Renal outcomes in mixed proliferative and membranous lupus nephritis (Class III/IV + V): A long-term observational study

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Abstract

Objectives: In this study, we aimed to assess the effect of combination of proliferative and membranous lesions (Class III + V or IV + V) on renal outcomes as an independent category distinct from Class III and IV.

Methods: We retrospectively analyzed 103 Japanese patients (14 male and 89 female) with Class III/IV LN, with or without Class V, who underwent renal biopsy and were treated at our institution. Renal endpoint was defined as doubling of serum creatinine or end-stage renal disease (ESRD).

Results: The number of patients in each group was as follows: pure Class III/IV, 81 patients and mixed Class III/IV + V, 22 patients. During a median follow-up period of 125.0 months, 10 patients developed renal endpoint: five had Class III/IV LN and five had a combination of Class III/IV + V. Kaplan–Meier analyses demonstrated that patients with mixed Class III/IV + V LN had significantly poorer renal outcomes than patients with Class III/IV LN. Multivariate Cox regression analyses identified serum creatinine, active and chronic lesions (A/C), and mixed Class III/IV + V as independent risk factors for poor renal outcomes.

Conclusions: This study demonstrated a combination of proliferative and membranous LN (ISN/RPS Class III/IV + V) predicts poor renal outcomes.

Introduction

Lupus nephritis (LN) continues to be a major contributor to morbidity and mortality in systemic lupus erythematosus (SLE) [1]. The pathological findings of LN are highly diverse and World Health Organization (WHO) classification was introduced to categorize the various morphological changes, especially focused on the glomerular lesions [2]. The WHO classification was shown not only to be distinctive on the basis of morphology, but also to have significant implications with regard to renal prognosis [3–7]. However, subsequent clinicopathologic studies have revealed the need for improved clarification using different categories and terminology [8].

The International Society of Nephrology (ISN)/Renal Pathology Society (RPS) classification of lupus nephritis (LN) was proposed more than a decade ago, aimed at improving categorization and precise terminology [8]. One of the major changes in the ISN/RPS classification was the subdivision of diffuse LN (Class IV) into segmental proliferative LN (Class IV-S) and diffuse global LN (Class IV-G). Several studies, including our previous study, have evaluated the effect of subdivision classifications on renal prognosis [9–15,16]. A recent meta-analysis of these studies concluded that the rates of serum creatinine doubling and end-stage renal disease (ESRD) did not significantly differ between patients with Class IV-S and patients with IV-G LN [17].

Another one of the major changes in the ISN/RPS classification was the removal of the diffuse membranous LN (Class V) subcategory [8]. In the 1982 WHO classification, Class V LN was subdivided into Class Va (pure membranous LN), Class Vb (associated with Class II), Class Vc (associated with Class III), and Class Vd (associated with Class IV) [2]. In the ISN/RPS classification, Class V was defined as membranous LN with or without mesangial alterations. When a diffusely distributed membranous lesion is associated with an active lesion of Class III or Class IV LN, both diagnoses are to be reported in the diagnostic line (i.e. Class III + V or Class IV + V) [8]. However, the effect of Class V lesions with concurrent Class III or IV lesions on renal survival remains unclear. In many reports using the ISN/RPS 2003 classification, including our previous study, proliferative LN (Class III or Class IV) combined with membranous lesions was classified as Class III or Class IV, respectively [10,12,14–16]. Further, recent LN guidelines and recommendations for LN have classified cases of Class III or Class IV LN, with or without Class V, as Class III/IV ± V and have recommended the same treatment strategy of combination of high-dose glucocorticoids and cyclophosphamide or mycophenolate mofetil for induction/initial therapy for the treatment of active lesions [18–20].

In the present study, we aimed to assess the effect of concurrent membranous lesions (Class V) with proliferative LN (Class III/IV) on renal outcomes by separating cases of Class III/IV + V LN from Class III/IV. The outcomes of 103 Japanese patients with...
Class III/IV ± V LN were analyzed retrospectively. Seventy-one of 103 patients had been assessed in a previous study [14], and 32 patients were newly recruited.

Patients and methods
Study design and patients

In this study, the data of Japanese patients who underwent renal biopsy and were treated for LN between 1976 and 2012 at the Department of Medicine of Gunma University Hospital were analyzed retrospectively. All patients fulfilled four or more of the American College of Rheumatology criteria for the classification of SLE [21]. Of the 143 patients with biopsy-proven LN (Class II to Class V), patients with Class III or IV LN, with or without Class V LN, according to the ISN/RPS 2003 criteria were included in this study. Patients with an observation period of less than one year were excluded, except patients who died or developed renal endpoint within one year of enrollment. In addition to 71 previously studied patients with Class III/IV ± V LN [14], 32 patients were newly included in this study, resulting in a total of 103 patients being included for analysis. Data from the first renal biopsy were used in patients that had received multiple renal biopsies at our department. This study was conducted in accordance with the principles of the Declaration of Helsinki and with the approval of the Epidemiologic Research Ethics Committee of Gunma University Faculty of Medicine.

Clinical and histological data

Collection of clinical data was performed in August 2014. The following clinical parameters were evaluated at the time of biopsy: sex, age, duration between SLE onset and biopsy, duration between renal onset and biopsy, systolic blood pressure, diastolic blood pressure, urinary protein, urinary occult blood, hemoglobin, serum creatinine, serum albumin, CH50, anti-DNA antibody. The ratio of urinary protein to urinary creatinine (g/gCr) of a spot urine sample was used to assess urinary protein excretion. Urinary protein excretion (g/day) was used in some old records where sample was used to assess urinary protein excretion. Urinary ratio of urinary protein to urinary creatinine (g/gCr) of a spot urine serum creatinine, serum albumin, CH50, anti-DNA antibody. The blood pressure, urinary protein, urinary occult blood, hemoglobin, between renal onset and biopsy, systolic blood pressure, diastolic blood pressure, sex, age, duration between SLE onset and biopsy, duration following clinical parameters were evaluated at the time of biopsy:

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Table 1. Baseline characteristics at renal biopsy.

| Baseline characteristics | Total patients (n = 103) | Pure Class III/IV (n = 81) | Mixed Class III/IV + V (n = 22) | p Value* |
|--------------------------|-------------------------|--------------------------|-------------------------------|---------|
| Female (number)          | 89 (86.4%)              | 70 (86.4%)               | 19 (86.4%)                    | 1.00    |
| Age (years)              | 33.0 (26.0–45.0)        | 35.0 (27.5–45.0)         | 29.5 (24.0–41.8)              | 0.29    |
| Duration between SLE onset and biopsy (months) | 8.0 (1.0–88.0) | 6.0 (1.0–72.5) | 68.5 (18.5–139.0) | 0.13 |
| Duration between renal onset and biopsy (months) | 2.0 (1.0–6.0) | 2.0 (1.0–4.5) | 4.0 (1.0–13.5) | 0.27 |
| Systolic blood pressure (mmHg) | 132.0 (120.0–147.5) | 131.0 (118.5–149.0) | 133.0 (123.5–145.0) | 0.80 |
| Diastolic blood pressure (mmHg) | 83.8 ± 16.2 | 84.2 ± 17.1 | 82.3 ± 13.1 | 0.64 |
| Urinary protein (g/gCr) | 3.2 (1.4–5.5) | 2.6 (1.2–4.8) | 5.0 (2.2–6.6) | 0.01 |
| Urinary occult blood (0–3) | 2.0 (1.0–3.0) | 2.0 (1.0–3.0) | 1.0 (0.5–2.3) | 0.07 |
| Hemoglobin (g/dl) | 10.9 ± 1.9 | 10.8 ± 2.0 | 11.4 ± 1.5 | 0.20 |
| Serum creatinine (mg/dl) | 0.80 (0.60–0.95) | 0.80 (0.60–1.00) | 0.66 (0.59–0.83) | 0.04 |
| Serum albumin (g/dl) | 2.9 ± 0.7 | 3.0 ± 0.7 | 2.4 ± 0.7 | 0.001 |
| CH50 (U/ml) | 12.0 (5.2–21.0) | 11.4 (10.0–18.1) | 20.4 (11.4–27.4) | 0.006 |
| Active/chronic (A/C) lesions (number) | 37 (35.9%) | 27 (33.3%) | 10 (45.5%) | 0.32 |
| Positive for anti–DNA antibody (number) | 85/102** (83.4%) | 70/80** (87.5%) | 15 (68.2%) | 0.05 |

Data are expressed as median (interquartile range), mean ± standard deviation, or number (percentage).
*Comparison between pure Class III/IV and mixed Class III/IV + V.
**One patient was not available for a test of anti-DNA antibody.

Statistical analyses

Data of continuous variables are expressed as mean ± standard deviation when normally distributed and medians (interquartile range, IQR) when not normally distributed. Categorical data are presented as numbers and percentages. Statistical analyses were performed using Student’s t-test or Mann–Whitney U-test for comparisons between groups. Categorical variables were analyzed by Fisher’s exact test. Renal survival was determined using the Kaplan–Meier method, with comparisons between groups performed using the log-rank test. To identify the risk factors for renal endpoint, univariate Cox regression analysis was initially performed. A forward stepwise multivariate Cox regression analysis was performed using significant variables identified by univariate analysis (p < 0.05). All statistical analyses were conducted using IBM SPSS statistics 22 (IBM SPSS, Tokyo, Japan). For all tests, a p value < 0.05 was considered statistically significant.

Results

Patients

Baseline patient characteristics at the time of renal biopsy are shown in Table 1. Of the 103 patients with Class III/IV ± V LN, 89 (86.4%) were female and the median patient age at time of renal biopsy was 33.0 years. The median follow-up period was 125.0 months. The median or mean value at the time of biopsy was as follows: systolic blood pressure, 132.0 mmHg; diastolic pressure, 83.8 mmHg; urinary protein, 3.2 g/gCr; urinary occult blood score, 2.0; hemoglobin, 10.9 g/dl; serum creatinine, 0.80 mg/dl; serum albumin, 2.9 g/dl; and CH50, 12.0 U/ml. Anti-DNA antibodies were present in 85 (83.3%) of the 102 patients with data available. Of the 103 patients with Class III/IV LN, the numbers of patients that did not reach the study endpoint were censored at the date of their last follow-up visit or death if death occurred prior to reaching doubling serum creatinine or ESRD.

Renal biopsy samples were histologically classified according to the ISN/RPS 2003 criteria. Class III ± V or Class IV ± V was defined as diffusely distributed membranous lesions (involving >50% of the tuft of >50% of the glomeruli by light microscopy or immunofluorescence) associated with active Class III or IV lesions [8]. Class III and Class IV LN were further subdivided into active lesions (A), active and chronic lesions (A/C), and chronic lesions (C). Class IV LN was subdivided into diffuse segmental LN (IV-S) and diffuse global LN (IV-G) [8].

Statistical analyses

Data of continuous variables are expressed as mean ± standard deviation when normally distributed and medians (interquartile range, IQR) when not normally distributed. Categorical data are presented as numbers and percentages. Statistical analyses were performed using Student’s t-test or Mann–Whitney U-test for comparisons between groups. Categorical variables were analyzed by Fisher’s exact test. Renal survival was determined using the Kaplan–Meier method, with comparisons between groups performed using the log-rank test. To identify the risk factors for renal endpoint, univariate Cox regression analysis was initially performed. A forward stepwise multivariate Cox regression analysis was performed using significant variables identified by univariate analysis (p < 0.05). All statistical analyses were conducted using IBM SPSS statistics 22 (IBM SPSS, Tokyo, Japan). For all tests, a p value < 0.05 was considered statistically significant.
with Class III/IV and mixed Class III/IV + V LN were 81 (78.6%) and 22 (21.4%), respectively. Significant differences in urinary protein levels ($p = 0.014$), serum creatinine ($p = 0.041$), serum albumin ($p = 0.001$), and CH50 ($p = 0.006$) were observed between patients with Class III/IV LN and patients with mixed Class III/IV + V LN. A significant difference in the presence of anti-DNA antibodies was observed between the two groups ($p = 0.049$).

Renal histology

As shown in Table 2, of the 81 (78.6%) patients with Class III/IV LN, 19 (18.4%) patients had Class III LN and 62 (60.2%) had Class IV LN. Of the 22 (21.4%) patients with mixed Class III/IV + V LN, eight (7.8%) patients had Class III + V LN, and 14 (13.6%) patients had Class IV + V LN. The detailed number and relative frequencies of each Subclass are also shown in Table 2.

Treatments

Treatments administered during the follow-up period are summarized in Table 3. All patients received glucocorticoid therapy. All patients with mixed Class III/IV + V LN were treated with glucocorticoids and immunosuppressants, whereas 23.5% patients with pure Class III/IV LN were treated with glucocorticoids alone ($p = 0.01$). No significant difference in the frequency of each immunosuppressant usage was observed between the groups, except for tacrolimus ($p = 0.001$) and tacrolimus plus mycophenolate mofetil ($p = 0.04$), which were more frequently used in patients with mixed Class III/IV + V LN.

Renal outcomes in each group

At the end of the follow-up period, 10 (9.7%) of 103 patients had reached renal endpoint: five patients with Class III/IV LN (6.2%) and five patients with mixed Class III/IV + V LN (22.7%; Table 4). Eight deaths occurred before doubling of serum creatinine levels and were censored from the study. We first determined renal outcomes in each class using Kaplan–Meier analysis. Figure 1(a) shows the overall renal outcomes during the follow-up period. The probabilities of sustained renal function at 5, 10, 15, and 20 years were 95.8%, 94.3%, 92.3%, and 76.9%, respectively. Kaplan–Meier curves demonstrated that patients with mixed Class III/IV + V LN had significantly poorer renal outcomes than patients with Class III/IV LN ($p = 0.002$, Figure 1b). We previously demonstrated that the A/C subcategory was associated with worse renal outcomes than the A subcategory in patients with Class IV-G LN [14]. In this study, this finding was corroborated by the observation of a significant difference in renal outcomes between the A and A/C subcategories in patients with Class III/IV ± V LN ($p = 0.001$, Figure 1c).

Predictors of renal outcome

Finally, we evaluated predictors of renal endpoint using univariate and multivariate Cox regression analyses (Table 5). Univariate Cox regression analysis indicated that male gender, serum creatinine, mixed Class III/IV + V LN, and (A/C) lesions were associated with poor renal outcomes ($p<0.05$). Multivariate Cox regression analysis using significant variables identified by univariate analysis demonstrated elevated serum creatinine [hazard ratio (HR), 4.66; 95% confidence interval (CI), 1.79–12.01; $p = 0.002$], (A/C) lesions (HR, 9.07; 95% CI, 1.80–45.69, $p = 0.008$), and mixed Class III/IV + V LN (HR, 2.13; 95% CI, 1.14–3.96, $p = 0.017$).

Table 2. Renal histology by the ISN/RPS 2003 classification.

| Group               | No. (%) | Class | No. (%) | Subclass | No. (%) |
|---------------------|---------|-------|---------|----------|---------|
| Pure Class III/IV   | 81 (78.6%) | III   | 19 (18.4%) | III (A) | 13 (12.6%) |
|                     |         |       |         | III (A/C) | 6 (5.8%) |
|                     |         |       |         | IV-S (A)  | 10 (9.7%) |
|                     |         |       |         | IV-S (A/C) | 5 (4.9%) |
|                     |         |       |         | IV-G (A)  | 31 (30.1%) |
|                     |         |       |         | IV-G (A/C) | 16 (15.5%) |
| Mixed Class III/IV + V | 22 (21.4%) | III + V | 8 (7.8%) | III(A)+V | 3 (2.9%) |
|                     |         |       |         | III(A/C)+V | 5 (4.9%) |
|                     |         |       |         | IV-S(A)+V  | 4 (3.9%) |
|                     |         |       |         | IV-G(A)+V  | 5 (4.9%) |
|                     |         |       |         | IV-G(A/C)+V | 5 (4.9%) |

Data are expressed as number of patients (%).

Table 3. Treatment during follow-up.

| Treatment | Pure Class III/IV | Mixed Class III/IV + V | $p$ Value |
|-----------|------------------|------------------------|-----------|
| Steroids alone | 19 (23.5%) | 0 (0%) | 0.01 |
| Any immunosuppressants | 62 (76.5%) | 22 (100%) | 0.01 |
| Oral cyclophosphamide | 31 (38.3%) | 8 (36.4%) | 1.00 |
| Intravenous cyclophosphamide | 15 (18.5%) | 7 (31.8%) | 0.24 |
| Azathioprine | 37 (45.7%) | 12 (54.5%) | 0.48 |
| Mizoribine | 22 (27.2%) | 8 (36.4%) | 0.43 |
| Cyclosporine A | 20 (24.7%) | 8 (36.4%) | 0.29 |
| Tacrolimus | 26 (32.1%) | 16 (72.7%) | 0.001 |
| Mycophenolate mofetil | 17 (21.0%) | 9 (40.9%) | 0.10 |
| Tacrolimus + Mycophenolate mofetil | 15 (18.5%) | 9 (40.9%) | 0.04 |

Data are expressed as number of patients (%).
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Table 5. Predictors of renal endpoint by univariate and multivariate Cox regression analyses

| Baseline characteristics | Hazard ratio (95% CI) | p Value |
|--------------------------|-----------------------|---------|
| **Univariate analysis**  |                       |         |
| Age                      | 1.00 (0.96 to 1.05)   | 0.93    |
| Male                     | 4.37 (1.23 to 15.53)  | 0.02    |
| Systolic blood pressure  | 1.01 (0.99 to 1.04)   | 0.41    |
| Diastolic blood pressure | 1.00 (0.96 to 1.05)   | 0.99    |
| Urinary protein          | 1.11 (0.99 to 1.21)   | 0.08    |
| Urinary occult blood     | 0.63 (0.35 to 1.15)   | 0.13    |
| Hemoglobin               | 0.94 (0.68 to 1.30)   | 0.70    |
| Serum albumin            | 0.64 (0.28 to 1.46)   | 0.29    |
| Serum creatinine         | 3.33 (1.52 to 7.31)   | 0.003   |
| CH50                     | 1.04 (1.00 to 1.09)   | 0.07    |
| Positive for anti to DNA antibody | 0.30 (0.08 to 1.08)  | 0.07    |
| Active and chronic lesions (A/C) | 6.86 (1.75 to 26.91) | 0.006   |
| Mixed Class III/IV + V   | 6.06 (1.70 to 21.54)  | 0.005   |
| **Multivariate analysis** |                       |         |
| Serum creatinine         | 4.66 (1.79 to 12.01)  | 0.002   |
| Active and chronic (A/C) lesions | 9.07 (1.80 to 45.69) | 0.008   |
| Mixed Class III/IV + V   | 22.13 (3.71 to 132.06)| 0.001   |

CI, 3.71–132.06; p = 0.001) as independent risk factors for progressive renal failure.

**Discussion**

We conducted a retrospective study to assess long-term renal outcomes in proliferative LN according to the ISN/RPS classification by separately analyzing patients with mixed Class III/IV + V LN and patients with Class III/IV LN alone. We identified mixed Class III/IV + V LN as an independent risk factor for poor renal outcomes by multivariable analysis in addition to increased serum creatinine levels, which were reported to be a predictor of poor renal survival by numerous previous LN studies [5,22–24], and (A/C) subclass, which was previously reported by our group [14].

Based on a modification of the previous WHO classification system [2], mixed proliferative and membranous LN were reported to be associated with poor outcomes [3,6]. Schwartz et al. examined the clinical outcomes of three histologic patterns of injury of severe LN: severe segmental LN, diffuse LN, and membranous LN associated with severe segmental LN or diffuse LN (MLN + PLN) [3]. Compared to patients with severe segmental LN or diffuse LN, patients with MLN + PLN had more adverse outcomes, including death or the occurrence of chronic renal failure. Subsequently, Najafi et al. demonstrated significantly poorer renal outcomes in patients with mixed proliferative and membranous LN (categorized as Class Vc with more than 50% glomeruli; Vc ≥ 50%) than in patients with Class IV LN [6].

However, limited data are available on Class III + V or IV + V of the ISN/RPS classification because Class III/IV + V has been so far analyzed in combination with pure Class III/IV + V in many studies [10,12,14–16]. In terms of the frequency of mixed Class III/IV + V, Guo et al. reported that 14 (20.9%) of 67 patients with Class III/IV LN had mixed Class III/IV + V LN. Similarly, Kono et al. found mixed Class III/IV + V LN in 18 (19.1%) out of 94 patients with Class III/IV LN. These values are comparable to the frequency observed in the present study (21.4%) [25,26]. We were unable to identify any previous studies comparing renal outcomes
between Class III/IV LN and mixed Class III/IV + V LN. However, a few studies have compared renal survival between Class V LN and mixed Class III/IV + V LN [23,27]. Okpechi et al. conducted a single center retrospective analysis of 42 patients with Class V LN or mixed Class III/IV + V LN. Patients with Class V LN were found to have significantly greater cumulative renal survival than patients with mixed Class III/IV + V LN [27]. In contrast, Moroni et al. compared the overall and renal survival rates between 67 patients with Class V LN and 36 patients with mixed Class III/IV + V LN and found no significant difference in the 10-year overall or renal survival rates [23]. Although patients with Class V LN were not included in the present study, renal endpoint was not observed in any of the 20 patients with pure Class V LN treated at our hospital over the same study period (Supplemental Figure 1).

The reason for poorer renal outcomes in patients with Class III/IV + V LN compared with Class III/IV LN remains unclear. Differences in the underlying immunological and inflammatory mechanisms between Class III/IV LN and mixed Class III/IV + V LN may partly explain this phenomenon. Indeed, several baseline characteristics were found to significantly differ between Class III/IV LN and mixed Class III/IV + V LN. Patients with mixed Class III/IV + V had significantly higher levels of proteinuria and CH50, lower levels of serum creatinine and serum albumin, and a lower frequency of the presence of anti-DNA antibodies. In addition, there were trends toward increased duration between SLE onset and renal biopsy and lower levels of urinary occult blood in patients with mixed Class III/IV + V compared with patients with Class III/IV LN alone, although these differences were not statistically significant. Hsieh et al. reported that only one patient had Class IV + V LN (0.8%), whereas 57 patients (43.5%) had Class IV LN in a study of renal histology in 131 LN patients with renal biopsies performed within three months of the first sign of renal disease [28]. Therefore, we propose that compared with Class III/IV LN, which often occurs rapidly due to acute inflammatory and immunological SLE activity and tends to be “nephritic,” mixed Class III/IV + V develops at a slower rate due to chronic inflammation and tends to be “nephrotic,” which may be associated with disease refractory to treatment in a proportion of patients. However, patients with Class III/IV + V LN likely consist of patients with mixed lesions that developed after transformation from either pure Class V or III/IV LN in addition to patients in whom proliferative and membranous lesions developed simultaneously. As the compositions of these patterns may influence renal outcomes, further studies including patients in whom repeated renal biopsies have been performed are required.

However, we also have to consider that patients with Class III/IV + V LN likely consist of not only patients in whom proliferative and membranous lesions simultaneously developed, but also those patients with mixed lesions which developed after transformation from either pure Class V or III/IV LN. Because in addition to patients in whom proliferative and membranous lesions developed simultaneously. As the compositions of these patterns LN classes may influence the renal outcomes, further study would be studies including patients in whom repeated renal biopsies have been performed are required to answer this question, by using the data of patients who received repeat renal biopsy.

This study had a number of limitations. First, the number of patients who developed renal endpoint was relatively low. Therefore, our study has limited power in determining independent factors for poor renal outcome and may have missed other independent risk factors. Second, treatment strategies differed during the long-term study period. In addition, calcineurin inhibitors were more frequently used in patients with mixed Class III/IV + V, which may indicate poor prognosis in this class is due to calcineurin inhibitor-induced nephrotoxicity. Indeed, three of the five patients with mixed Class III/IV + V who reached renal endpoint had received calcineurin inhibitors. However, with the exception of one patient who was treated with calcineurin inhibitors for a total of nine years due to repeated flares of LN, the duration of calcineurin inhibitor use was less than seven months in the remaining patients. Clinical courses of these two patients showed that chronic renal failure was not caused by calcineurin inhibitors. Finally, this was a single-center study and all patients were Japanese. In LN, race and ethnicity are considered important factors in therapy response rates and long-term renal outcomes [29].

In summary, this single-center retrospective study of 103 Japanese patients with proliferative LN (Class III/IV) over a median follow-up period of 125.0 months after renal biopsy demonstrate mixed proliferative and membranous LN (Class III/IV + V) as an independent risk factor for poor renal outcomes. Further studies are required to validate these findings at other institutions, particularly in other racial and ethnic groups. In addition, in order to confirm that these findings hold true in Japanese ethnicity, a multicenter study with a large number of patients is desired to be performed in the future in Japan.

Conflict of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.
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