-specific autoantibodies, and the subsequent IC formation may incite glomerular damage, leading to proteinuria and hematuria [18]. Gharavi et al. [29] reported that blood relatives of IgAN patients had increased serum Gd-IgA1 levels even in the absence of nephropathy, suggesting that additional events may be required for complete IgAN progression. Thus, IC formation with Gd-IgA1 and glycan-specific IgG antibody may be one of the second 'hit' events [18, 20]. It is generally known that higher molecular ICs have a higher phlogogenic capacity via the activation of Fc receptors [37]; hence, serum IgA/IgG-IC levels may correlate with severity of glomerular damage leading to proteinuria better than Gd-IgA1 alone. These facts are consistent with present findings in a cross-sectional analysis that serum levels of IgA/IgG-IC were more correlated with severity of urinary abnormalities than those of Gd-IgA1.

In conclusion, we showed in this study that disease activity assessment by hematuria and proteinuria correlated with changes in serum Gd-IgA1 and IgA/IgG-IC levels in most IgAN patients, providing novel value for these new noninvasive and real-time disease activity markers. Although further validation with a larger cohort will be required, clinical application, such as IgAN activity score or risk score, with these markers as principal components could be extremely useful for guiding the therapeutic approaches applicable in all stages of IgAN.

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Conflict of interest The authors have declared that no conflict of interest exists.

References

1. D'Amico G. Natural history of idiopathic IgA nephropathy: role of clinical and histological prognostic factors. Am J Kidney Dis. 2000;36:227–37.
2. Chauveau D, Droz D. Follow-up evaluation of the first patients with IgA nephropathy described at Necker Hospital. Contrib Nephrol. 1993;104:1–5.
3. Szeto CC, Lai FM, To KF, et al. The natural history of immunoglobulin A nephropathy among patients with hematuria and minimal proteinuria. Am J Med. 2001;110:434–7.
4. Shen P, He L, Li Y, et al. Natural history and prognostic factors of IgA nephropathy presented with isolated microscopic hematuria in Chinese patients. Nephron Clin Pract. 2007;106:c157–61.
5. Imai H, Miura N. A treatment dilemma in adult immunoglobulin A nephropathy: what is the appropriate target, preservation of kidney function or induction of clinical remission? Clin Exp Nephrol. 2011;16:195–201.
6. Donadio JV, Grande JP. IgA Nephropathy. N Engl J Med. 2002;347:738–48.
7. Maeda A, Gohda T, Funabiki K, et al. Significance of serum IgA levels and serum IgA/C3 ratio in diagnostic analysis of patients with IgA nephropathy. J Clin Lab Anal. 2003;17:73–6.
8. Nakayama K, Ohsawa I, Maeda-Ohtani A, et al. Prediction of diagnosis of immunoglobulin A nephropathy prior to renal biopsy and correlation with urinary sediment findings and prognostic grading. J Clin Lab Anal. 2008;22:114–8.
9. Wakai K, Kawamura T, Endoh M, et al. A scoring system to predict renal outcome in IgA nephropathy: from a nationwide prospective study. Nephrol Dial Transplant. 2006;21:2800–8.
10. Goto M, Wakai K, Kawamura T, et al. A scoring system to predict renal outcome in IgA nephropathy: a nationwide 10-year prospective cohort study. Nephrol Dial Transplant. 2009;24:3068–74.
11. Nair R, Walker PD. Is IgA nephropathy the commonest primary glomerulopathy among young adults in the USA? Kidney Int. 2006;69:1455–8.
12. Kitagawa T. Lessons learned from the Japanese nephritis screening study. Pediatr Nephrol. 1988;2:256–63.
13. Yamagata K, Iseki K, Nitta K, et al. Chronic kidney disease perspectives in Japan and the importance of urinalysis screening. Clin Exp Nephrol. 2008;12:1–8.

14. Katafuchi R, Ninomiya T, Nagata M, et al. Validation study of oxford classification of IgA nephropathy: the significance of extracapillary proliferation. Clin J Am Soc Nephrol. 2011;6: 2806–13.
15. Shima Y, Nakanishi K, Hama T, et al. Validity of the Oxford classification of IgA nephropathy in children. Pediatr Nephrol. 2012;27:783–92.
16. Moldoveanu Z, Wyatt RJ, Lee JY, et al. Patients with IgA nephropathy have increased serum galactose-deficient IgA1 levels. Kidney Int. 2007;71:1148–54.
17. Suzuki H, Moldoveanu Z, Hall S, et al. IgA1-secreting cell lines from patients with IgA nephropathy produce aberrantly glycosylated IgA1. J Clin Invest. 2008;118:629–39.
18. Suzuki H, Fan R, Zhang Z, et al. Aberrantly glycosylated IgA1 in IgA nephropathy patients is recognized by IgG antibodies with restricted heterogeneity. J Clin Invest. 2009;119:1668–77.
19. Novak J, Julian BA, Tomana M, et al. IgA glycosylation and IgA immune complexes in the pathogenesis of IgA nephropathy. Semin Nephrol. 2008;28:78–87.
20. Suzuki H, Kiryluk K, Novak J, et al. The pathophysiology of IgA nephropathy. J Am Soc Nephrol. 2011;22:1795–803.
21. Berthoux F, Suzuki H, Thibaudin L, et al. Autoantibodies targeting galactose-deficient IgA1 associate with progression of IgA nephropathy. J Am Soc Nephrol. 2012;23:1579–87.
22. Ieiri N, Hotta O, Sato T, et al. Significance of the duration of nephropathy for achieving clinical remission in patients with IgA nephropathy treated by tonsillectomy and steroid pulse therapy. Clin Exp Nephrol. 2012;16:122–9.
23. Hotta O, Furuta T, Chiba S, et al. Regression of IgA nephropathy: a repeat biopsy study. Am J Kidney Dis. 2002;39:493–502.
24. Tomana M, Novak J, Julian BA, et al. Circulating immune complexes in IgA nephropathy consist of IgA1 with galactose-deficient hinge region and antiglycan antibodies. J Clin Invest. 1999;104:73–81.
25. Matousovic K, Novak J, Yanagihara T, et al. IgA1-containing immune complexes in the urine of IgA nephropathy patients. Nephrol Dial Transplant. 2006;21:2478–84.
26. Hollander M, Wolfe DA. Nonparametric statistical methods. 2nd ed. Hoboken: Wiley-Interscience; 1999.
27. Clarkson AR, Seymour AE, Thompson AJ, et al. IgA nephropathy: a syndrome of uniform morphology, diverse clinical features and uncertain prognosis. Clin Nephrol. 1977;8:459–71.
28. D'Amico G. The commonest glomerulonephritis in the world: IgA nephropathy. Q J Med. 1987;64:709–27.
29. Gharavi AG, Moldoveanu Z, Wyatt RJ, et al. Aberrant IgA1 glycosylation is inherited in familial and sporadic IgA nephropathy. J Am Soc Nephrol. 2008;19:1008–14.
30. Suzuki H, Suzuki Y, Narita I, et al. Toll-like receptor 9 affects severity of IgA nephropathy. J Am Soc Nephrol. 2008;19: 2384–95.
31. Kajiyama T, Suzuki Y, Kihara M, et al. Different pathological roles of toll-like receptor 9 on mucosal B cells and dendritic cells in murine IgA nephropathy. Clin Dev Immunol. 2011;2011: 819646. doi:10.1155/2011/819646
32. Sato D, Suzuki Y, Kano T, et al. Tonsillar TLR9 expression and efficacy of tonsillectomy with steroid pulse therapy in IgA nephropathy patients. Nephrol Dial Transplant. 2012;27:1090–7.
33. Suzuki Y, Suzuki H, Nakata J, et al. Pathological role of tonsillar B cells in IgA nephropathy. Clin Dev Immunol. 2011;2011:639074. doi:10.1155/2011/639074
34. Jackson S. Immunoglobulin-antiimmunoglobulin interactions and immune complexes in IgA nephropathy. Am J Kidney Dis. 1988;12:425–9.
35. Czerkinsky C, Koopman WJ, Jackson S, et al. Circulating immune complexes and immunoglobulin A rheumatoid factor in patients with mesangial immunoglobulin A nephropathies. J Clin Invest. 1986;77:1931–8.
36. González-Cabrero J, Egido J, Sancho J, et al. Presence of shared idiotypes in serum and immune complexes in patients with IgA nephropathy. Clin Exp Immunol. 1987;68:694–702.
37. Nimmerjahn F, Ravetch JV. Fc-receptors as regulators of immunity. Adv Immunol. 2007;96:179–204.

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ORIGINAL ARTICLE

Nationwide survey on current treatments for IgA nephropathy in Japan

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Abstract
Background A wide variety of treatments, including tonsillectomy and steroid pulse therapy (TSP), are performed for the various stages of IgA nephropathy (IgAN) in Japan. However, the current status of treatments for IgAN patients in Japan is still unclear. The objective of the present study was to investigate the current status of treatments for IgAN patients.
Methods A nationwide survey was conducted in 2008 by sending questionnaires to the 1,194 teaching hospitals of the Japanese Society of Nephrology (JSN) via Progressive Renal Diseases Research, Research on intractable disease, from the Ministry of Health, Labour and Welfare of Japan.
Results Among the total 376 hospitals (31.4 %) that responded, 188 hospitals (66.2 % in the internal medicine departments) performed TSP, out of which 137 hospitals (61.4 %) had begun to perform TSP in the period from 2004 to 2008. The following two major steroid pulse protocols in TSP were used: (1) three cycles over 3 consecutive weeks and (2) three cycles every 2 months. Approximately 68 % of pediatric hospitals (68 hospitals) performed combination therapy with prednisolone, azathioprine, heparin-warfarin and dipyridamole. The clinical remission rates for hematuria and proteinuria after TSP tended to be higher than those following other corticosteroid therapies. Almost all hospitals prescribed antiplatelet agents and renin angiotensin system inhibitor (RAS-I).
Conclusion In addition to popular treatments such as antiplatelet agents and RAS-I, TSP is becoming a standard treatment for adult IgAN patients in Japan.

Keywords IgA nephropathy · Nationwide survey · Questionnaire survey · Tonsillectomy and steroid pulse therapy

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Introduction

IgA nephropathy (IgAN) is the most common primary chronic glomerulonephritis in the world, and is recognized as one of the major causes of end-stage kidney disease (ESKD) [1–5]. Although IgAN was initially believed to represent a benign condition, recent studies [6] have shown that 30–40 % of patients progress to ESKD within 10–25 years from its apparent onset. Therefore, treatment strategies to decrease the risk of IgAN progressing to ESKD would have substantial health benefits [7]. However, disease-specific therapy for IgAN patients has not been established because the pathogenesis of IgAN is still a matter of debate.

As annual check-ups including urinalysis are well established in Japan, patients in various stages of IgAN can be managed and are provided a wide variety of treatments. Oral corticosteroid, steroid pulse therapy, tonsillectomy and steroid pulse therapy (TSP), antihypertensive agents, immunosuppressants, antiplatelet agents and anticoagulants are listed in the regional guidelines of Japan [8].

Corticosteroid therapy is now a popular treatment for IgAN patients after being first reported by Kobayashi [9]. Although the clinical value of intravenous steroid pulse therapy was demonstrated by Pozzi et al [10], no consensus exists for the corticosteroid dose and administration route (oral or intravenous infusion). TSP has recently become a popular standard treatment in Japan. However, the current status of IgAN treatment in Japan is still unclear because no nationwide study has been conducted. Thus, we conducted a nationwide survey using a questionnaire through the Progressive Renal Diseases Research, Research on intractable disease, from the Ministry of Health, Labour and Welfare of Japan.

Methods

We sent questionnaires by mail to 1,194 hospitals (Internal Medicine, 803; Pediatrics, 391), which are teaching hospitals in the Japanese Society of Nephrology (JSN), between October 30 and December 27 in 2008. The questionnaire covered treatment details provided for IgAN and their outcomes (Table 1).

Results

A total of 376 hospitals (31.4 %) (Internal Medicine 284; Pediatrics 92) responded. The mean number of beds in these hospitals was 581.

Tonsillectomy and steroid pulse therapy (TSP)

A total of 188 internal medicine hospitals (66.2 %) stated that they had performed TSP. Steroid pulse therapy was always combined with tonsillectomy in 72 (38.3 %) hospitals. The starting year for TSP is shown in Fig. 1. The annual number of patients who received TSP treatment ranged widely from 1 to 200 patients per year. Steroid pulse therapy using 500–1,000 mg/day (or 20–30 mg/kg/day) methylprednisolone (m-PSL) was performed using the following two major protocols; (1) three times over 3 consecutive weeks (47.8 %), and (2) three times every 2 months (18.9 %). The number of steroid pulses varied at each hospital (24 hospitals, once; 12 hospitals, twice; 92 hospitals, three times). In total, 179 hospitals (80.2 %) did not change the protocol for each patient. Almost all facilities prescribed oral prednisolone after the steroid pulse therapy. A total of 141 hospitals (63.2 %) had criteria for tapering oral prednisolone. The most cited indication for the therapy was the histological findings (164 hospitals, 87.2 %), and other indications were proteinuria grade (156 hospitals, 83.0 %), disease activity (104 hospitals, 55.3 %), hematuria grade (56 hospitals, 29.8 %) and duration from onset (38 hospitals, 20.2 %). In addition, 109 hospitals (48.9 %) performed TSP if the patients wanted and the doctors judged to need the treatment. Figures 2 and 3 show the clinical remission rates for hematuria and proteinuria. The most frequent remission rate ranged from 60 to 80 %. Table 3 shows the routine examination before TSP, concomitant drugs and adverse effects.

Steroid pulse therapy without tonsillectomy

A total of 192 hospitals (51.1 %) performed steroid pulse therapy without tonsillectomy (Table 2). Most of the hospitals (183 hospitals, 95.3 %) performed steroid pulse therapy for less than 10 patients annually. Only six hospitals performed steroid pulse therapy for more than 11 patients per year. The main protocol of steroid pulse therapy was 500–1,000 mg/day m-PSL for 3 consecutive days. The number of times steroid pulses were varied among hospitals (34 hospitals, once; 31 hospitals twice; 65 hospitals, three times). The most cited indication for this therapy was histological findings and proteinuria grade (137 hospitals, 71.4 %), and other indications were disease activity (97 hospitals, 50.5 %), hematuria grade (30 hospitals; 15.6 %) and duration from onset (22 hospitals, 11.5 %). All hospitals prescribed oral prednisolone after the steroid pulse therapy. In total, 102 hospitals (53.1 %) had criteria for tapering oral prednisolone. Although the clinical remission rate for hematuria ranged between 60 and 80 % (Fig. 2), the remission rate for proteinuria was ranged between 0 and 20 % (Fig. 3). Table 3 shows the routine examinations performed before the steroid pulse therapy without tonsillectomy, concomitant drugs and adverse effects.

Oral corticosteroid monotherapy (including combination therapy)

A total of 184 hospitals (48.9 %) performed oral corticosteroid monotherapy (Table 2). Most of the hospitals (149, 81.0 %) performed this therapy for less than 10 patients annually, and only 10 hospitals performed it for more than 11 patients. The most frequent initial dose in the internal medicine and pediatric departments was 21–40 and 1–2 mg/kg/day, respectively. The most frequent duration of medication was 24 months (54 hospitals, 28.7 %), and the duration of medication varied in each hospital. Seventy-four hospitals (40.2 %) had tapering criteria, and

Table 1 Questionnaire

Q1 Check all the following treatments used in your hospital
a. Tonsillectomy and steroid pulse therapy (TSP)
b. Steroid therapy (steroid pulse without tonsillectomy or oral steroid monotherapy)
c. Antiplatelet agents
d. Angiotensin-converting enzyme inhibitor (ACE-I) or angiotensin receptor blocker (ARB)
e. Other therapies

Q2 Tonsillectomy and steroid pulse therapy (TSP)
Q2-1 When did you start TSP for IgA nephropathy patients in your hospital?
Q2-2 To how many patients did your department administer TSP during the last year?
Q2-3 What is the standard protocol for TSP in your hospital?
Q2-4 Do you have a treatment criteria?
If you answered yes, please check the following criteria.
a. Amount of proteinuria
b. Degree of hematuria
c. Histological grade 1, 2, 3, 4*
d. Activity
e. Duration of onset
Q2-5 If the patient wants this treatment, do you perform it in all cases?
Q2-6 Do you modify the treatment protocol in each patient?
If you answered yes, how did you modify the protocol?
Q2-7 On an average, how long do you use oral steroids after steroid pulse therapy?
Q2-8 What are the criteria of the tapering oral steroid?
Q2-9 What is the remission rate of hematuria one year after treatment?
Q2-10 What is the remission rate of proteinuria one year after treatment?
Q2-11 Do you perform routine examinations during the time of steroid therapy?
a. General blood examination (blood cell counts, biochemistry, fasting blood sugar)
b. Blood pressure
c. Upper gastrointestinal endoscopy
d. Bone densitometry
e. Bone metabolism makers
f. Ophthalmologic examination
Q2-12 What concomitant drugs do you use?
(e.g., H2 blocker, proton-pump inhibitor, bisphosphonates, vitamin D3, vitamin K2, antiplatelet agents)
Q2-13 What adverse effects have you ever experienced because of this treatment?

Q3 Steroid therapy
(steroid pulse therapy without tonsillectomy and oral corticosteroid monotherapy)
Steroid pulse therapy without tonsillectomy
Q3-1,2,3 (same as Q2-2,3,4)
Q3-4 What is after treatment of steroid pulse therapy?
Q3-5,6,7,8,9,10 (same as Q2-8,9,10,11,12,13)
Oral corticosteroid monotherapy
Q3-11 to 20 (same as Q3-1 to Q3-10)

Q4 Antiplatelet agents
Q4-1 (same as Q2-4)
Q4-2 How often do you use antiplatelet agents?
a. 75%–100%, b. 50%–74%, c. 25%–49%, d. 0%–24%
Q4-3 When do you discontinue?
a. Before surgery
b. Appearance of adverse effects
c. Disappearance of hematuria
d. Disappearance of proteinuria
Q4-4 Do you perform routine examinations at the time of using the antiplatelet agents?
Q4-5 What drugs do you use for concomitant therapy?
Q4-6 What adverse effects have you ever experienced due to this treatment?

Q5 Angiotensin-converting enzyme inhibitor (ACE-I) or angiotensin receptor blocker (ARB)
Q5-1 (same as Q4-2)
Q5-2 Do you have a specific criteria for their use?
Q5-3 (If you answered yes in Q5-2)
In what cases do you use ACE-I/ARB?
a. Urinary findings and hypertension
b. Only proteinuria
c. Only hypertension
Q5-4 What is the rate of ACE-I or ARB use?
Q5-5 If you can separate the use of ACE-I and ARB, what are the criteria for that?
a. Nothing particular
b. Drug price
c. Adverse effect
d. Dose
e. Others
Q5-6 Do you use combination therapy with ACE-I andARB?
Q5-7 Do you have an indication of the concurrent therapy?
a. Nothing particular
b. Urinary protein
c. Blood pressure
d. Others
Q5-8 How many doses do you use ?
Q5-9 (same as Q4-6)

1 good prognosis group, *2* relatively good prognosis group, *3* relatively poor prognosis group, *4* poor prognosis group

*Criteria for histological grading from IgA nephropathy (IgAN) clinical guidelines in Japan

68 hospitals (68.5 % in pediatric hospitals) provided a combination therapy of prednisolone, azathioprine, heparin-warfarin and dipyridamole. The most cited indication for this therapy was the proteinuria grade (140 hospitals; 76.1 %). Other indications included histological findings (129 hospitals, 70.1 %), disease activity (93 hospitals, 50.5 %), hematuria grade (31 hospitals, 16.8 %) and duration from onset (19 hospitals, 10.3 %). The most frequent clinical remission rate of hematuria was 40–60 % (Fig. 2), and that of proteinuria was 0–20 % (Fig. 3). Table 3 shows the routine examinations performed before oral corticosteroid monotherapy, concomitant drugs and adverse effects.

Antiplatelet agents

A total of 351 hospitals (93.4 %) prescribed antiplatelet agents (Table 2). The majority of hospitals (188; 53.6 %) prescribed the antiplatelet agents in all cases. The prescription rate in each hospital is shown in Fig. 4. The main reason for discontinuation was scheduled surgery (313 hospitals, 89.3 %). The routine examination before this treatment was mainly a general blood examination. Major adverse effects were headache and gastrointestinal symptoms.

Renin-angiotensin system inhibitor (RAS-I)

A total of 371 hospitals (98.7 %) prescribed RAS-I (Table 2), but 226 hospitals (60.1 %) did not have criteria for this treatment. The prescription rate is shown in Fig. 5. Most hospitals did not have clear criteria for the choice between angiotensin-converting enzyme inhibitor (ACE-I) and angiotensin receptor blocker (ARB), and 218 hospitals (58.8 %) prescribed concurrently ACE-I and ARB. The most indicated criteria for the combination was proteinuria

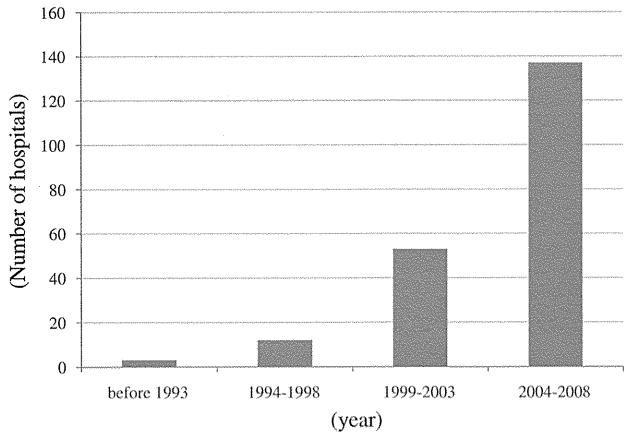

Fig. 1 Starting year for tonsillectomy and steroid pulse therapy (TSP). TSP spread rapidly in Japan from 2004 to 2008

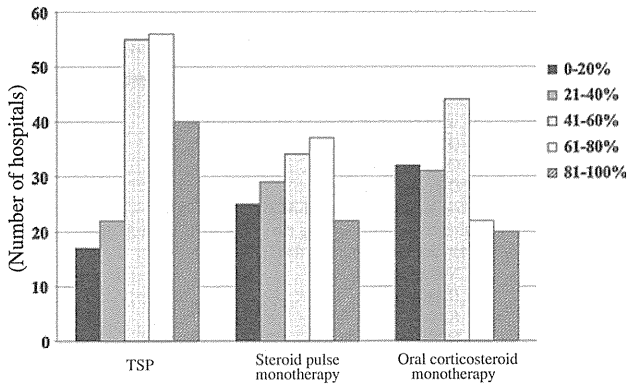

Fig. 2 Clinical remission rate for hematuria based on treatment. The clinical remission rate for hematuria in many hospitals using TSP was higher than that after steroid pulse without tonsillectomy or oral corticosteroid monotherapy

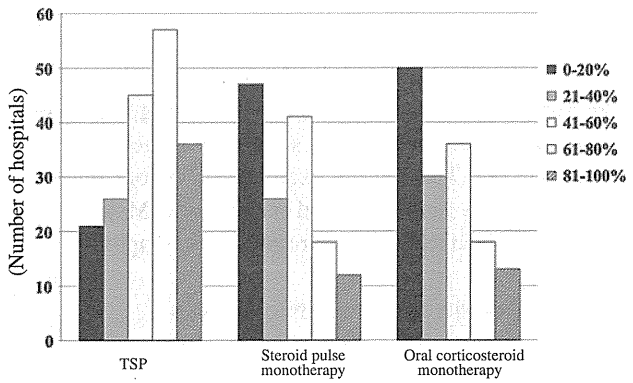

Fig. 3 Clinical remission rate of proteinuria based on the treatment. The clinical remission rate for proteinuria using TSP was higher than that using steroid pulse without tonsillectomy or oral corticosteroid monotherapy

(160 hospitals, 73.4 %) and blood pressure (94 hospitals, 43.1 %). Adverse effects include hyperkalemia, elevation of serum creatinine, hypotension, dizziness and dry cough.

Table 2 Number of hospitals for each treatment

	Total (%) $n = 376$	Internal medicine (%) $n = 284$	Pediatrics (%) $n = 92$
TSP	223 (59.3)	188 (66.2)	35 (38.0)
Steroid pulse monotherapy	192 (51.1)	159 (56.0)	33 (35.9)
Oral corticosteroid monotherapy[a]	184 (48.9)	156 (54.9)	28 (30.4)
Antiplatelet agents	351 (93.4)	275 (96.8)	76 (82.6)
RAS-I	371 (98.7)	283 (99.6)	88 (95.7)

TSP tonsillectomy and steroid pulse therapy, *RAS-I* renin–angiotensin system inhibitor

[a] Including combination therapy (prednisolone, azathioprine, heparin-warfarin, and dipyridamole)

Discussion

A wide variety of treatments for IgAN exist in Japan because various stages of disease can be observed and managed. The current treatment situation has been unclear until now because no nationwide study has been conducted regarding IgAN treatment. The present study assessed the precise situation of treatment for IgAN in Japan.

TSP was first reported by Hotta et al. [11] in 2001. Many clinical studies on TSP have been reported from Japan since 2001 [12–14]. Miura et al. conducted a multicenter retrospective cohort study, and reported that TSP was effective for patients with early-stage IgAN if performed within 5 years of onset and for those who have daily proteinuria <1.1 g and serum creatinine <1.5 mg/dl [15]. However, the details of TSP (protocols, indication, clinical remission rate, etc.) varied in each report, and the current TSP situation was thus unclear. Our results show that almost 70 % of internal medicine hospitals performed TSP. Almost 40 % of hospitals always added combined steroid pulse therapy with tonsillectomy. Moreover, almost 60 % hospitals began TSP in the period between 2004 and 2008 (Fig. 1), indicating that TSP spread through Japan quickly and has become the major therapeutic approach for IgAN in the last decade. We also observed that the clinical remission rates for both hematuria and proteinuria following TSP tended to be higher than those resulting from steroid pulse without tonsillectomy or oral corticosteroid monotherapy (Figs. 2, 3). This may be one of the main reasons for the quick spread of this therapy in Japan. In previous reports, TSP protocols have varied. In particular, the number of steroid pulses given during TSP varied in each report [11–13]. Our results showed that there are two major protocols for TSP in Japan. One is a protocol in which the steroid pulses are administrated three times, with a steroid pulse every week, on the basis of the original report by Hotta et al. [11]. Another is in which steroid

Table 3 Routine examinations, concomitant drugs, and adverse effects for each treatment

	Routine examination (hospitals, %)	Concomitant drugs (hospitals, %)	Adverse effects (hospitals, %)
TSP	General blood examination (221, 99.1), Blood pressure (202, 90.6), Ophthalmologic examination (108, 48.4), Bone densitometry (107, 48.0), Upper gastrointestinal endoscopy (40, 17.9), Bone metabolism maker (20, 9.0)	H2 blocker or proton-pump inhibitor (207, 92.8), Antiplatelet agent (157, 70.4), Vitamin D3 (91, 40.8), Vitamin K2 (15, 6.7)	Steroid-induced diabetes (32, 14.3), Steroid-induced psychosis (17, 7.6), Moon face (12, 5.4), Steroid osteoporosis (6, 2.7), Postoperative pain (6, 2.7), Bleeding (5, 2.2), Loss of taste (3, 1.3)
Steroid pulse monotherapy	General blood examination (147, 76.6), Blood pressure (135, 70.3), Ophthalmologic examination (75, 39.0), Bone densitometry (74, 38.5), Upper gastrointestinal endoscopy (28, 14.6), Bone metabolism maker (16, 8.3)	H2 blocker or proton-pump inhibitor (137, 71.4), Antiplatelet agent (22, 11.5), Vitamin K2 (13, 6.8)	Steroid-induced diabetes (13, 6.8), Steroid-induced cataract (7, 3.6), Pneumonia (5, 2.6), Moon face (4, 2.1), Central obesity (4, 2.1)
Oral corticosteroid monotherapy*	General blood examination (128, 69.6), Blood pressure (116, 63.0), Bone densitometry (56, 30.4), Ophthalmologic examination (55, 29.9), Upper gastrointestinal endoscopy (20, 10.9), Bone metabolism maker (15, 8.2)	H2 blockers or proton-pump inhibitors (111, 60.3), bisphosphonates (74, 40.2), Vitamin D3 (56, 30.4), Antiplatelet agents (26, 14.1), Vitamin K2 (9, 4.9)	Steroid-induced diabetes (11, 6.0), Steroid-induced cataract (5, 2.7), Steroid-induced psychosis (4, 2.1), Moon face (3, 1.6), Steroid-induced osteoporosis (3, 1.6)

*Including combination therapy (prednisolone, azathioprine, heparin-warfarin, and dipyridamole) TSP, tonsillectomy and steroid pulse therapy

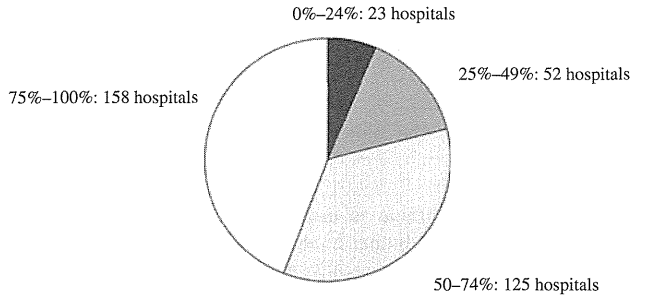

Fig. 4 Prescription rate for antiplatelet agents in each hospital. Almost 40 % of the hospitals prescribed for 75–100 % patients in their hospital

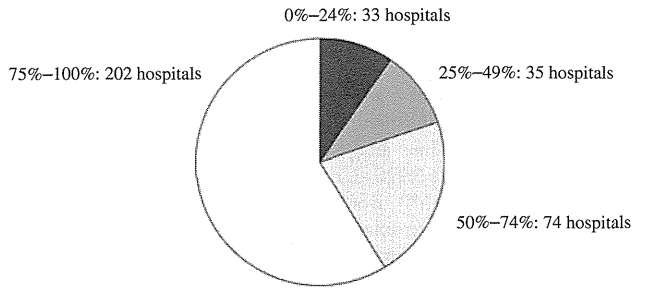

Fig. 5 Prescription rate for renin-angiotensin system inhibitors in each hospital. More than 50 % hospitals prescribed for 75–100 % patients in each hospital

pulses are administrated three times every 2 months, based on previous report by Pozzi et al. [10]. We did not find a clear difference in clinical efficacy between two methods.

The Japanese Pediatric IgA Nephropathy Treatment Study Group advocated combination therapy for childhood IgAN in their 2008 guideline [16]. A number of studies by Japanese groups [17–19] have reported beneficial outcomes in childhood IgAN using the combination therapy with prednisolone, azathioprine, heparin-warfarin and dipyridamole. The rationale for this treatment is as follows; (1) corticosteroids and immunosuppressive agents reduce serum IgA production and minimize the abnormal immune response and inflammatory events following glomerular IgA deposition, and (2) heparin-warfarin and dipyridamole are used to inhibit the mediators of glomerular damage [17]. Our results demonstrated that 68 hospitals (68.5 % of pediatric hospitals) performed the combination therapy, suggesting that combination therapy is a standard therapy for pediatric IgAN in Japan. Pozzi et al. [20] recently demonstrated that clinical outcomes in adults are not different between treatment with corticosteroids alone and corticosteroids with oral azathioprine. In contrast, Kamei et al. [21] reported that the combination therapy improves the long-term outcome in childhood IgAN. Because these two studies enrolled different populations, this difference may provide a clue of the indications for this treatment.

A recent meta-analysis showed that the use of corticosteroids IgAN patients is associated with a significant decrease in the risk of ESKD and urinary protein excretion [22]. Although the practice pattern (with or without tonsillectomy, immunosuppressants, etc) was not standardized, almost 70 % hospitals were found to perform corticosteroid therapy. Consequently, one can conclude that corticosteroid therapy has become standard in Japan. In particular, TSP and combination therapy are popular in internal medicine and pediatric departments, respectively.

Intraglomerular coagulation, either through local activation of blood coagulation or impaired removal by the fibrinolytic system, has been proposed as one of the factors causing glomerular injury in IgAN [23]. Previous studies including meta-analysis [24–27] reported beneficial effects of anti-platelet agents for IgAN. Therefore, antiplatelet agents are listed in the Japanese regional guidelines [8]. In fact, the national health insurance covers dipyridamole for glomerulonephritis and dilazep hydrochloride for IgAN. On the contrary, Fleoge et al. [28] did not recommend using antiplatelet agents in patients with IgAN because most studies on antiplatelet agents are often combined with immunosuppressants and were retrospective and nonrandomized. Moreover, the Kidney Disease: Improving Global Outcomes (KDIGO) Clinical Practice Guideline for Glomerulonephritis concluded that there is no benefit for antiplatelet agents alone because patients received other concomitant therapies in Japanese studies [29]. Our results suggest that almost all Japanese hospitals (351, 93.4 %) prescribed antiplatelet agents for IgAN. It is thought that Japanese nephrologists prescribe these drugs based on previous studies and for compliance with regional guidelines. In future, we need to confirm the effects of antiplatelet agents in a large cohort study from Japan.

RAS-I is effective for glomerular hypertension, podocyte injury and tubulointerstitial injury, and thus is prescribed for glomerulonephritis. Amelioration of glomerular injury and fibrosis by ARB has been demonstrated in animal models of IgAN [30]. Because several studies, including randomized controlled trials [31–33], have reported the effectiveness of RAS-I for IgAN, recent guidelines [29] recommend this therapy for IgAN. Tomino et al. [34] and Moriyama et al. [35] reported the beneficial effects of IgAN in Japan. Furthermore, our results revealed that almost all hospitals (371, 98.7 %) prescribed RAS-I for IgAN, indicating that RAS-I is a popular treatment in Japan. The combination of ACE-I and ARB has antiproteinuric effects greater than monotherapy in normotensive IgAN [36]. The present study revealed that 218 hospitals (58.8 %) prescribed ACE-I and ARB concurrently. The indications for concurrent use are proteinuria and blood pressure, suggesting that they aim to renoprotect through antiproteinuric effects.

Our study has several limitations. First, there was a possibility of selection bias. The response rate was only 31.4 % of 1,194 hospitals. Those hospitals tended to be large hospitals; thus, our results could not reflect the treatments in small hospitals and clinics. Second, a possibility for measurement bias regarding clinical efficacy existed. Because we did not strictly define "clinical remission" in this study, treatment efficacy depended on the judgment of each hospital. Third, the questionnaire asked about all treatments in each hospital; thus, we could not analyze and estimate the priority of the treatments. Fourth, the questionnaire surveyed all IgAN stages, but it is well known that IgAN has a heterogeneous disease course; therefore, treatments may depend on stage. In future, we need to conduct an investigation of the treatments for each stage of IgAN.

In conclusion, corticosteroid therapy, along with antiplatelet agents and RAS-I therapy, has become a standard treatment for IgAN in Japan. Although we observed that the corticosteroid therapy protocol varied, TSP is becoming a standard treatment, at least for adult IgAN. Further studies are required to compare the efficacy of each treatment and to determine the standard therapy for each stage of IgAN.

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Conflict of interest The authors have declared that no Conflict of interest exists.

References

1. D'Amico G. The commonest glomerulonephritis in the world: IgA nephropathy. Q J Med. 1987;245:709–27.
2. Schena FP. A retrospective analysis of the natural history of primary IgA nephropathy worldwide. Am J Med. 1990;89:209–15.
3. Li L-S, Liu Z-H. Epidemiologic data of renal diseases from a single unit in China: analysis based on 13,519 renal biopsies. Kidney Int. 2004;66:920–3.
4. Simon P, Ramee MP, Boulahrouz R, Stanescu C, Charasse C, Ang KS, et al. Epidemiologic data of primary glomerular diseases in western France. Kidney Int. 2004;66:905–8.
5. Barratt J, Feehally J. IgA nephropathy. J Am Soc Nephrol. 2005; 16:2088–97.
6. D'Amico G. Natural history of idiopathic IgA nephropathy and factors predictive of disease outcome. Semin Nephrol. 2004;24: 179–96.

7. Barratt J, Feehally J. Treatment of IgA nephropathy. Kidney Int. 2006;69:1934–8.
8. Progressive Renal Diseases Research, Research on intractable disease, from the Ministry of Health Labors and Welfare of Japan. Clinical guides for Immunoglobulin A (IgA) nephropathy in Japan, third version. Nihon Jinzo Gakkai shi 2011; 53:123–35.
9. Kobayashi Y, Fujii K, Hiki Y, Tateno S. Steroid therapy in IgA nephropathy: a prospective pilot study in moderate proteinuric cases. Q J Med. 1986;61:935–43.
10. Pozzi C, Bolasco PG, Fogazzi GB, Andrulli S, Altieri P, Ponticelli C, et al. Corticosteroids in IgA nephropathy: a randomised controlled trial. Lancet. 1999;353:883–7.
11. Hotta O, Miyazaki M, Furuta T, Tomioka S, Chiba S, Horigome I, et al. Tonsillectomy and steroid pulse therapy significantly impact on clinical remission in patients with IgA nephropathy. Am J Kidney Dis. 2001;38:736–43.
12. Sato M, Hotta O, Tomioka S, Chiba S, Miyazaki M, Noshiro H, et al. Cohort study of advanced IgA nephropathy: efficacy and limitations of corticosteroids with tonsillectomy. Nephron Clin Pract. 2003;93:c137–45.
13. Komatsu H, Fujimoto S, Hara S, Sato Y, Yamada K, Kitamura K. Effect of tonsillectomy plus steroid pulse therapy on clinical remission of IgA nephropathy: a controlled study. Clin J Am Soc Nephrol. 2008;3:1301–7.
14. Kawaguchi T, Ieiri N, Yamazaki S, Hayashino Y, Gillespie B, Miyazaki M, et al. Clinical effectiveness of steroid pulse therapy combined with tonsillectomy in patients with immunoglobulin A nephropathy presenting glomerular haematuria and minimal proteinuria. Nephrology. 2010;15:116–23.
15. Miura N, Imai H, Kikuchi S, Hayashi S, Endoh M, Kawamura T, et al. Tonsillectomy and steroid pulse (TSP) therapy for patients with IgA nephropathy: a nationwide survey of TSP therapy in Japan and an analysis of the predictive factors for resistance to TSP therapy. Clin Exp Nephrol. 2009;13:460–6.
16. Yoshikawa N, Igarashi T, Ishikura K, Kaku Y, Nakazato H, Kamei K, et al. Guidelines for the treatment of childhood IgA nephropathy. Nihon Jinzo Gakkai shi. 2008;50:31–41.
17. Yoshikawa N, Ito H, Sakai T. A controlled trial of combined therapy for newly diagnosed severe childhood IgA nephropathy. J Am Soc Nephrol. 1999;10:101–9.
18. Yoshikawa N, Honda M, Iijima K, Awazu M, Hattori S, Nakanishi K, et al. Steroid treatment for severe childhood IgA nephropathy: a randomized, controlled trial. Clin J Am Soc Nephrol. 2006;1:511–7.
19. Yoshikawa N, Honda M, Iijima K, Awazu M, Hattori S, Nakanishi K, et al. Combination therapy with mizoribine for severe childhood IgA nephropathy: a pilot study. Pediatr Nephrol. 2008;23:757–63.
20. Pozzi C, Andrulli S, Pani A, Scaini P, Del Vecchio L, Fogazzi G, et al. Addition of azathioprine to corticosteroids does not benefit patients with IgA nephropathy. J Am Soc Nephrol. 2010;21: 1783–90.
21. Kamei K, Nakanishi K, Ito S, Saito M, Sako M, Ishikura K, et al. Long term results of a randomized controlled trial in childhood IgA nephropathy. Clin J Am Soc Nephrol. 2011;6:1301–7.
22. Samuels JA, Strippoli GF, Craig JC, Schena FP, Molony DA. Immunosuppressive treatments for immunoglobulin A nephropathy: a meta-analysis of randomized controlled trials. Nephrology (Carlton). 2004;9:177–85.
23. Barratt J, Feehally J, Lai KN (ed): Recent Advances in IgA Nephropathy. 1st ed. World Scientific Pub Co Inc; 2009: Chapter 24 "Other non-immunomodulatory agents".
24. Chan MK, Kwan SY, Chan KW, Yeung CK. Controlled trial of antiplatelet agents in mesangial IgA glomerulonephritis. Am J Kidney Dis. 1987;9:417–21.
25. Lee GS, Choong HL, Chiang GSC, Woo KT. Three year randomized controlled trial of dipyridamole and low-dose warfarin in patients with IgA nephropathy and renal impairment. Nephrology (Carlton). 1997;3:117–21.
26. Tomino Y. Long term effects of dilazep hydrochloride, an antiplatelet drug, on patients with IgA nephropathy—reports of 5-year treatment. Curr. Top. Pharmacol. 2007;11:45–9.
27. Taji Y, Kuwahara T, Shikata S, Morimoto T. Meta-analysis of antiplatelet therapy for IgA nephropathy. Clin Exp Nephrol. 2006;10:268–73.
28. Floege J, Eitner F. Current therapy for IgA nephropathy. J Am Soc Nephrol. 2011;22:1785–94.
29. Kidney Disease: Improving Global Outcomes (KDIGO) Glomerulonephritis Work Group. KDIGO Clinical Practice Guideline for Glomerulonephritis. Kidney Int Suppl. 2012;2: 139–274.
30. Suzuki Y, Thang NT, Horikoshi S, Shirato I, Nakamura S, Kimura M, et al. Effect of valsartan, an angiotensin II AT 1 receptor blocker, on the glomerular fibrosis of IgA nephropathy in ddY mice. Nephron. 2000;86:374–5.
31. Li PK-T, Leung CB, Chow KM, Cheng YL, Fung SK-S, Mak SK, et al. Hong Kong study using valsartan in IgA nephropathy (HKVIN): a double blind, randomized, placebo-controlled study. Am J Kidney Dis. 2006;47:751–60.
32. Coppo R, Peruzzi L, Amore A, Piccoli A, Cochat P, Stone R, et al. IgACE: a placebo-controlled, randomized trial of angiotensin-converting enzyme inhibitors in children and young people with IgA nephropathy and moderate proteinuria. J Am Soc Nephrol. 2007;18:1880–8.
33. Praga M, Gutiérrez E, González E, Morales E, Hernández E. Treatment of IgA nephropathy with ACE Inhibitors: a randomized and controlled trial. J Am Soc Nephrol. 2003;14:1578–83.
34. Tomino Y, Kawamura T, Kimura K, Endoh M, Hosoya T, Horikoshi S, et al. Antiproteinuric effect of olmesartan in patients with IgA nephropathy. J Nephrol. 2009;2:224–31.
35. Moriyama T, Amamiya N, Ochi A, Tsuruta Y, Shimizu A, Kojima C, et al. Long-term beneficial effects of angiotensin-converting enzyme inhibitor and angiotensin receptor blocker therapy for patients with advanced immunoglobulin A nephropathy and impaired renal function. Clin Exp Nephrol. 2011;15: 700–7.
36. Russo D, Minutolo R, Pisani A, Esposito R, Signoriello G, Andreucci M, et al. Coadministration of losartan and enalapril exerts additive antiproteinuric effect in IgA nephropathy. Am J Kidney Dis. 2001;38:18–25.

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