

## PAPER

# Delayed lupus nephritis in the course of systemic lupus erythematosus is associated with a poorer treatment response: a multicentre, retrospective cohort study in Japan

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**Objective:** The objective of this study was to investigate possible differences in treatment responses between two categories for the onset of lupus nephritis. **Methods:** We performed a multicentre, retrospective cohort study of class III–V lupus nephritis patients diagnosed between 1997 and 2014. The renal responses to initial induction therapy were compared between patients who developed lupus nephritis within one year from diagnosis of systemic lupus erythematosus (early (E-) LN) and the remainder (delayed (D-) LN) using the Kaplan–Meier method. We determined the predictors of renal response as well as renal flares and long-term renal outcomes using multivariate Cox regression analyses. **Results:** A total of 107 E-LN and 70 D-LN patients were followed up for a median of 10.2 years. Log-rank tests showed a lower cumulative incidence of complete response in D-LN compared with E-LN patients. Multivariate analysis identified D-LN (hazard ratio (HR) 0.48, 95% confidence interval (CI) 0.33–0.70), nephrotic syndrome at baseline, and a chronicity index greater than 2 as negative predictors of complete response. D-LN patients were more likely to experience renal flares. D-LN (HR 2.54, 95% CI 1.10–5.83) and decreased renal function were significant predictors of chronic kidney disease at baseline. **Conclusion:** D-LN was a predictor of poorer treatment outcomes, in addition to renal histology and severity of nephritis at lupus nephritis onset. *Lupus* (2019) 28, 1062–1073.

**Key words:** Lupus nephritis; delayed lupus nephritis; systemic lupus erythematosus; treatment response

## Introduction

Lupus nephritis (LN) is a common manifestation of systemic lupus erythematosus (SLE) and remains a major contributor to significant morbidity and mortality.<sup>1–3</sup> Despite recent advances, a significant number of LN patients still develop irreversible renal damage.<sup>2–4</sup> The initial renal response to

induction therapy is considered important in the management of LN, because an insufficient response often leads to relapses and a poor renal prognosis, as well as systemic damage accrual.<sup>5–10</sup> Detailed data analysis from the Euro-Lupus Nephritis Trial demonstrated that the renal response after 6 or 12 months of treatment predicted long-term renal outcomes.<sup>11–13</sup> Therefore, identification of the baseline features that predict early treatment responses would be significant in the clinical setting. Previous studies have described several prognostic factors in LN such as decreased renal function at baseline, nephrotic syndrome, class IV in the International Society of Nephrology/Renal Pathology Society

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(ISN/RPS) 2003 classification, and chronicity on histology.<sup>2, 14–21</sup> Despite these findings, it remains challenging to predict accurately which LN patients will respond to initial treatment because of its heterogeneous nature.

Although a renal disorder may be the first manifestation of SLE and most commonly occurs within one year of SLE diagnosis, it can also develop later in the course of the disease.<sup>22, 23</sup> We previously reported a potentially poorer renal outcome in LN that developed later after SLE onset, termed delayed lupus nephritis (D-LN), compared with LN that was present at SLE onset.<sup>24</sup> However, our earlier single-centre study was limited by a small sample size and lack of detailed clinicopathological analyses, making it difficult to develop broader conclusions. The objective of the current multicentre study was to examine possible differences in treatment responses between these two categories for LN onset and to investigate whether D-LN was a predictor of poorer treatment outcomes in addition to currently reported prognostic factors.

## Methods

### *Patients*

This was a multicentre, retrospective cohort study of Japanese patients diagnosed with LN between 1997 and 2014 in the National Center for Global Health and Medicine, The University of Tokyo Hospital and Tohoku University Hospital. During that period, 257 patients newly fulfilled the American College of Rheumatology (ACR) criteria for a renal disorder,<sup>25</sup> with LN confirmed by a renal biopsy. All these patients also met the ACR classification criteria for SLE<sup>25, 26</sup> before or at diagnosis of LN. The inclusion criteria for the study were: (a) patients with class III, III+V, IV, IV+V, or V LN according to the ISN/RPS 2003 criteria;<sup>17</sup> (b) patients aged 18 years or more at LN diagnosis; (c) patients observed for at least 3 years from LN diagnosis; (d) patients without any other coexisting kidney disease at baseline; and (e) patients who underwent renal biopsy and received initial induction therapy within 6 months from their first symptom of renal involvement (proteinuria with a spot urine protein:creatinine ratio (UPCR) of greater than 0.5, glomerular haematuria and/or cellular casts). We excluded 19, 19, 12, three and 27 patients who did not meet the above criteria, respectively, leaving 177 patients for inclusion in the study.

The study was conducted in accordance with the principles of the Declaration of Helsinki. The ethics committees of the National Center for Global Health and Medicine, The University of Tokyo Faculty of Medicine and Tohoku University Faculty of Medicine approved the study (approval numbers 002264-01, 11751-2 and 2017-1-966, respectively). Patient consent was obtained by opt-out procedures at each facility.

### *Data collection*

Clinicopathological information and the therapeutic regimen at onset of LN and throughout the course of the disease were collected from hospital records. All renal biopsies at LN onset were classified histologically according to the ISN/RPS 2003 criteria.<sup>17</sup> The National Institute of Health activity indices (AIs) and chronicity indices (CIs)<sup>15</sup> were scored for all the histology samples. Sequential serological and urinary data at intervals of 1–3 months after initial induction therapy, until either December 2017 or their last visit were collected to evaluate the renal response to induction therapy, relapses and long-term renal outcomes.

### *Definition*

The definitions of the response to treatment and renal flares were based on the Joint European League Against Rheumatism and European Renal Association–European Dialysis and Transplant Association (EULAR/ERA–EDTA) recommendations for LN.<sup>9</sup> With reference to recent studies,<sup>12</sup> we defined good long-term renal outcome as a serum creatinine level of 1.0 mg/dl or less and chronic kidney disease (CKD) as a serum creatinine greater than 1.0 mg/dl with an estimated glomerular filtration rate of less than 60 ml/min/1.73m<sup>2</sup>, confirmed by at least three determinations. In this study, we divided the LN patients into two groups according to the interval between their SLE and LN diagnosis. We defined patients who developed LN within one year from their SLE diagnosis as the early-LN (E-LN) group, while the delayed-LN (D-LN) group included patients who developed renal involvement more than one year after SLE diagnosis.

### *Statistical analysis*

To examine differences in the categorical and continuous variables, the chi-square test, Student's *t*-test and the Mann–Whitney U test were performed as appropriate. The Kaplan–Meier

method was used to compare the cumulative incidence in the E-LN and D-LN groups for complete response (CR) over 24 months from initial induction therapy, and also renal relapse-free and CKD-free survival over 20 years after initial treatment. The cumulative incidence curves and survival curves were tested using the log-rank test. Multivariate Cox and logistic regression analyses were used to determine whether D-LN was a predictor of CR, renal flares and long-term renal outcomes. In addition to the factors significant in the univariate analysis, factors such as treatment options for both induction and maintenance therapy, hospital facilities the patients attended and the period of LN diagnosis were included in the multivariate models. For the regression analyses to evaluate the predictors of long-term renal outcomes, the age at LN diagnosis, gender and body mass index were also used as covariates in the multivariate models. Mediation analysis with a generalized structural equation model was carried out to evaluate the association between baseline variables at LN onset, intermediate variables after treatment and eventual long-term renal outcomes. A *P* value less than 0.05 was considered statistically significant. Because this study was exploratory research, multiplicity was not taken into account. Imputation methods were not required because missing data for all variables were less than 10% of the total number. All data were analyzed using Stata 15.1. More detailed methods are described in the Supplementary material.

## Results

### *Baseline clinical and pathological characteristics and treatment regimens*

Of the 177 patients in the study, 107 and 70 were classified into the E-LN and D-LN groups, respectively. The median duration of follow-up from LN diagnosis was 11.0 years in the E-LN group and 9.1 years in the D-LN group. Table 1 shows the baseline clinicopathological features and treatment regimens for LN onset in the two groups. The D-LN patients were younger at SLE diagnosis (*P* < 0.001). The extent of urinary protein did not differ between the two groups, while higher serum creatinine (*P* = 0.006), lower haemoglobin (*P* = 0.010), lower serum C3 levels (*P* = 0.018), and higher Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) scores (*P* < 0.001) were observed in the

**Table 1** Baseline clinicopathological characteristics and treatment regimens of E-LN and D-LN patients

| Parameters                                   | E-LN<br>(n = 107) | D-LN<br>(n = 70) | P value |
|--|-------------------|------------------|---------|
| Gender (% female)                            | 86 (80.4)         | 59 (84.3)        | 0.508   |
| Age at SLE diagnosis, years                  | 36.0 ± 12.5       | 28.8 ± 12.5      | <0.001  |
| Age at LN diagnosis, years                   | 36.0 ± 12.5       | 37.7 ± 13.5      | 0.425   |
| BMI  | 21.1 ± 2.9        | 22.0 ± 3.1       | 0.073   |
| Systolic blood pressure, mmHg                | 132.6 ± 25.3      | 128.1 ± 21.7     | 0.216   |
| Diastolic blood pressure, mmHg               | 76.7 ± 16.3       | 76.9 ± 14.6      | 0.934   |
| Hypertension (%)                             | 42 (39.3)         | 20 (28.6)        | 0.145   |
| Haematuria (%)                               | 84 (78.5)         | 47 (67.1)        | 0.092   |
| Urinary protein/creatinine ratio, g/gCr      | 3.9 ± 3.3         | 3.6 ± 3.2        | 0.256   |
| Serum albumin, g/dl                          | 2.8 ± 0.6         | 2.9 ± 0.6        | 0.093   |
| Nephrotic syndrome (%)                       | 46 (43.0)         | 21 (30.0)        | 0.081   |
| Serum creatinine, mg/dl                      | 1.0 ± 0.5         | 0.8 ± 0.5        | 0.006   |
| Haemoglobin, g/dl                            | 10.2 ± 2.0        | 11.0 ± 2.3       | 0.010   |
| Serum C3, mg/dl                              | 42.2 ± 23.1       | 50.9 ± 24.3      | 0.018   |
| Serum C4, mg/dl                              | 7.6 ± 6.6         | 7.9 ± 5.6        | 0.183   |
| Range of anti-dsDNA antibodies titre         |                   |                  | 0.124   |
| <30, IU/ml (%)                               | 33 (30.8)         | 20 (28.6)        |         |
| 30–<80, IU/ml (%)                            | 14 (13.1)         | 19 (27.1)        |         |
| 80–<200, IU/ml (%)                           | 16 (15.0)         | 9 (12.9)         |         |
| 200–, IU/ml (%)                              | 44 (41.1)         | 22 (31.4)        |         |
| SLEDAI score                                 | 19.7 ± 6.6        | 15.8 ± 5.0       | <0.001  |
| ISN/RPS classification                       |                   |                  |         |
| III (%)                                      | 17 (15.9)         | 8 (11.4)         | 0.405   |
| III+V (%)                                    | 7 (6.5)           | 12 (17.1)        | 0.026   |
| IV (%)                                       | 49 (45.8)         | 16 (22.9)        | 0.002   |
| IV+V (%)                                     | 14 (13.1)         | 19 (27.1)        | 0.019   |
| V (%)  | 20 (18.7)         | 15 (21.4)        | 0.655   |
| Activity index                               | 5.8 ± 3.8         | 4.8 ± 3.0        | 0.138   |
| Endocapillary hypercellularity <sup>a</sup>  | 1.8 ± 1.1         | 1.4 ± 1.1        | 0.019   |
| Leukocyte infiltration <sup>a</sup>          | 0.6 ± 0.6         | 0.5 ± 0.6        | 0.411   |
| Subendothelial hyaline deposits <sup>a</sup> | 0.9 ± 1.0         | 0.6 ± 0.9        | 0.043   |
| Fibrinoid necrosis/karyorrhexis <sup>a</sup> | 0.4 ± 0.6         | 0.3 ± 0.5        | 0.973   |
| Cellular crescents <sup>a</sup>              | 0.4 ± 0.7         | 0.3 ± 0.5        | 0.676   |
| Interstitial inflammation <sup>a</sup>       | 0.9 ± 0.7         | 0.9 ± 0.7        | 0.695   |
| Activity index > 7 (%)                       | 30 (28.0)         | 14 (20.0)        | 0.226   |
| Chronicity index                             | 1.4 ± 1.3         | 1.5 ± 1.1        | 0.322   |
| Glomerular sclerosis <sup>a</sup>            | 0.3 ± 0.5         | 0.4 ± 0.6        | 0.252   |
| Fibrous crescents <sup>a</sup>               | 0.2 ± 0.5         | 0.1 ± 0.3        | 0.288   |
| Tubular atrophy <sup>a</sup>                 | 0.3 ± 0.5         | 0.4 ± 0.5        | 0.174   |
| Interstitial fibrosis <sup>a</sup>           | 0.6 ± 0.6         | 0.6 ± 0.6        | 0.853   |
| Chronicity index > 2 (%)                     | 17 (15.9)         | 11 (15.7)        | 0.975   |
| Induction therapy                            |                   |                  |         |
| Daily oral PSL dose, mg <sup>b</sup>         | 51.5 ± 10.6       | 48.3 ± 11.1      | 0.027   |
| Intravenous mPSL pulse (%)                   | 57 (53.3)         | 31 (44.3)        | 0.242   |
| CYC (%)                                      | 33 (30.8)         | 16 (22.9)        | 0.246   |
| MMF (%)                                      | 13 (12.1)         | 10 (14.3)        | 0.679   |
| TAC (%)                                      | 14 (13.1)         | 12 (17.1)        | 0.456   |
| CyA (%)                                      | 4 (3.7)           | 7 (10.0)         | 0.092   |
| Other immunosuppressants (%) <sup>c</sup>    | 15 (14.0)         | 12 (17.1)        | 0.572   |
| Cumulative dose of IVCY, g                   | 4.8 ± 1.6         | 4.7 ± 1.9        | 0.457   |
| Maintenance therapy                          |                   |                  |         |
| AZA (%)                                      | 17 (15.9)         | 10 (14.3)        | 0.772   |
| MMF (%)                                      | 14 (13.1)         | 14 (20.0)        | 0.218   |
| TAC (%)                                      | 29 (27.1)         | 15 (21.4)        | 0.393   |
| CyA (%)                                      | 9 (8.4)           | 9 (12.9)         | 0.339   |

(continued)



**Table 1** Continued

| Parameters                 | E-LN<br>(n = 107) | D-LN<br>(n = 70) | P value |
|----------------------------|-------------------|------------------|---------|
| MZR (%)                    | 17 (15.9)         | 8 (11.4)         | 0.405   |
| ACE inhibitors or ARBs (%) | 65 (60.7)         | 35 (50.0)        | 0.158   |

Values are expressed as mean  $\pm$  SD unless otherwise indicated.

<sup>a</sup>The extent of each parameter was expressed as scores of 0–3.

<sup>b</sup>Glucocorticoid dose was expressed as prednisolone or equivalent.

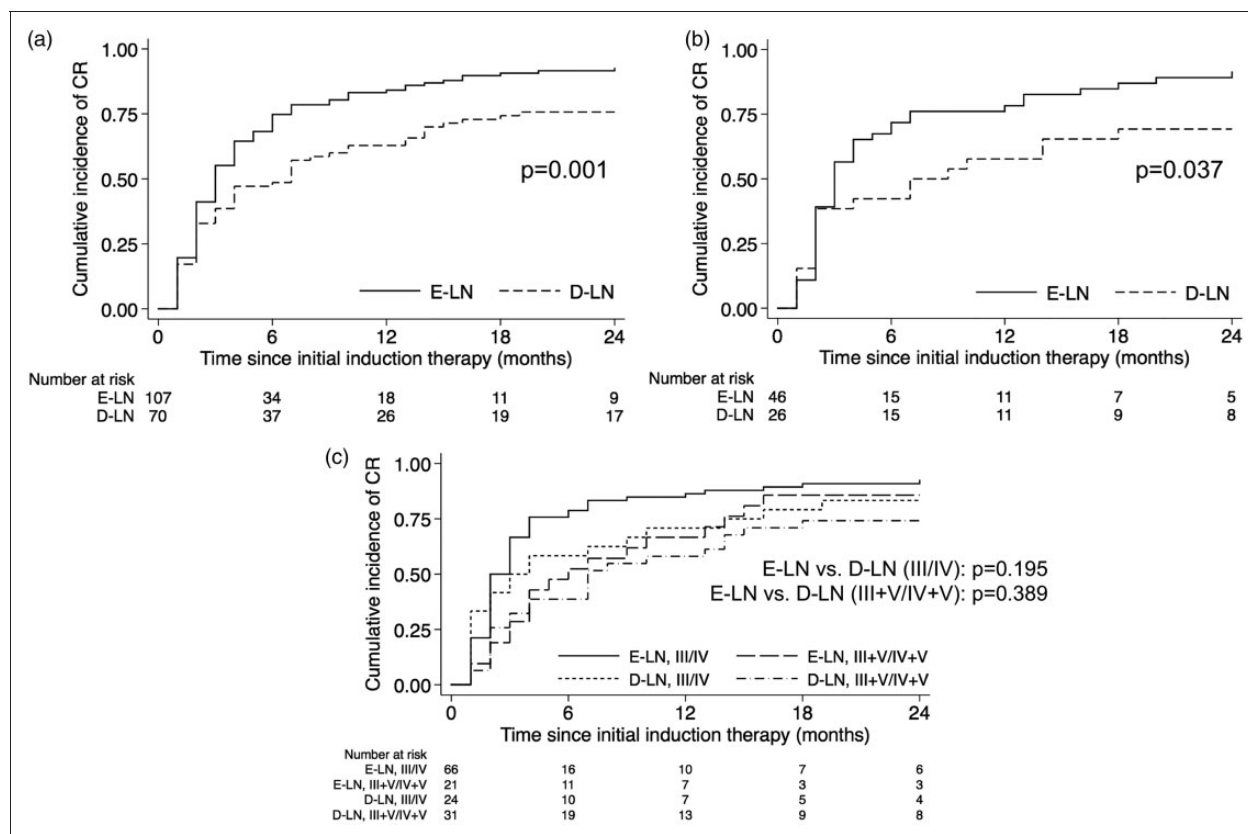
<sup>c</sup>Including MZR and AZA.

E-LN: early lupus nephritis; D-LN: delayed lupus nephritis; SLE: systemic lupus erythematosus; LN: lupus nephritis; BMI: body mass index; SLEDAI: Systemic Lupus Erythematosus Disease Activity Index; ISN/RPS: International Society of Nephrology/Renal Pathology Society; PSL: prednisolone; mPSL: methylprednisolone; CYC: cyclophosphamide; MMF: mycophenolate mofetil; TAC: tacrolimus; CyA: cyclosporine; IVCY: intravenous cyclophosphamide; AZA: azathioprine; MZR: mizoribine; ACE: angiotensin-converting enzyme; ARBs: angiotensin receptor blockers; SD: standard deviation.

E-LN patients. There were more patients with pure class IV nephritis in the E-LN group ( $P=0.002$ ), whereas mixed classes III+V and IV+V LN were more prevalent in the D-LN group ( $P=0.026$  and  $P=0.019$ , respectively). The total frequency of class IV and IV+V was comparable between the two groups (58.9% vs. 50.0%,  $P=0.245$ ). Although the total AI and CI scores did not differ significantly between the two groups, the extent of endocapillary hypercellularity and subendothelial hyaline deposits was greater in the E-LN group ( $P=0.019$  and  $P=0.043$ , respectively). After LN diagnosis, all patients received glucocorticoid therapy at an initial dose of 0.5–1.0 mg equivalent prednisolone/kg/day, with or without intravenous methylprednisolone pulse therapy. The initial dose of prednisolone was higher in the E-LN group, although this difference was numerically small ( $51.5 \pm 10.6$  vs.  $48.3 \pm 11.1$  mg/day,  $P=0.027$ ). There was no significant difference in the treatment options either in the induction or the maintenance phases. The clinical course in the D-LN patients prior to manifestation of LN is summarized in Supplementary Table 1. The majority of the initial clinical symptoms at SLE diagnosis were mild manifestations such as malar rash or arthritis. Until LN had developed, 17 (24.3%) patients experienced extra-renal flares with one or more organs involved or haematological disorders. As a result, 25 (35.7%) patients had a history of treatment with more than 30 mg/day of prednisolone and 30 (42.9%) had received immunosuppressive treatment before LN diagnosis. Fifty-six (80.0%) patients received maintenance glucocorticoids with a mean dose of 6.3 mg/day of prednisolone at their first renal manifestation.

### Renal response to initial induction therapy in E-LN and D-LN patients and predictors of treatment response

We next focused on differences in the renal response in E-LN and D-LN patients. Figure 1(a) shows the cumulative incidence of CR in the two groups over the 24-month period after initial induction therapy. Log-rank test analysis showed a significantly lower cumulative incidence of CR after induction in the D-LN group ( $P=0.001$ ). The cumulative incidence rates of CR at 6 and 12 months in the E-LN and D-LN groups were 74.8% (95% confidence interval (CI) 66.3–82.5%) versus 48.6% (95% CI 37.6–60.8%) and 84.1% (95% CI 76.6–90.3%) versus 62.9% (95% CI 51.7–74.0%), respectively. Table 2 shows a comparison of the baseline clinicopathological features for both the E-LN and D-LN patients who achieved CR at 6 months (responders) and those who did not (non-responders). In the E-LN group, the non-responders were more likely to present with hypertension ( $P=0.045$ ), haematuria ( $P=0.002$ ), nephrotic syndrome ( $P<0.001$ ), decreased baseline renal function ( $P=0.002$ ), a lower prevalence of pure class III ( $P=0.009$ ), a higher prevalence of class IV+V ( $P=0.022$ ) and greater total AI and CI scores ( $P=0.011$  and  $P=0.003$ , respectively). In the D-LN group, the non-responders were characterized by severe proteinuria ( $P=0.001$ ), higher SLEDAI scores at LN onset ( $P=0.014$ ), a lower prevalence of pure class III ( $P=0.019$ ) and a higher prevalence of class IV+V ( $P=0.023$ ). Baseline renal function did not differ significantly between the responders and non-responders in the D-LN group. There was no significant difference in the induction treatment regimens between the responders and non-responders, with the exception of a higher frequency of pulse steroid therapy in E-LN non-responders ( $P=0.012$ ). When the comparison was performed dependent on the response at 12 months, similar results were obtained (Supplementary Table 2). We next evaluated the predictors of CR using Cox regression models (Table 3 and Supplementary Table 3). In the univariate analysis, D-LN (hazard ratio (HR) 0.60, 95% CI 0.43–0.84,  $P=0.003$ ), nephrotic syndrome (HR 0.57, 95% CI 0.41–0.80,  $P=0.001$ ), serum creatinine greater than 1.0 mg/dl (HR 0.61, 95% CI 0.41–0.90,  $P=0.014$ ), class IV+V (HR 0.49, 95% CI 0.31–0.78,  $P=0.003$ ), an AI greater than 7 (HR 0.57, 95% CI 0.39–0.85,  $P=0.005$ ) and a CI greater than 2 (HR 0.44, 95% CI 0.26–0.73,  $P=0.001$ ) at baseline were associated significantly with a poorer renal



**Figure 1** Cumulative incidence of CR over 24 months from initial induction therapy between E-LN and D-LN groups in all patients (a), and those who received cyclophosphamide or mycophenolate mofetil as induction therapy (b). Also shown is the cumulative incidence of CR between the E-LN and D-LN groups with class III/IV or class III+V/IV+V (c). Unadjusted HR (D-LN to E-LN) (95% CI): (a) 0.60 (0.43–0.84),  $P=0.003$ ; (b) 0.58 (0.33–1.02),  $P=0.057$ ; (c) 0.74 (0.44–1.23),  $P=0.250$  in class III/IV and 0.77 (0.42–1.43),  $P=0.413$  in class III+V/IV+V. Multivariate adjusted HR (D-LN to E-LN) (95% CI): (a) 0.48 (0.33–0.70),  $P<0.001$ ; (b) 0.54 (0.30–0.99),  $P=0.047$ ; (c) 0.68 (0.39–1.18),  $P=0.175$  in class III/IV and 0.44 (0.20–1.01),  $P=0.053$  in class III+V/IV+V. CR: complete response; E-LN: early lupus nephritis; D-LN: delayed lupus nephritis; HR: hazard ratio; CI: confidence interval.

response. Multivariate analysis demonstrated that D-LN (HR 0.48, 95% CI 0.33–0.70,  $P<0.001$ ), nephrotic syndrome (HR 0.56, 95% CI 0.39–0.81,  $P=0.002$ ) and a CI greater than 2 (HR 0.57, 95% CI 0.33–0.99,  $P=0.048$ ) were independent negative predictors of CR. When we substituted cellular crescents, fibrous crescents and interstitial fibrosis at renal biopsy, which were also associated with a poorer response in the univariate analysis, for the total AI and CI scores in the multivariate model, D-LN (HR 0.49, 95% CI 0.33–0.71,  $P<0.001$ ) and nephrotic syndrome (HR 0.53, 95% CI 0.37–0.75,  $P<0.001$ ) were identified as negative predictors of CR (Supplementary Table 4). Considering the potential influence on the treatment responses of the relatively heterogeneous induction regimens used in this cohort, we performed an additional analysis only in patients who received cyclophosphamide or mycophenolate mofetil as induction

therapy (Figure 1(b)). A significantly poorer treatment response was also observed in the D-LN patients who received standard induction therapy compared with E-LN ( $P=0.037$ ) even after multivariate adjustment (HR 0.54, 95% CI 0.30–0.99,  $P=0.047$ ). Because a higher prevalence of LN with class III+V/IV+V was observed in the D-LN group (19.6% vs. 44.3%,  $P<0.001$ ), we also investigated the treatment response in E-LN and D-LN patients grouped according to class III/IV (pure proliferative) LN and class III+V/IV+V (mixed proliferative and membranous) LN (Figure 1(c)). In both subgroups, D-LN patients showed poorer treatment responses compared with E-LN patients, although the differences were not statistically significant (log rank  $P=0.195$  in class III/IV and  $P=0.389$  in class III+V/IV+V; multivariate adjusted HR 0.68, 95% CI 0.39–1.18,

**Table 2** Baseline clinicopathological characteristics of the responders and non-responders at 6 months from initial induction therapy in E-LN and D-LN patients

| Parameters                                   | E-LN (n = 107)      |                         |         | D-LN (n = 70)       |                         |         |
|--|---------------------|-------------------------|---------|---------------------|-------------------------|---------|
|  | Responders (n = 80) | Non-responders (n = 27) | P value | Responders (n = 34) | Non-responders (n = 36) | P value |
| Gender (% female)                            | 63 (78.8)           | 23 (85.2)               | 0.467   | 28 (82.4)           | 31 (86.1)               | 0.666   |
| Age at SLE diagnosis, years                  | 36.3 ± 12.9         | 35.0 ± 11.6             | 0.752   | 30.3 ± 13.9         | 27.4 ± 11.0             | 0.518   |
| Age at LN diagnosis, years                   | 36.3 ± 12.9         | 35.0 ± 11.6             | 0.744   | 39.9 ± 14.1         | 35.6 ± 12.7             | 0.196   |
| BMI  | 20.9 ± 2.9          | 21.7 ± 3.1              | 0.209   | 22.4 ± 3.2          | 21.6 ± 3.0              | 0.294   |
| Hypertension (%)                             | 27 (33.8)           | 15 (55.6)               | 0.045   | 9 (26.5)            | 11 (30.6)               | 0.705   |
| Haematuria (%)                               | 57 (71.3)           | 27 (100.0)              | 0.002   | 21 (61.8)           | 26 (72.2)               | 0.352   |
| Urinary protein/creatinine ratio, g/gCr      | 3.4 ± 3.2           | 5.7 ± 3.1               | <0.001  | 2.6 ± 2.8           | 4.4 ± 3.4               | 0.001   |
| Serum albumin, g/dl                          | 2.9 ± 0.5           | 2.5 ± 0.6               | 0.004   | 3.0 ± 0.6           | 2.8 ± 0.6               | 0.166   |
| Nephrotic syndrome (%)                       | 26 (32.5)           | 20 (74.1)               | <0.001  | 7 (20.6)            | 14 (38.9)               | 0.095   |
| Serum creatinine, mg/dl                      | 0.8 ± 0.4           | 1.3 ± 0.7               | 0.002   | 0.7 ± 0.3           | 0.9 ± 0.6               | 0.151   |
| Haemoglobin, g/dl                            | 10.3 ± 2.1          | 9.6 ± 1.7               | 0.125   | 11.2 ± 2.3          | 10.9 ± 2.4              | 0.531   |
| Serum C3, mg/dl                              | 41.1 ± 22.3         | 45.6 ± 25.7             | 0.391   | 53.3 ± 29.1         | 48.6 ± 18.8             | 0.421   |
| Serum C4, mg/dl                              | 7.8 ± 6.7           | 7.2 ± 6.4               | 0.858   | 8.5 ± 6.3           | 7.4 ± 5.0               | 0.834   |
| Serum anti-dsDNA antibodies ≥ 80 IU/ml (%)   | 46 (57.5)           | 14 (51.9)               | 0.609   | 15 (44.1)           | 16 (44.4)               | 0.978   |
| SLEDAI score                                 | 19.5 ± 7.0          | 20.2 ± 5.2              | 0.630   | 14.3 ± 4.5          | 17.2 ± 5.0              | 0.014   |
| ISN/RPS classification                       |                     |                         |         |                     |                         |         |
| III (%)                                      | 17 (21.3)           | 0 (0.0)                 | 0.009   | 7 (20.6)            | 1 (2.8)                 | 0.019   |
| III+V (%)                                    | 4 (5.0)             | 3 (11.1)                | 0.267   | 7 (20.6)            | 5 (13.9)                | 0.457   |
| IV (%)                                       | 35 (43.8)           | 14 (51.9)               | 0.465   | 7 (20.6)            | 9 (25.0)                | 0.660   |
| IV+V (%)                                     | 7 (8.8)             | 7 (25.9)                | 0.022   | 5 (14.7)            | 14 (38.9)               | 0.023   |
| V (%)  | 17 (21.3)           | 3 (11.1)                | 0.243   | 8 (23.5)            | 7 (19.4)                | 0.677   |
| Activity index                               | 5.2 ± 3.4           | 7.6 ± 4.4               | 0.011   | 4.3 ± 2.6           | 5.3 ± 3.3               | 0.160   |
| Endocapillary hypercellularity <sup>a</sup>  | 1.7 ± 1.1           | 2.2 ± 1.0               | 0.050   | 1.3 ± 1.1           | 1.6 ± 1.0               | 0.179   |
| Leukocyte infiltration <sup>a</sup>          | 0.6 ± 0.6           | 0.8 ± 0.6               | 0.143   | 0.5 ± 0.5           | 0.6 ± 0.6               | 0.734   |
| Subendothelial hyaline deposits <sup>a</sup> | 0.8 ± 0.9           | 1.1 ± 1.1               | 0.219   | 0.4 ± 0.7           | 0.8 ± 1.1               | 0.101   |
| Fibrinoid necrosis/karyorrhexis <sup>a</sup> | 0.3 ± 0.5           | 0.5 ± 0.8               | 0.433   | 0.3 ± 0.5           | 0.3 ± 0.5               | 0.726   |
| Cellular crescents <sup>a</sup>              | 0.4 ± 0.6           | 0.6 ± 0.9               | 0.182   | 0.3 ± 0.5           | 0.4 ± 0.6               | 0.186   |
| Interstitial inflammation <sup>a</sup>       | 0.7 ± 0.6           | 1.3 ± 0.8               | <0.001  | 1.0 ± 0.8           | 0.8 ± 0.6               | 0.351   |
| Chronicity index                             | 1.2 ± 1.1           | 2.0 ± 1.4               | 0.003   | 1.2 ± 0.9           | 1.7 ± 1.2               | 0.098   |
| Glomerular sclerosis <sup>a</sup>            | 0.2 ± 0.5           | 0.3 ± 0.5               | 0.482   | 0.3 ± 0.4           | 0.4 ± 0.7               | 0.294   |
| Fibrous crescents <sup>a</sup>               | 0.2 ± 0.4           | 0.3 ± 0.6               | 0.090   | 0.1 ± 0.2           | 0.2 ± 0.4               | 0.093   |
| Tubular atrophy <sup>a</sup>                 | 0.3 ± 0.4           | 0.4 ± 0.6               | 0.168   | 0.4 ± 0.5           | 0.4 ± 0.5               | 0.956   |
| Interstitial fibrosis <sup>a</sup>           | 0.5 ± 0.6           | 0.9 ± 0.5               | 0.002   | 0.5 ± 0.6           | 0.7 ± 0.6               | 0.152   |
| Induction therapy                            |                     |                         |         |                     |                         |         |
| Daily oral PSL dose, mg <sup>b</sup>         | 50.9 ± 10.6         | 53.3 ± 10.4             | 0.172   | 50.7 ± 11.9         | 46.0 ± 9.8              | 0.072   |
| Intravenous mPSL pulse (%)                   | 37 (46.3)           | 20 (74.1)               | 0.012   | 14 (41.2)           | 17 (47.2)               | 0.611   |
| CYC (%)                                      | 23 (28.8)           | 10 (37.0)               | 0.420   | 6 (17.6)            | 10 (27.8)               | 0.313   |
| MMF (%)                                      | 10 (12.5)           | 3 (11.1)                | 0.849   | 5 (14.7)            | 5 (13.9)                | 0.922   |
| TAC (%)                                      | 13 (16.3)           | 1 (3.7)                 | 0.095   | 8 (23.5)            | 4 (11.1)                | 0.168   |
| CyA (%)                                      | 2 (2.5)             | 2 (7.4)                 | 0.245   | 2 (5.9)             | 5 (13.9)                | 0.264   |
| Other immunosuppressants (%) <sup>c</sup>    | 11 (13.8)           | 4 (14.8)                | 0.890   | 5 (14.7)            | 7 (19.4)                | 0.599   |
| Cumulative dose of IVCY, g                   | 4.9 ± 1.5           | 4.5 ± 1.9               | 0.658   | 4.5 ± 0.4           | 4.8 ± 2.5               | 0.698   |
| ACE inhibitors or ARBs (%)                   | 45 (56.3)           | 20 (74.1)               | 0.101   | 18 (52.9)           | 17 (47.2)               | 0.632   |

Values are expressed as mean ± SD unless otherwise indicated.

<sup>a</sup>The extent of each parameter was expressed as scores of 0–3.

<sup>b</sup>Glucocorticoid dose was expressed as prednisolone or equivalent.

<sup>c</sup>Including MZR and AZA.

E-LN: early lupus nephritis; D-LN: delayed lupus nephritis; LN: lupus nephritis; BMI: body mass index; SLEDAI: Systemic Lupus Erythematosus Disease Activity Index; ISN/RPS: International Society of Nephrology/Renal Pathology Society; PSL: prednisolone; mPSL: methylprednisolone; CYC: cyclophosphamide; MMF: mycophenolate mofetil; TAC: tacrolimus; CyA: cyclosporine; IVCY: intravenous cyclophosphamide; ACE: angiotensin-converting enzyme; ARBs: angiotensin receptor blockers; SD: standard deviation; MZR: mizoribine; AZA: azathioprine.

$P=0.175$  in class III/IV and HR 0.44, 95% CI 0.20–1.01,  $P=0.053$  in class III+V/IV+V).

*Relapse-free survival in E-LN and D-LN patients and predictors of renal flares*

Figure 2(a) compares renal relapse-free survival over the 20-year period after initial induction

**Table 3** Predictors of renal response, renal flares and long-term renal outcomes analyzed by multivariate Cox or logistic regression models

| Variables  | Univariate analysis |         | Multivariate analysis |         |
|--|---------------------|---------|-----------------------|---------|
|  | HR (95% CI)         | P value | HR (95% CI)           | P value |
| <b>Predictors of CR<sup>a</sup></b>  |                     |         |                       |         |
| D-LN   | 0.60 (0.43–0.84)    | 0.003   | 0.48 (0.33–0.70)      | <0.001  |
| Nephrotic syndrome at baseline   | 0.57 (0.41–0.80)    | 0.001   | 0.56 (0.39–0.81)      | 0.002   |
| Serum creatinine > 1.0 mg/dl at baseline                                       | 0.61 (0.41–0.90)    | 0.014   | 0.67 (0.41–1.10)      | 0.113   |
| ISN/RPS classification   |                     |         |                       |         |
| Class IV   | 0.81 (0.57–1.15)    | 0.247   | 1.14 (0.74–1.76)      | 0.552   |
| Class IV+V   | 0.49 (0.31–0.78)    | 0.003   | 0.86 (0.50–1.47)      | 0.579   |
| Others (reference)   | –                   | –       | –                     | –       |
| Activity index > 7   | 0.57 (0.39–0.85)    | 0.005   | 0.74 (0.45–1.22)      | 0.241   |
| Chronicity index > 2   | 0.44 (0.26–0.73)    | 0.001   | 0.57 (0.33–0.99)      | 0.048   |
| <b>Predictors of renal flares<sup>b</sup></b>                                  |                     |         |                       |         |
| D-LN   | 2.10 (1.23–3.59)    | 0.007   | 1.71 (0.95–3.08)      | 0.072   |
| Age at SLE diagnosis   | 0.98 (0.95–1.00)    | 0.038   | 0.99 (0.96–1.01)      | 0.242   |
| Failure to achieve CR at 6 months  | 2.13 (1.25–3.64)    | 0.006   | 2.15 (1.18–3.90)      | 0.012   |
| <b>Predictors of CKD (baseline variables only)<sup>c</sup></b>                 |                     |         |                       |         |
| D-LN   | 1.70 (0.81–3.57)    | 0.160   | 2.54 (1.10–5.83)      | 0.028   |
| Hypertension at baseline   | 2.18 (1.04–4.58)    | 0.040   | 1.51 (0.60–3.80)      | 0.378   |
| Serum creatinine > 1.0 mg/dl at baseline                                       | 4.06 (1.93–8.55)    | <0.001  | 5.51 (1.86–16.34)     | 0.002   |
| Activity index > 7   | 2.28 (1.08–4.83)    | 0.031   | 1.09 (0.42–2.77)      | 0.865   |
| Chronicity index > 2   | 3.49 (1.61–7.57)    | 0.002   | 0.94 (0.31–2.89)      | 0.915   |
| <b>Predictors of CKD (including intermediate outcome measures)<sup>d</sup></b> |                     |         |                       |         |
| D-LN   | 1.66 (0.74–3.74)    | 0.221   | 1.71 (0.52–5.67)      | 0.377   |
| Hypertension at baseline   | 2.50 (1.10–5.68)    | 0.028   | 1.66 (0.52–5.28)      | 0.387   |
| Serum creatinine > 1.0 mg/dl at baseline                                       | 4.32 (1.85–10.08)   | 0.001   | 6.19 (1.40–27.42)     | 0.016   |
| Activity index > 7   | 2.74 (1.18–6.38)    | 0.019   | 1.90 (0.53–6.83)      | 0.324   |
| Chronicity index > 2   | 4.04 (1.62–10.11)   | 0.003   | 0.77 (0.18–3.29)      | 0.727   |
| Failure to achieve CR at 6 months  | 6.16 (2.52–15.06)   | <0.001  | 5.83 (1.68–20.30)     | 0.006   |
| Renal flare  | 4.19 (1.81–9.67)    | 0.001   | 6.09 (1.83–20.23)     | 0.003   |

<sup>a</sup>See Supplementary Table 3 for more details.

<sup>b</sup>One patient who experienced a renal flare until 6 months from initial induction therapy was excluded from the analysis. See Supplementary Table 5 for more details.

<sup>c</sup>See Supplementary Table 7 for more details.

<sup>d</sup>See Supplementary Table 8 for more details.

CR: complete response; HR: hazard ratio; CI: confidence interval; D-LN: delayed lupus nephritis; ISN/RPS: International Society of Nephrology/Renal Pathology Society; CKD: chronic kidney disease; OR: odds ratio.

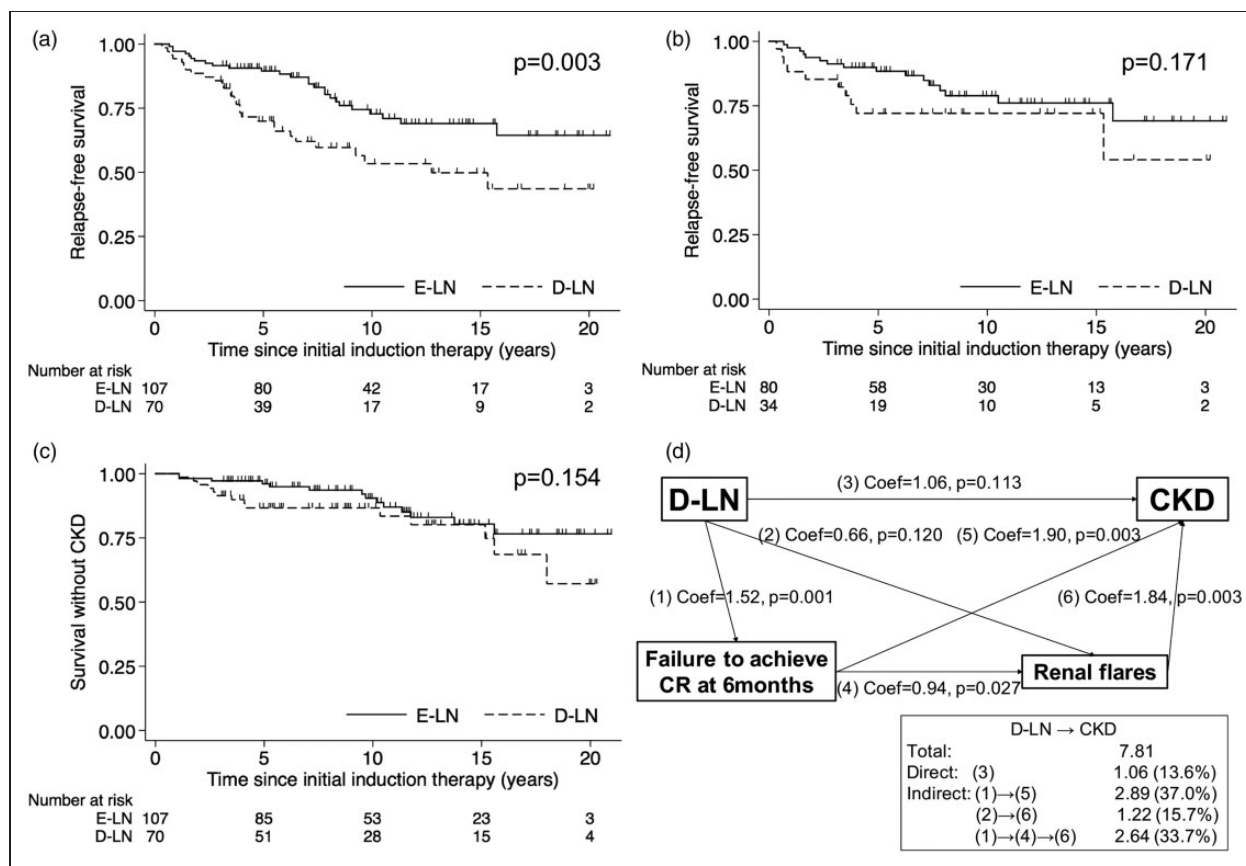
therapy between the E-LN and D-LN groups. Compared to the E-LN patients, those with D-LN were more likely to experience renal flares (log rank  $P=0.003$ ; HR 2.10, 95% CI 1.23–3.59,  $P=0.007$ ). The estimated relapse-free survival rates at 5 years following initial treatment in the E-LN and D-LN groups were 89.5% (95% CI 81.8–94.1%) versus 69.9% (95% CI 57.3–79.5%). On the basis that a poorer treatment response in D-LN patients could result in more frequent flares, we next evaluated renal relapse-free survival only in those who achieved CR within 6 months (Figure 2(b)). Although the difference did not reach statistical significance, LN relapses were relatively more frequent in D-LN compared with E-LN patients (log rank  $P=0.171$ ; HR 1.73, 95% CI 0.78–3.81,  $P=0.176$ ). In the multivariate analysis,

failure to achieve CR at 6 months (HR 2.15, 95% CI 1.18–3.90,  $P=0.012$ ) predicted subsequent renal flares (Table 3 and Supplementary Table 5). D-LN narrowly failed to retain its statistical significance after multivariate adjustment (HR 1.71, 95% CI 0.95–3.08,  $P=0.072$ ). When we substituted the renal response status at 12 months for that at 6 months, D-LN (HR 1.86, 95% CI 1.01–3.45,  $P=0.047$ ) was identified as a significant predictor of renal flares (Supplementary Table 6).

#### *Renal outcomes in E-LN and D-LN patients and predictors of long-term renal damage*

We next investigated the long-term renal outcomes between the two groups. Whereas 93 E-LN (86.9%) and 56 D-LN (80.0%) patients had preserved renal function, 14 E-LN (13.1%) and 14 D-LN (20.0%)





**Figure 2** Renal relapse-free survival over 20 years from initial induction therapy between the E-LN and D-LN groups in all patients (a), and those who achieved CR within 6 months (b). Also shown is survival without CKD over 20 years from initial induction therapy between the E-LN and D-LN groups in all patients (c) and the associations among D-LN, renal response status at 6 months, renal flares and long-term renal outcomes analyzed using mediation analysis with a generalized structural equation model (d) (see Supplementary Table 10 and Supplementary Figure 1, for more details). Unadjusted HR (D-LN to E-LN) (95% CI) (a) 2.10 (1.23–3.59),  $P=0.007$ ; (b) 1.73 (0.78–3.81),  $P=0.176$ ; (c) 1.70 (0.81–3.57),  $P=0.160$ . Multivariate adjusted HR (D-LN to E-LN) (95% CI) (a) 1.71 (0.95–3.08),  $P=0.072$ ; (b) 1.68 (0.70–4.05),  $P=0.247$ ; (c) 2.54 (1.10–5.83),  $P=0.028$ . E-LN: early lupus nephritis; D-LN: delayed lupus nephritis; CR: complete response; CKD: chronic kidney disease; HR: hazard ratio; CI: confidence interval; Coef: coefficient value.

patients developed CKD. Figure 2(c) shows CKD-free survival between the two groups. CKD was relatively common in D-LN compared with E-LN patients over the 20-year period following initial treatment, although this difference was not statistically significant (log rank  $P=0.154$ ; HR 1.70, 95% CI 0.81–3.57,  $P=0.160$ ). To determine the predictors of CKD, we first conducted Cox regression analysis limited to baseline variables at LN onset (Table 3 and Supplementary Table 7). After multivariate adjustment, D-LN (HR 2.54, 95% CI 1.10–5.83,  $P=0.028$ ) and baseline serum creatinine greater than 1.0 mg/dl (HR 5.51, 95% CI 1.86–16.34,  $P=0.002$ ) were identified as predictors of CKD. Logistic regression analysis was then performed to evaluate the influence on eventual long-term renal outcomes of the intermediate outcome measures including renal response status at

6 months and renal flares (Table 3 and Supplementary Table 8). Multivariate analysis identified baseline serum creatinine greater than 1.0 mg/dl (odds ratio (OR) 6.19, 95% CI 1.40–27.42,  $P=0.016$ ), failure to achieve CR at 6 months (OR 5.83, 95% CI 1.68–20.30,  $P=0.006$ ) and renal flares (OR 6.09, 95% CI 1.83–20.23,  $P=0.003$ ) as independent predictors of CKD. When we substituted the renal response status at 12 months for that at 6 months, the results were almost similar (Supplementary Table 9). Because these intermediate outcome measures were statistically intermediate variables in the regression model, we next performed mediation analysis with a generalized structural equation model (Figure 2(d), Supplementary Table 10 and Supplementary Figure 1). The coefficient values for the direct and indirect associations between D-LN and CKD were



1.06 (13.6%) and 6.75 (86.4%), respectively. These results suggested that the development of CKD in D-LN patients was attributable mainly to the failure to achieve CR at 6 months and renal flares.

#### Clinical and serological features throughout the course in E-LN and D-LN patients

Finally, we examined the clinical features such as organ involvement due to SLE throughout the course and compared serum autoantibody profiles between E-LN and D-LN patients (Table 4). While the duration of follow-up of SLE was longer in D-LN patients ( $P < 0.001$ ), serositis was more frequent in E-LN patients ( $P = 0.006$ ). There were no significant differences in the other clinical manifestations between the two groups. Of note, regarding serology, anti-Sm and anti-RNP antibodies were significantly more prevalent in D-LN than in E-LN patients ( $P = 0.009$  and  $P < 0.001$ , respectively), whereas the frequencies of the other autoantibodies examined were similar between the two groups.

## Discussion

In this study, poorer treatment responses were observed in the D-LN group compared with the

E-LN group. Our analyses demonstrated that a poor renal response to initial treatment and renal flares were associated strongly with future renal damage, consistent with the previous literature,<sup>6–8, 11, 12, 27, 28</sup> and that the relatively worse long-term renal outcome in D-LN was due mainly to failure to achieve a sustained remission in these patients. We also found that previously reported prognostic factors, such as decreased baseline renal function, nephrotic syndrome, class IV/IV+V and higher AI and CI scores<sup>2, 14–21</sup> were associated with a poor renal response. However, some of these parameters did not retain statistical significance in our multivariate analysis. Apart from our previous report,<sup>24</sup> only a few studies have examined the association between the time of LN development in the course of SLE and renal prognosis, with the results obtained remaining controversial.<sup>8, 29</sup> However, all of these previous studies lacked detailed clinicopathological comparisons between E-LN and D-LN. In the present study, detailed clinicopathological investigation showed that, in addition to currently reported prognostic factors, D-LN may be a potential predictor of poorer treatment outcomes.

As shown in Table 1, the severity of nephritis at LN onset was almost comparable between the two groups, with the exception that higher serum

**Table 4** Clinical characteristics throughout the disease course and autoantibodies profiles in E-LN and D-LN patients

| Parameters   | E-LN (n = 107)    | D-LN (n = 70)     | P value |
|--|-------------------|-------------------|---------|
| Follow-up duration from SLE diagnosis, years, median (IQR) | 11.0 (6.3–14.5)   | 18.0 (12.8–24.3)  | <0.001  |
| Follow-up duration from LN diagnosis, years, median (IQR)  | 11.0 (6.3–14.5)   | 9.1 (5.4–15.5)    | 0.629   |
| Clinical manifestations                                    |                   |                   |         |
| Malar rash (%)   | 56 (52.3)         | 46 (65.7)         | 0.078   |
| Arthritis (%)  | 60 (56.1)         | 48 (68.6)         | 0.096   |
| Myositis (%)   | 6 (5.6)           | 6 (8.6)           | 0.443   |
| Serositis (%)  | 37 (34.6)         | 11 (15.7)         | 0.006   |
| Neuropsychiatric manifestation (%)                         | 12 (11.2)         | 11 (15.7)         | 0.384   |
| Autoimmune haemolytic anaemia (%)                          | 15 (14.0)         | 11 (15.7)         | 0.755   |
| Thrombocytopenia (%)                                       | 29 (27.1)         | 23 (32.9)         | 0.411   |
| Haemophagocytic lymphohistiocytosis (%)                    | 5 (4.7)           | 5 (7.1)           | 0.486   |
| Thrombotic microangiopathy (%)                             | 1 (0.9)           | 3 (4.3)           | 0.142   |
| Pulmonary hypertension (%)                                 | 1 (0.9)           | 3 (4.3)           | 0.142   |
| Diffuse alveolar haemorrhage (%)                           | 3 (2.8)           | 1 (1.4)           | 0.547   |
| Gastrointestinal system involvement (%)                    | 8 (7.5)           | 5 (7.1)           | 0.934   |
| Autoantibodies, positive/negative (%) <sup>a</sup>         |                   |                   |         |
| Anti-dsDNA (%)   | 88/19 (82.2/17.8) | 60/10 (85.7/14.3) | 0.542   |
| Anti-Sm (%)  | 31/76 (29.0/71.0) | 33/35 (48.5/51.5) | 0.009   |
| Anti-RNP (%)   | 39/68 (36.4/63.6) | 48/20 (70.6/29.4) | <0.001  |
| Anti-Ro (%)  | 68/39 (63.6/36.4) | 42/24 (63.6/36.4) | 0.991   |
| Anti-La (%)  | 21/86 (19.6/80.4) | 11/55 (16.7/83.3) | 0.626   |
| Anti-β2 glycoprotein I (%)                                 | 23/82 (21.9/78.1) | 12/56 (17.6/82.4) | 0.496   |
| Lupus anticoagulant (%)                                    | 23/80 (22.3/77.7) | 13/48 (21.3/78.7) | 0.879   |

<sup>a</sup>Only the data measured before or at LN diagnosis were collected.

E-LN: early lupus nephritis; D-LN: delayed lupus nephritis; IQR: interquartile range; LN: lupus nephritis.

creatinine levels were observed in E-LN patients. In Table 2 and Supplementary Table 2, the extent of proteinuria and renal function at baseline were both associated with the renal response to initial therapy in the E-LN group. On the other hand, in the D-LN group, the non-responders were characterized only with severe proteinuria, and the differences in baseline renal function were not remarkable between the responders and non-responders at 6 and 12 months after treatment. These results suggest that baseline renal function may be less informative for predicting renal response particularly in D-LN patients.

This study also investigated the histological characteristics of the E-LN and D-LN patients. Although previous studies have described that features such as crescent formation and chronic tubulointerstitial changes could have prognostic value in LN,<sup>8, 16, 30</sup> the extent of these parameters at baseline did not differ between the two groups. With regard to the ISN/RPS classification, we observed a higher prevalence of mixed proliferative and membranous LN (class III+V/IV+V) in the D-LN group than in the E-LN group. Based on this finding, we next examined the renal response to initial induction therapy in E-LN and D-LN patients with class III/IV or class III+V/IV+V (Figure 1(c)). In these analyses, both histological subgroups showed a similar tendency towards poorer treatment responses in D-LN patients compared with E-LN patients, although these differences were not statistically significant probably because of the small sample size in each subgroup. Although it is known that some LN patients with membranous lesions may exhibit persistent proteinuria and treatment resistance,<sup>31, 32</sup> the results of our study suggested that the poorer treatment response observed in D-LN patients could not simply be explained by only a higher prevalence of class III+V/IV+V LN in these patients.

In our study, multivariate analyses identified D-LN as a predictor of a poorer treatment response independent of renal histology and the severity of nephritis at baseline. This suggested it is necessary to carry out further investigations other than clinicopathological analyses at LN onset. In this cohort, the D-LN group had an earlier onset of SLE compared with the E-LN group (Table 1). Some genetic studies in SLE have demonstrated that an earlier onset of SLE may be associated with a higher genetic risk and more severe disease phenotype.<sup>33, 34</sup> Because genetic data were not available in this retrospective cohort study, we instead sought to analyze possible differences in

clinical and serological features between the two groups (Table 4). With the exception that serositis was more frequent in E-LN patients, we failed to detect any significant differences in the frequencies of other organ involvement between the two groups. On the other hand, we found a higher prevalence of anti-Sm and anti-RNP antibodies in D-LN patients (48.5% and 70.6%, respectively). Anti-Sm and anti-RNP antibodies are generally detectable in 5–30% and 25–47% of SLE patients,<sup>35</sup> with some studies reporting a higher prevalence of these antibodies in LN patients.<sup>36,37</sup> Although the association of these autoantibodies with delayed renal involvement appears to be conflicting,<sup>38–40</sup> our findings suggested that D-LN may be characterized by these specific autoantibody profiles. Despite recent advances in the understanding of the pathogenesis of SLE, it remains challenging to predict accurately which LN patients will develop treatment resistance due to its heterogeneous nature. Although we found some possible heterogeneity in the E-LN and D-LN groups in terms of epidemiology and serology, further research is needed to reveal the potential mechanism behind different treatment responses between these two onset categories of LN.

There were some limitations in this study. First of all, it was a retrospective study. Therapeutic regimens were not standardized in the study cohort and we could not obtain clinical information such as extra-renal damage accrual in some patients. Second, the study population was limited to Japanese patients. It is known that the prevalence and the clinical courses of LN may vary considerably between different ethnicities.<sup>23, 37, 41</sup> Although there has been one study from Asia to support our findings,<sup>8</sup> another retrospective study from Brazil and Italy reported comparable long-term renal outcomes between early-onset and late-onset LN.<sup>29</sup> However, the latter study only included a small number of patients. The results of the present study need to be validated in a prospective study on a larger multi-ethnic population receiving standardized treatment protocols. Finally, it should be noted that it may be difficult to confirm whether all of the D-LN patients had first developed LN later after SLE onset, because the onset of nephritis can sometimes be subtle or even silent.<sup>42, 43</sup> However, the present study at least indicated that clinically delayed-onset LN may have poorer treatment outcomes than early-onset LN, and that this finding could be helpful for the management of LN in the clinical setting.

In conclusion, this study observed poorer treatment responses in D-LN patients than in E-LN patients. D-LN was a predictor of poorer treatment outcomes, in addition to currently reported clinicopathological prognostic factors. Failure to achieve a sustained remission was shown to be the leading cause of long-term renal damage in D-LN patients. We also found that an earlier SLE onset, and anti-Sm and anti-RNP antibodies were characteristic of the D-LN group. Our findings suggested that the time of LN development in the course of SLE may be useful to predict a renal response to induction therapy and that D-LN patients should be carefully followed up to improve their clinical course and outcomes.


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### References

- 1 Cervera R, Khamashta MA, Font J, *et al.* Morbidity and mortality in systemic lupus erythematosus during a 10-year period: a comparison of early and late manifestations in a cohort of 1,000 patients. *Medicine (Baltimore)* 2003; 82: 299–308.
- 2 Faurschou M, Dreyer L, Kamper AL, Starklint H, Jacobsen S. Long-term mortality and renal outcome in a cohort of 100 patients with lupus nephritis. *Arthritis Care Res* 2010; 62: 873–880.
- 3 Hahn BH, McMahon MA, Wilkinson A, *et al.* American College of Rheumatology guidelines for screening, treatment, and management of lupus nephritis. *Arthritis Care Res* 2012; 64: 797–808.
- 4 Tektonidou MG, Dasgupta A, Ward MM. Risk of end-stage renal disease in patients with lupus nephritis, 1970–2015: a systematic review and Bayesian meta-analysis. *Arthritis Rheum* 2016; 68: 1432–1441.
- 5 Illei GG, Takada K, Parkin D, *et al.* Renal flares are common in patients with severe proliferative lupus nephritis treated with pulse immunosuppressive therapy: long-term followup of a cohort of 145 patients participating in randomized controlled studies. *Arthritis Rheum* 2002; 46: 995–1002.
- 6 Mok CC, Ying KY, Tang S, *et al.* Predictors and outcome of renal flares after successful cyclophosphamide treatment for diffuse proliferative lupus glomerulonephritis. *Arthritis Rheum* 2004; 50: 2559–2568.
- 7 Chen YE, Korbet SM, Katz RS, Schwartz MM, Lewis EJ; Collaborative Study Group. Value of a complete or partial remission in severe lupus nephritis. *Clin J Am Soc Nephrol* 2008; 3: 46–53.
- 8 Moon SJ, Kwok SK, Ju JH, *et al.* Predictors of chronic kidney disease in Korean patients with lupus nephritis. *J Rheumatol* 2011; 38: 2588–2597.
- 9 Bertias GK, Tektonidou M, Amoura Z, *et al.* The Joint European League Against Rheumatism and European Renal Association–European Dialysis and Transplant Association (EULAR/ERA–EDTA) recommendations for the management of adult and paediatric lupus nephritis. *Ann Rheum Dis* 2012; 71: 1771–1782.
- 10 Hanaoka H, Yamada H, Kiyokawa T, *et al.* Lack of partial renal response by 12 weeks after induction therapy predicts poor renal response and systemic damage accrual in lupus nephritis class III or IV. *Arthritis Res Ther* 2017; 19: 4–13.
- 11 Houssiau FA, Vasconcelos C, D’Cruz D, *et al.* Early response to immunosuppressive therapy predicts good renal outcome in lupus nephritis: lessons from long-term follow up of patients in the Euro-Lupus Nephritis Trial. *Arthritis Rheum* 2004; 50: 3934–3940.
- 12 Dall’Era M, Cisternas MG, Smilek DE, *et al.* Predictors of long-term renal outcome in lupus nephritis trials: lessons learned from the Euro-Lupus Nephritis cohort. *Arthritis Rheum* 2015; 67: 1305–1313.
- 13 Houssiau FA, Vasconcelos C, D’Cruz D, *et al.* Immunosuppressive therapy in lupus nephritis: the Euro-Lupus Nephritis Trial, a randomized trial of low-dose versus high-dose intravenous cyclophosphamide. *Arthritis Rheum* 2002; 46: 2121–2131.
- 14 Dall’Era M, Stone D, Levesque V, Cisternas M, Wofsy D. Identification of biomarkers that predict response to treatment of lupus nephritis with mycophenolate mofetil or pulse cyclophosphamide. *Arthritis Care Res* 2011; 63: 351–357.
- 15 Austin HA, Muenz LR, Joyce KM, *et al.* Prognostic factors in lupus nephritis. Contribution of renal histologic data. *Am J Med* 1983; 75: 382–391.
- 16 Austin HA, Boumpas DT, Vaughan EM, Balow JE. Predicting renal outcomes in severe lupus nephritis: contributions of clinical and histologic data. *Kidney Int* 1994; 45: 544–550.
- 17 Weening JJ, D’Agati VD, Schwartz MM, *et al.* The classification of glomerulonephritis in systemic lupus erythematosus revised. *J Am Soc Nephrol* 2004; 15: 241–250.
- 18 Yokoyama H, Wada T, Hara A, *et al.* The outcome and a new ISN/RPS 2003 classification of lupus nephritis in Japanese. *Kidney Int* 2004; 66: 2382–2388.
- 19 Contreras G, Pardo V, Cely C, *et al.* Factors associated with poor outcomes in patients with lupus nephritis. *Lupus* 2005; 14: 890–895.

- 20 Hiramatsu N, Kuroiwa T, Ikeuchi H, *et al.* Revised classification of lupus nephritis is valuable in predicting renal outcome with an indication of the proportion of glomeruli affected by chronic lesions. *Rheumatology (Oxford)* 2008; 47: 702–707.
- 21 Kono M, Yasuda S, Kato M, *et al.* Long-term outcome in Japanese patients with lupus nephritis. *Lupus* 2014; 23: 1124–1132.
- 22 Jacobsen S, Petersen J, Ullman S, *et al.* A multicentre study of 513 Danish patients with systemic lupus erythematosus. I. Disease manifestations and analyses of clinical subsets. *Clin Rheumatol* 1998; 17: 468–477.
- 23 Seligman VA, Lum RF, Olson JL, Li H, Criswell LA. Demographic differences in the development of lupus nephritis: a retrospective analysis. *Am J Med* 2002; 112: 726–729.
- 24 Takahashi Y, Mizoue T, Suzuki A, *et al.* Time of initial appearance of renal symptoms in the course of systemic lupus erythematosus as a prognostic factor for lupus nephritis. *Mod Rheumatol* 2009; 19: 293–301.
- 25 Tan EM, Cohen AS, Fries JF, *et al.* The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1982; 25: 1271–1277.
- 26 Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1997; 40: 1725.
- 27 Moroni G, Quaglini S, Maccario M, Banfi G, Ponticelli C. “Nephritic flares” are predictors of bad long-term renal outcome in lupus nephritis. *Kidney Int* 1996; 50: 2047–2053.
- 28 Gibson KL, Gipson DS, Massengill SA, *et al.* Predictors of relapse and end stage kidney disease in proliferative lupus nephritis: focus on children, adolescents, and young adults. *Clin J Am Soc Nephrol* 2009; 4: 1962–1967.
- 29 Ugolini-Lopes MR, Santos LPS, Stagnaro C, Seguro LPC, Mosca M, Bonfá E. Late-onset biopsy-proven lupus nephritis without other associated autoimmune diseases: severity and long-term outcome. *Lupus* 2019; 28: 123–128.
- 30 Cai F, Han F, Wang H, *et al.* The crescentic implication of renal outcomes in proliferative lupus nephritis. *J Rheumatol* 2018; 45: 513–520.
- 31 Najafi CC, Korbet SM, Lewis EJ, Schwartz MM, Reichlin M, Evans J; Lupus Nephritis Collaborative Study Group. Significance of histologic patterns of glomerular injury upon long-term prognosis in severe lupus glomerulonephritis. *Kidney Int* 2001; 59: 2156–2163.
- 32 Mok CC. Membranous nephropathy in systemic lupus erythematosus: a therapeutic enigma. *Nat Rev Nephrol* 2009; 5: 212–220.
- 33 Webb R, Kelly JA, Somers EC, *et al.* Early disease onset is predicted by a higher genetic risk for lupus and is associated with a more severe phenotype in lupus patients. *Ann Rheum Dis* 2011; 70: 151–156.
- 34 Taylor KE, Chung SA, Graham RR, *et al.* Risk alleles for systemic lupus erythematosus in a large case-control collection and associations with clinical subphenotypes. *PLoS Genet* 2011; 7: e1001311.
- 35 Migliorini P, Baldini C, Rocchi V, Bombardieri S. Anti-Sm and anti-RNP antibodies. *Autoimmunity* 2005; 38: 47–54.
- 36 Alba P, Bento L, Cuadrado MJ, *et al.* Anti-dsDNA, anti-Sm antibodies, and the lupus anticoagulant: significant factors associated with lupus nephritis. *Ann Rheum Dis* 2003; 62: 556–560.
- 37 Bastian HM, Roseman JM, McGwin G Jr, *et al.* Systemic lupus erythematosus in three ethnic groups. XII. Risk factors for lupus nephritis after diagnosis. *Lupus* 2002; 11: 152–160.
- 38 Homma M, Mimori T, Takeda Y, *et al.* Autoantibodies to the Sm antigen: immunological approach to clinical aspects of systemic lupus erythematosus. *J Rheumatol Suppl* 1987; 14: 188–193.
- 39 Varela DC, Quintana G, Somers EC, *et al.* Delayed lupus nephritis. *Ann Rheum Dis* 2008; 67: 1044–1046.
- 40 Kwon OC, Lee JS, Ghang B, *et al.* Predicting eventual development of lupus nephritis at the time of diagnosis of systemic lupus erythematosus. *Semin Arthritis Rheum* 2018; 48: 462–466.
- 41 Lewis MJ, Jawad AS. The effect of ethnicity and genetic ancestry on the epidemiology, clinical features and outcome of systemic lupus erythematosus. *Rheumatology (Oxford)* 2017; 56: i67–i77.
- 42 Zabaleta-Lanz ME, Munoz LE, Tapanes FJ, *et al.* Further description of early clinically silent lupus nephritis. *Lupus* 2006; 15: 845–851.
- 43 Wakasugi D, Gono T, Kawaguchi Y, *et al.* Frequency of class III and IV nephritis in systemic lupus erythematosus without clinical renal involvement: an analysis of predictive measures. *J Rheumatol* 2012; 39: 79–85.