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ORIGINAL ARTICLE

Biopsy timing and Oxford classification variables in Childhood/Adolescent IgA nephropathy

Yuko Shima · Koichi Nakanishi · Taketsugu Hama · Masashi Sato · Hironobu Mukaiyama · Hiroko Togawa · Ryojiro Tanaka · Hiroshi Kaito · Kandai Nozu · Kazumoto Iijima · Norishige Yoshikawa

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Abstract
Background Although the Oxford classification of IgA nephropathy appears valid, we found crescents were significantly related to renal outcome in our cohort, whereas segmental glomerulosclerosis (S) was not. The timing of renal biopsy may significantly affect the variables in the Oxford classification.
Method The relationship between biopsy timing and pathological variables (mesangial hypercellularity score [M], endocapillary hypercellularity [E], S, tubular atrophy/interstitial fibrosis [T], crescents, and global glomerulosclerosis [G]) was analyzed retrospectively in 250 children with IgA nephropathy.
Results The median time from disease onset to renal biopsy was 5.1 months (interquartile range, 2.7–15.4). M ($\rho=-0.26$, $P<0.0001$), E ($\rho=-0.34$, $P<0.0001$), and crescents ($\rho=-0.14$, $P=0.023$) showed significant negative correlations, and S ($\rho=0.15$, $P=0.018$) and G ($\rho=0.25$, $P<0.0001$) showed significant positive correlations with time to biopsy (Spearman test). M, E, and crescents differed significantly in renal biopsies obtained before and after 3 years from onset (Wilcoxon test). Most crescents (92.9 %) were cellular/fibrocellular and were acute lesions. As crescents formed early after disease onset and decreased over time, they may be prognostic for acute phase, but not for chronic phase disease.
Conclusions Renal biopsy timing may alter the significance of variables used in the Oxford classification.

Keywords Glomerulosclerosis · Crescents · Correlation · Onset · Diagnosis

Introduction

IgA nephropathy (IgAN) is the most common glomerulonephritis worldwide and the main cause of end-stage renal disease in patients of all ages with primary glomerular disease [1, 2]. Pathological findings have been shown to depend on the timing of the biopsy. For example, mesangial and endocapillary proliferation were high in renal biopsies obtained from patients with early-stage disease, whereas segmental and global sclerosis were found in late-stage biopsy samples [3].

In 2009, the Oxford classification of IgAN was formulated. This classification system was expected to be highly reproducible and highly predictive of renal outcome. Indeed, four variables—mesangial hypercellularity score (M), endocapillary hypercellularity (E), segmental glomerulosclerosis or adhesion (S), and tubular atorophy/interstitial fibrosis (T)—were subsequently shown to be independent predictors of renal outcome in adults [4, 5] and children [6].

We previously confirmed the validity of the Oxford classification of IgAN in children [7]. In our study, M, E, T and crescents were significantly associated with renal outcome. We hypothesized that pathological changes over time might affect variables of the Oxford classification, and that the latter might depend on the timing of the biopsy. Thus, differences in the significance of pathological variables between the original

Y. Shima · K. Nakanishi (✉) · T. Hama · M. Sato · H. Mukaiyama · H. Togawa · N. Yoshikawa
Department of Pediatrics, Wakayama Medical University, Wakayama, Japan
e-mail: knakanis@wakayama-med.ac.jp

R. Tanaka
Kobe Children's Hospital, Department of Nephrology, Kobe, Hyogo, Japan

H. Kaito · K. Nozu · K. Iijima
Department of Pediatrics, Kobe University Graduate School of Medicine, Kobe, Hyogo, Japan

Oxford classification and our data may have been due, at least in part, to differences in the timing of the renal biopsy.

To clarify the changes over time in each of the variables included in the Oxford classification, we analyzed the correlation between each variable and the time from IgAN onset to the renal biopsy.

Methods

Patients

The study was performed in accordance with the principles of the Declaration of Helsinki and was approved by regional research ethics boards. This study involved the retrospective investigation of clinical records from 491 consecutive children and adolescents, aged <20 years, newly diagnosed with IgAN at Kobe University and Wakayama Medical University hospitals between June 1972 and December 2004 and who underwent routine renal biopsies before the start of treatment. Of these 491 patients, 153 with proteinuria <0.5 g/day/1.73 m^2 at biopsy and 88 without sufficient data were excluded. Thus, our study cohort included 250 children with proteinuria ≥0.5 g/day/1.73 m^2 at biopsy, matching the inclusion criteria for IgAN of the original Oxford cohort. The pathological variables in these patients were fully evaluated according to the Oxford classification.

The diagnosis of IgAN was based on the presence of IgA as the sole or predominant immunoglobulin in the glomerular mesangium without systemic disease [8]. All diagnoses of IgAN were confirmed by a single investigator (N.Y.) using the same criteria throughout the entire study period. Renal biopsies were obtained from children with persistent proteinuria, with or without hematuria, before the start of treatment. Hematuria was defined as five or more red blood cells per high-power field in a properly collected and centrifuged urine specimen. Proteinuria was defined as a positive dipstick reading of ≥1+, urinary protein ≥0.2 g/m^2/day, or a urinary protein to creatinine ratio (uP/Cr) ≥0.2 g/g. No patients received any angiotensin converting enzyme inhibitors/angiotensin receptor blockers prior to biopsy.

Clinical data

Clinical parameters collected at biopsy included sex, age, time from onset to renal biopsy, weight, height, systolic and diastolic blood pressure, serum creatinine concentration and proteinuria. Proteinuria was expressed in grams per day per 1.73 m^2. When 24-h urine protein was not available, uP/Cr was considered an estimate of 24-h protein excretion, adjusted for body surface area. Proteinuria >3.0 g/day per 1.73 m^2 was considered to be in the nephrotic range, as was uP/Cr >3.0 g/g [5, 6]. Blood pressure criteria were according to the Guidelines for Drug Therapy in Pediatric Patients with Cardiovascular Diseases (JCS2012, http://www.j-circ.or.jp/guideline/pdf/JCS2012_sachi_h.pdf, Japanese). Mean arterial pressure was defined as two-thirds of the diastolic pressure plus a third of the systolic pressure. The estimated glomerular filtration rate (eGFR) was determined using the Schwartz formula and the constants 0.55 and 0.7 for adolescent boys [9]. To avoid sudden artificial changes in eGFR and to simplify the estimation of renal function, all patients were evaluated solely by the Schwartz equation. We determined the end-point of renal outcome as≥stage III chronic kidney disease (CKD, eGFR <60 ml/min/1.73 m^2), because stage III CKD is recognized as an "irreversible status".

Pathological data

Based on the Oxford classifications, seven pathological variables were evaluated: M, E, S, T, crescents, global glomerulosclerosis (G), and arterial intimal thickening (A).

Statistical methods

All statistical analyses were performed using JMP ver. 10 software (SAS Institute Japan, Tokyo, Japan). Continuous data were compared using the Mann–Whitney *U* test, and categorical data using the Fisher's exact test. Correlations between pathological variables and clinical presentations were evaluated by Spearman's rank correlation coefficient analysis. After adjusting for the effect of proteinuria at biopsy, a generalized linear model using a logarithm link function was used to formulate a regression model predicting pathological features. A correlation between the renal biopsy timing and the renal outcome was examined using Cox regression analysis. All *P* values were two-tailed and *P* values less than 0.05 were considered statistically significant.

Results

Clinical findings

The clinical findings of the 250 children in our cohort are shown in Table 1. Of these children, 201 (80.4 %) were found to have IgAN by a school screening program. The median time from disease onset to renal biopsy was 5.1 months (interquartile range, 2.7–15.4 months), with most patients diagnosed during the early stage of IgAN (Fig. 1).

Pathological findings

Histology slides from all patients were reviewed and scored for the seven pathological variables included in the Oxford

Table 1 Clinical findings at biopsy

Findings	*n*=250
Age (years)	11.5±3.3
Duration from onset to renal biopsy (months)	5.1 (2.7-15.4)
Onset mode with school screening	201 (80.4 %)
Female	115 (46.0)%
Mean arterial pressure (mmHg)	79±12
Estimated glomerular filtration rate (ml/min/1.73 m^2)	108±24
Proteinuria (g/day/1.73 m^2)	1.4 (0.9-2.8)
Nephrotic syndrome	15 (6.0 %)
Previous gross hematuria	130 (52.0 %)
Initial treatment	
No treatment	39 (15.6 %)
Antiplatelet and/or anticoagulant	29 (11.6 %)
Prednisolone, (± antiplatelet and/or anticoagulant)	28 (11.2 %)
Prednisolone+immunosuppressant (±Antiplatelet and/or anticoagulant)	71 (28.4 %)
Chinese herb (Sairei-to)	26 (10.4 %)
ACEI and/or ARB	17 (6.8 %)
Unknown	40 (16.0 %)

Data are shown as mean±standard deviation, median (interquartile range), or number (percentage)

ACEI angiotensin-converting enzyme inhibitor, *ARB* angiotensin II receptor blocker

classification. The median number of glomeruli per biopsy was 22 (interquartile range, 15–32). The median (interquartile range) of M and T, and of the ratios of glomeruli showing E, S, crescents, and G were 0.70 (0.22–1.00), 5.0 % (0.0–5.0 %), 10.0 % (0.0–25.0 %), 0.0 % (0.0–7.0 %), 9.9 % (0.0–21.0 %), and 0.0 % (0.0–0.0 %), respectively. The frequency distributions of selected pathological findings are shown in Fig. 2.

Of the 250 children in this cohort, 105 (41.8 %) had M <0.5, 71 (28.4 %) had no E, 68 (27.2 %) had no crescents, and none had A. Most (92.9 %) of the crescents were cellular or fibrocellular, indicative of acute lesions.

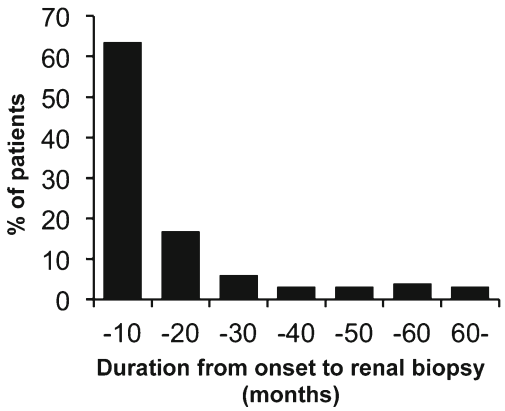

Fig 1 Frequency of duration from IgA nephropathy onset to renal biopsy in patients

Correlations between pathological features and time from onset to renal biopsy and proteinuria at biopsy

Correlations between each pathological feature of the Oxford classification and time from onset to renal biopsy are shown in Table 2 and Fig. 3. M (ρ=−0.26), E (ρ=−0.34), and crescents (ρ=−0.14) showed significant negative correlations with time from disease onset to biopsy, with all three tending to decrease over time, despite weak tendencies. In contrast, S (ρ=0.15) and G (ρ=0.25) showed significant positive correlations with time to biopsy, tending to increase over time, despite weak tendencies. T was not correlated with time to biopsy.

Correlations between each pathological feature of the Oxford classification and proteinuria at time of renal biopsy are shown in Table 2. Proteinuria at biopsy was associated with all variables, except for S and G.

Comparisons of each pathological feature in patients with renal biopsies obtained before and after 1 year and before and after 3 years from disease onset are shown in Fig. 4. Biopsies taken before and after 1 year showed significant differences in M (P=0.02), E (P=0.009), S (P=0.02), and G (P<0.0001), and biopsies taken before and after 3 years showed significant differences in M (P=0.02), E (P=0.04), and crescents (P=0.008).

Correlations between eGFR at biopsy and renal outcome, and time from onset to renal biopsy and proteinuria at biopsy

There was no significant correlation between eGFR at biopsy and time from onset to renal biopsy (ρ=−0.027, P=0.69). The time from onset to renal biopsy was not a significant predictor for renal outcome (hazard ratio [per month], 0.98; 95 % confidence interval, 0.94-1.01, P=0.29).

Generalized linear models for pathological features

Analyses of the associations between the data used to formulate generalized linear models and pathological features are shown in Table 3. The time from disease onset to renal biopsy was significantly related to all pathological features except T after adjusting for proteinuria at biopsy.

Discussion

This study showed weak but significant relationships between pathological features in patients with IgAN and the time from disease onset to biopsy. This study was unique, in that it assessed patients identified during a school urinary screening program by the Japanese government [10, 11]. Since 1974, all

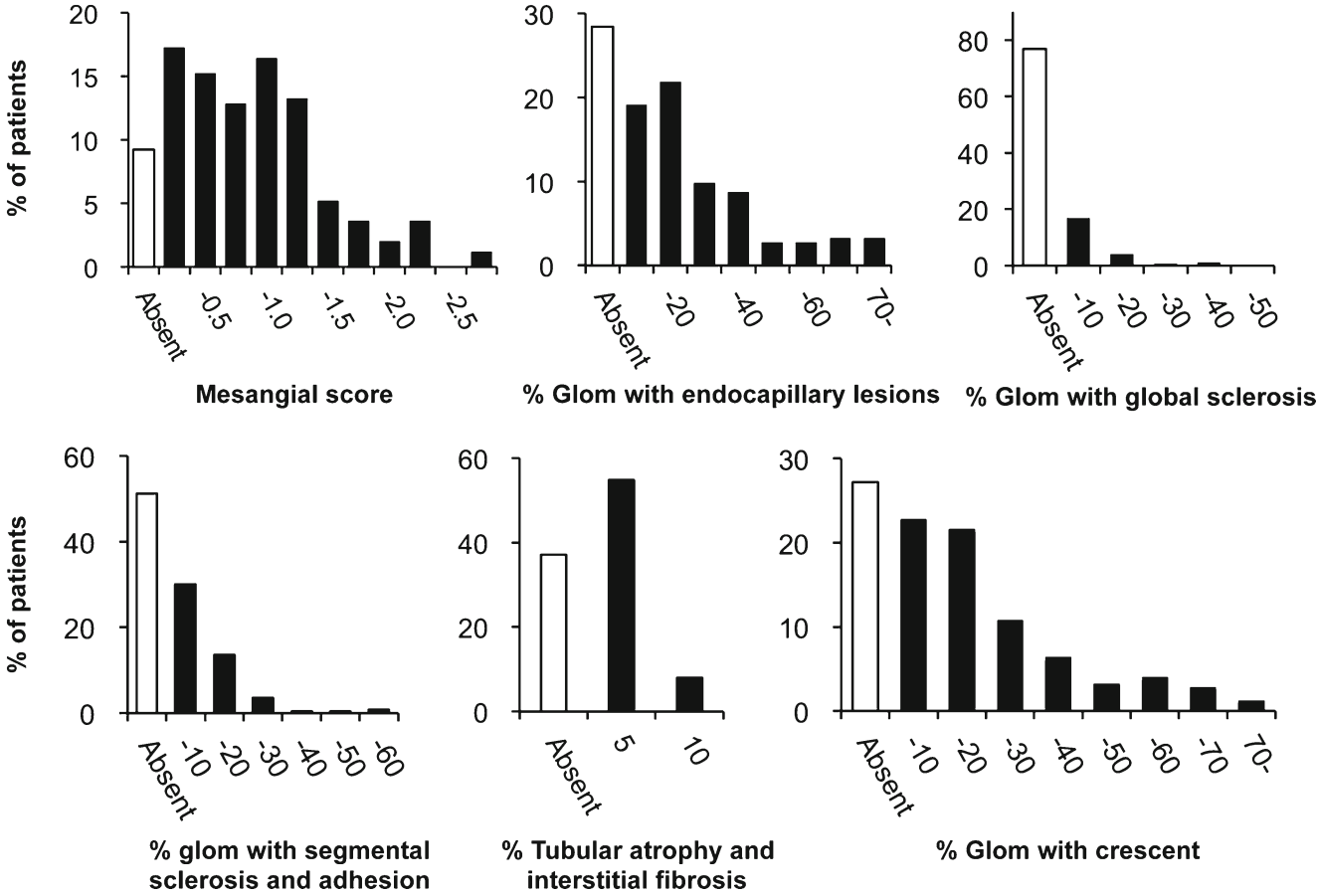

Fig 2 Frequency of pathological features

children in Japan between the ages of 6 and 18 years are screened annually, allowing the diagnosis of IgAN early during the course of disease. The annual urinary examination can therefore determine the onset times of IgAN within 1 year. It is important to notice that these correlations may be different in countries without routine school urine examinations.

The present study clarified that a shorter time from IgAN onset to renal biopsy was associated with higher glomerular ratios of M, E and crescents, and lower ratios of S and G. These findings indicate that acute lesions, such as M and E, reflect disease activity, with shorter times from onset to biopsy generally indicating more severe disease activity. Crescents also seem to reflect disease activity, because crescents were acute lesions in our cohort. In contrast, chronic lesions such as S and G tend to be absent early in the course of disease, even in patients with severe acute lesions. Changes over time in each patient were not assessed in this study. Rather, this study focused on the duration from onset to diagnosis, with disease course after diagnosis not considered. Although acute lesions early in the course of disease reflect disease prognosis to some

Table 2 Spearman's rank correlation coefficients between pathological variables and time from onset to renal biopsy and proteinuria at biopsy

	M ρ *P*	E *ρ* *P*	S ρ *P*	T *ρ* *P*	C ρ *P*	G *ρ* *P*
Duration from onset to renal biopsy (months)	−0.26	−0.34	0.15	0.11	−0.14	0.25
	<0.0001	<0.0001	0.018	0.075	0.023	<0.0001
Proteinuria at biopsy (g/day/1.73 m^2)	0.31	0.45	0.047	0.14	0.17	−0.0085
	<0.0001	<0.0001	0.46	0.029	0.007	0.90

M mesangial hypercellularity score, *E* endocapillary hypercellularity in percentage of glomeruli, *S* segmental glomerulosclerosis in percentage of glomeruli, *T* tubular atrophy/interstitial fibrosis in percentage, *C* crescents in percentage of glomeruli, *G* global glomerulosclerosis in percentage of glomeruli

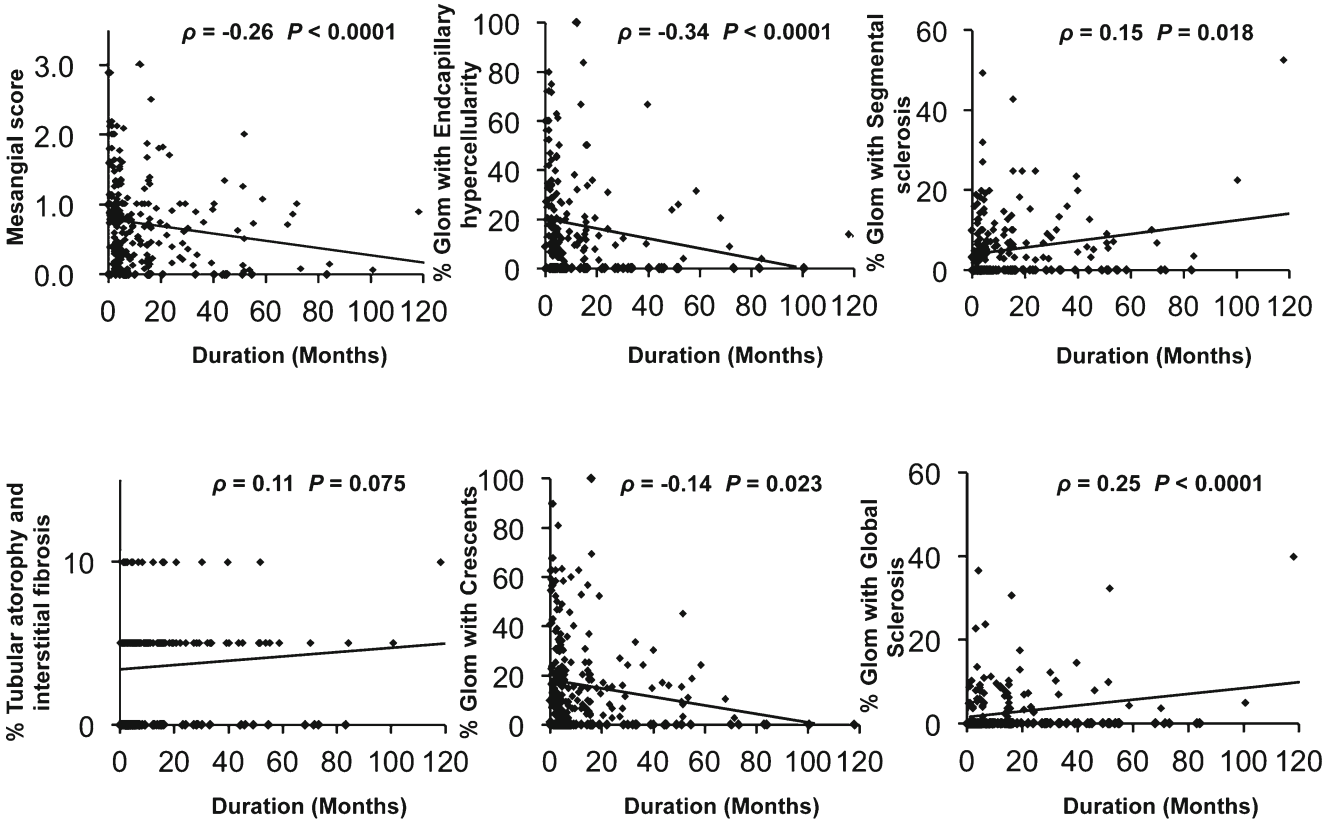

Fig 3 Correlation between each pathological feature and time from disease onset to biopsy. The *line* represents a regression line for tendency

extent, these lesions can be modified by adequate treatment, such that they no longer tend to be prognostic factors. In contrast, chronic lesions are difficult to modify with any treatments and may therefore be prognostic. Taken together, our results suggest that the prognostic value of each pathological lesion may be partially due to biopsy timing.

The criteria used to assess renal biopsies were of great importance. To standardize our results, all biopsy samples

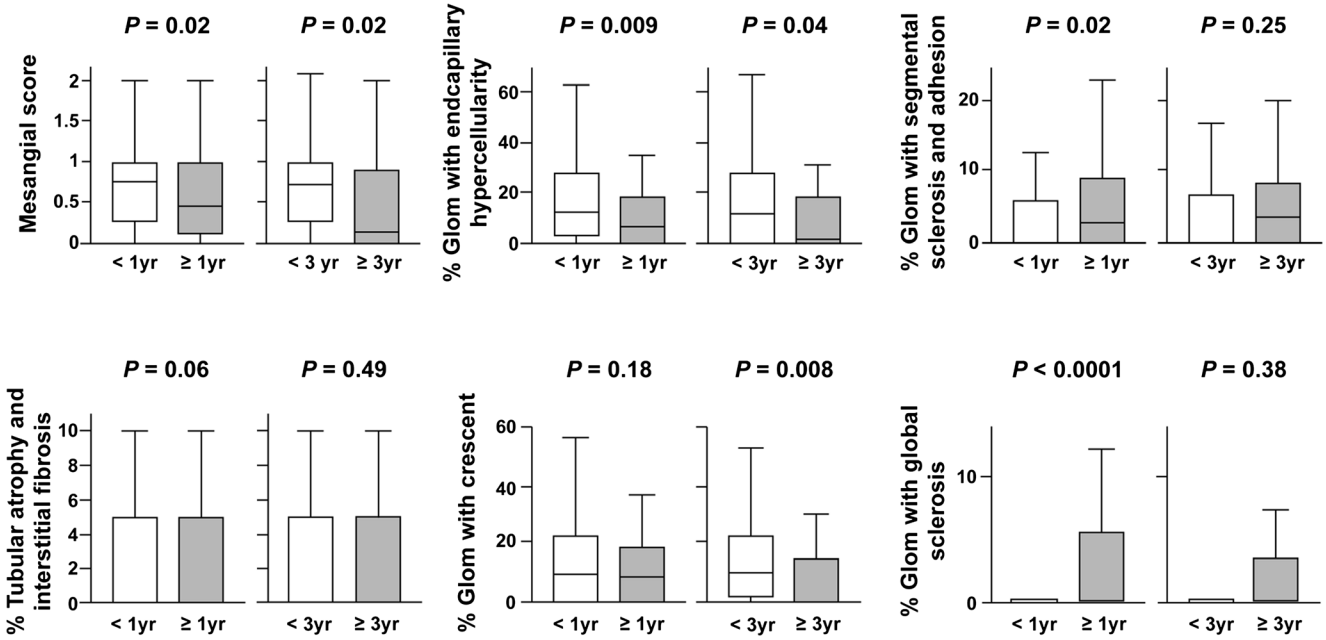

Fig 4 Pathological features in biopsy samples taken before and after 1 and 3 years from onset of IgA nephropathy. Shown is a Tukey box-and-whisker graph, where the *line within the box* represents the median and the *box* represents the interquartile range

Table 3 Generalized linear models for pathological features

		Estimate	SE	Likelihood ratio χ^2	*P*
M	Duration from onset to renal biopsy (months)	−0.0073	0.0033	4.339	0.037
	Proteinuria (g/day/1.73 m^2)	0.093	0.032	10.63	0.001
E	Duration from onset to renal biopsy (months)	−0.014	0.0035	12.81	0.0003
	Proteinuria (g/day/1.73 m^2)	0.20	0.044	28.71	<0.0001
S	Duration from onset to renal biopsy (months)	0.011	0.0032	15.25	<0.0001
	Proteinuria (g/day/1.73 m^2)	−0.055	0.043	1.574	0.21
T	Duration from onset to renal biopsy (months)	0.0034	0.0035	0.9845	0.32
	Proteinuria (g/day/1.73 m^2)	0.037	0.035	1.171	0.28
C	Duration from onset to renal biopsy (months)	−0.017	0.0040	14.81	0.0001
	Proteinuria (g/day/1.73 m^2)	0.10	0.035	10.83	0.001
G	Duration from onset to renal biopsy (months)	0.019	0.0039	37.20	<0.0001
	Proteinuria (g/day/1.73 m^2)	0.047	0.030	2.616	0.11

M mesangial hypercellularity score, *E* endocapillary hypercellularity in percentage of glomeruli, *S* segmental glomerulosclerosis in percentage of glomeruli, *T* tubular atrophy/interstitial fibrosis in percentage, *C* crescents in percentage of glomeruli, *G* global glomerulosclerosis in percentage of glomeruli; *SE* standard error

were evaluated using the same criteria throughout the entire study period. Despite the use of the same biopsy criteria, disease severity at biopsy varied because of various factors. One marker of disease severity is proteinuria at diagnosis [12, 13]. However, it may not be a significant long-term predictor, because it can be modified by treatment. We therefore analyzed the relationships between pathological features and time from disease onset to biopsy after adjusting for proteinuria at diagnosis. Even after this adjustment, however, the time from IgAN onset to diagnosis was significantly associated with pathological features.

To match the inclusion criteria for the initial Oxford cohort, this study included only those children with proteinuria ≥0.5 g/day/1.73 m^2 at biopsy. However, when we included our entire patient cohort, consisting of more than 400 children with IgAN, we observed similar relationships between pathological features and the duration from onset to biopsy (data not shown). This finding further suggests that these relationships are independent of proteinuria at the time of diagnosis.

Our previous analyses were not consistent with the original Oxford classification analyses, because crescents and S differed in their ability to predict disease outcomes [7]. Extracapillary proliferation has also shown prognostic significance in adults within the IgAN cohort [14]. Extracapillary proliferation (crescents) did not emerge from the Oxford classification as a predictor probably because patients with severe IgAN (eGFR <30 ml/min per 1.73 m^2) were excluded. Because few of these patients were present in our cohort, it cannot explain the differences in prognostic significance of crescents.

We found that biopsy samples obtained before and after 1 year following disease onset differed significantly in M, E, S and G, and those taken before and after 3 years differed significantly in M, E and crescents. The difference observed between the 1-and 3-year cutoffs may reflect the characteristics of each pathological feature. Notably, our finding that crescents differed significantly only before and after 3 years, may suggest that the predictive value of crescents decreases over time, whereas the differences in S and G at the 1-year cutoff suggest that S and G become more stable after 1 year.

This study had several limitations. For example, our data have not yet been validated in other patient cohorts. Moreover, it has not been determined whether these findings are unique to children or are common to adults. Further investigations are required to clarify these limitations.

In conclusion, our data suggest that each variable of the Oxford classification of IgAN was influenced by the time from disease onset to renal biopsy, and that differences in the significance of S and crescents between the original Oxford classification and our data may be due, at least in part, to differences in the timing of renal biopsy.

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Disclosures None

References

1. Donadio JV, Grande JP (2002) IgA nephropathy. N Engl J Med 347: 738–748
2. Alexopoulos E (2004) Treatment of primary IgA nephropathy. Kidney Int 65:341–355

3. Yoshikawa N, Iijima K, Maehara K, Yoshiara S, Yoshiya K, Matsuo T, Okada S (1987) Mesangial changes in IgA nephropathy in children. Kidney Int 32:585–589
4. Working Group of the International IgA Nephropathy Network and the Renal Pathology Society, Cattran DC, Coppo R, Cook HT, Feehally J, Roberts IS, Troyanov S, Alpers CE, Amore A, Barratt J, Berthoux F, Bonsib S, Bruijn JA, D'Agati V, D'Amico G, Emancipator S, Emma F, Ferrario F, Fervenza FC, Florquin S, Fogo A, Geddes CC, Groene HJ, Haas M, Herzenberg AM, Hill PA, Hogg RJ, Hsu SI, Jennette JC, Joh K, Julian BA, Kawamura T, Lai FM, Leung CB, Li LS, Li PK, Liu ZH, Mackinnon B, Mezzano S, Schena FP, Tomino Y, Walker PD, Wang H, Weening JJ, Yoshikawa N, Zhang H (2009) The Oxford classification of IgA nephropathy: rationale, clinicopathological correlations, and classification. Kidney Int 76:534–545
5. Working Group of the International IgA Nephropathy Network and the Renal Pathology Society, Roberts IS, Cook HT, Troyanov S, Alpers CE, Amore A, Barratt J, Berthoux F, Bonsib S, Bruijn JA, Cattran DC, Coppo R, D'Agati V, D'Amico G, Emancipator S, Emma F, Feehally J, Ferrario F, Fervenza FC, Florquin S, Fogo A, Geddes CC, Groene HJ, Haas M, Herzenberg AM, Hill PA, Hogg RJ, Hsu SI, Jennette JC, Joh K, Julian BA, Kawamura T, Lai FM, Li LS, Li PK, Liu ZH, Mackinnon B, Mezzano S, Schena FP, Tomino Y, Walker PD, Wang H, Weening JJ, Yoshikawa N, Zhang H (2009) The Oxford classification of IgA nephropathy: pathology definitions, correlations, and reproducibility. Kidney Int 76:546–556
6. Coppo R, Troyanov S, Camilla R, Hogg RJ, Cattran DC, Cook HT, Feehally J, Roberts IS, Amore A, Alpers CE, Barratt J, Berthoux F, Bonsib S, Bruijn JA, D'Agati V, D'Amico G, Emancipator SN, Emma F, Ferrario F, Fervenza FC, Florquin S, Fogo AB, Geddes CC, Groene HJ, Haas M, Herzenberg AM, Hill PA, Hsu SI, Jennette JC, Joh K, Julian BA, Kawamura T, Lai FM, Li LS, Li PK, Liu ZH, Mezzano S, Schena FP, Tomino Y, Walker PD, Wang H, Weening JJ, Yoshikawa N, Zhang H (2010) The Oxford IgA nephropathy clinicopathological classification is valid for children as well as adults. Kidney Int 77:921–927
7. Shima Y, Nakanishi K, Hama T, Mukaiyama H, Togawa H, Hashimura Y, Kaito H, Sako M, Iijima K, Yoshikawa N (2012) Validity of the Oxford classification of IgA nephropathy in children. Pediatr Nephrol 27:783–792
8. Yoshikawa N, Ito H, Yoshiara S, Nakahara C, Yoshiya K, Hasegawa O, Matsuo T (1987) Clinical course of IgA nephropathy in children. J Pediatr 110:555–560
9. Schwartz GJ, Haycock GB, Edelmann CM Jr, Spitzer A (1976) A simple estimate of glomerular filtration rate in children derived from body length and plasma creatinine. Pediatrics 58:259–263
10. Murakami M, Yamamoto H, Ueda Y, Murakami K, Yamauchi K (1991) Urinary screening of elementary and junior high-school children over a 13-year period in Tokyo. Pediatr Nephrol 5:50–53
11. Murakami M, Hayakawa M, Yanagihara T, Hukunaga Y (2005) Proteinuria screening for children. Kidney Int Suppl 94:S23–S27
12. Yoshikawa N, Ito H, Nakamura H (1992) Prognostic indicators in childhood IgA nephropathy. Nephron 60:60–67
13. D'Amico G (2004) Natural history of idiopathic IgA nephropathy and factors predictive of disease outcome. Semin Nephrol 24:179–196
14. Katafuchi R, Ninomiya T, Nagata M, Mitsuiki K, Hirakata H (2011) Validation study of oxford classification of IgA nephropathy: the significance of extracapillary proliferation. Clin J Am Soc Nephrol 6:2806–2813