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Japan College of Rheumatology
MODERN RHEUMATOLOGY

http://informahealthcare.com/mor
ISSN 1439-7595 (print), 1439-7609 (online)

Mod Rheumatol, 2014; 24(3): 448–452

DOI: 10.3109/14397595.2013.843745

informa
healthcare

ORIGINAL ARTICLE

Psychological stress in a Japanese population with systemic lupus erythematosus: Finding from KYSS study

Hiroki Takahashi[1], Masakazu Washio[2], Chikako Kiyohara[3], Yoshifumi Tada[4], Toyoko Asami[5], Saburo Ide[2], Tatsuya Atsumi[6], Gen Kobashi[7], Motohisa Yamamoto[1], and Takahiko Horiuchi[8]
the Kyushu Sapporo SLE (KYSS) Study Group

[1]*First Department of Internal Medicine, Sapporo Medical University School of Medicine, Sapporo, Japan,* [2]*Department of Community Health and Clinical Epidemiology, St. Mary's College, Kurume, Japan,* [3]*Department of Preventive Medicine, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan,* [4]*Department of Internal Medicine, Faculty of Medicine, Saga University, Saga, Japan,* [5]*Rehabilitation Center, Saga University Hospital, Saga, Japan,* [6]*Department of Medicine II, Hokkaido University Graduate School of Medicine, Sapporo, Japan,* [7]*Molecular Biostatistics Research Team, Research Center for Charged Particle Therapy, National Institute of Radiological Science, Chiba, Japan, and* [8]*Department of Medicine and Biosystemic Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan*

Abstract

Objectives. Daily psychological stress has been proposed as a risk factor for systemic lupus erythematosus (SLE) in Western countries. However, there is little information about the relationship between daily psychological stress and the risk of SLE in a Japanese population. We examined the association between SLE and daily psychological stress.

Methods. A case–control study was conducted to examine the relationship between daily psychological stress and SLE in Japanese females. The participants were 160 female SLE patients and 660 female volunteers. Unconditional logistic regression was used to compute OR and 95% confidence interval (CI), with adjustment for several covariates.

Results. Smoking (OR = 2.59; 95% CI, 1.74–3.86), walking (OR = 1.75; 95% CI, 1.81–2.56) and daily psychological stress (OR = 1.88; 95% CI, 1.14–3.10) were increased in patients with SLE after adjusting for age, region and all factors. Smokers with daily psychological stress (OR = 4.70; 95% CI = 2.53–8.77) were more prevalent than nonsmokers without daily psychological stress in SLE. The multiplicative interaction measures between smoking status and daily psychological stress did not reach statistical significance.

Conclusions. The present study suggests the possibility that daily psychological stress as well as smoking might be associated with an increased risk of SLE.

Keywords

Epidemiology, Psychological stress, Risk factors, Smoking, SLE

History

Received 19 December 2012
Accepted 9 May 2013
Published online 31 October 2013

Introduction

The Japanese Ministry of Health and Welfare designated systemic lupus erythematosus (SLE) as an intractable disease because there is no established way to prevent or cure it [1]. SLE is a prototypic systemic autoimmune disease characterized by a broad spectrum of clinical manifestations and diverse immunological disorders [2–4]. Although genetic factors play a role in the development of SLE, environmental factors may also play a role in the development of SLE [2,4]. Thus, interactions between susceptibility genes and environmental factors are responsible for the development of SLE.

Many factors have been proposed as causing or preventing the development of SLE [5–11]. Among these factors, smoking is demonstrated as a risk factor for SLE [5–9,11]. Daily psychological stress is also reported to be related to SLE disease activity [12,13], suggesting that daily psychological factors might be associated with SLE. Furthermore, daily psychological stress is associated with smoking [14]. In our previous study [11], however, daily psychological stress failed to show any meaningful relation to the development of SLE although smoking increased the risk of SLE. This may be partly explained by the small number of SLE patients. Thus, we have continued this case–control study in order to increase the number of SLE patients. The present study was conducted to investigate the influence of daily psychological stress as well as of smoking and other lifestyle factors on SLE.

Correspondence to: Hiroki Takahashi, MD, First Department of Internal Medicine, Sapporo Medical University School of Medicine, S-1, W-16, Chuou-ku, Sapporo 060-8543, Japan. Tel: 81-11-611-2111. Fax: 81-11-611-2282. E-mail: htakahas@sapmed.ac.jp

Methods

Profile of Kyushu Sapporo Systemic lupus erythematosus (KYSS) study

Kyushu Sapporo Systemic lupus erythematosus (KYSS) study was a case–control study in order to evaluate factors associated with SLE, which was supported by a Grant for Research on Measures for Intractable Diseases from the Japanese Ministry of Health, Labour and Welfare. Study methods and ethical issues have been described elsewhere [11]. Briefly, cases were recruited from 2002 in Kyushu (i.e. southwestern edge of Japan with a temperate climate) and from 2004 in Hokkaido (i.e. northern edge of Japan with a subarctic climate). The patients had been followed up at the

Table 1. Characteristics of SLE patients and controls.

	SLE patients $n = 160$	Controls $n = 660$
Regions		
Kyushu	94 (58.8%)	393 (59.6%)
Hokkaido	66 (41.3%)	267 (40.5%)
Gender		
Females	160 (100%)	660 (100%)
Males	0 (0%)	0 (0%)
Age (years old)		
10–19	28 (17.5%)	80 (12.1%)
20–29	56 (35.0%)	278 (42.1%)
30–39	35 (21.9%)	69 (10.5%)
40–49	20 (12.5%)	102 (15.5%)
50–59	10 (6.3%)	127 (19.2%)
60+	11 (6.9%)	4 (0.6%)
Mean ± SD (years old)	32.3 ± 13.8	32.8 ± 14.1

rheumatology clinic of Kyushu University Hospital and Saga University Hospital in Kyushu, and Sapporo Medical University Hospital and Hokkaido University Hospital in Hokkaido. All patients fulfilled the American College of Rheumatology (ACR) 1982 and 1997 revised criteria for SLE [15]. Controls were recruited from nursing college students and care workers in nursing homes in Kyushu, while population controls were recruited from participants of a health checkup in a town in Hokkaido.

Patients and controls completed self-administered questionnaires about their lifestyles before SLE diagnosis and about their current lifestyles, respectively. The patients and controls provided written informed consent to participate in the study and then completed self-administered questionnaires about their lifestyles before SLE diagnosis and current lifestyles, respectively. The self-administered questionnaire provided data about habits such as smoking, alcohol consumption, walking and sleeping. Questions about daily psychological stress determined whether respondents had experienced stress at home or at work before being diagnosed with SLE. The answers "always" and "sometimes" were classified as feeling stress, and "seldom" and "no" as not having stress.

The present study was approved by the institutional review boards of Kyushu University Graduate school of Medical Sciences, Saga Medical School, St. Mary's College, Sapporo Medical University and Hokkaido University School of Medicine.

Subjects and methods in the present study

A self-administered questionnaire was obtained from 362 female SLE patients and 660 female controls. In the present study, we excluded 202 patients treated for SLE more than 10 years. Thus, cases were 160 female patients and controls were 660 female volunteers.

Table 1 displays the region (Kyushu or Hokkaido) and age distributions of the two patients group (age at the diagnosis of SLE) and the control group (age at the survey). Region and age distributions did not differ between two groups.

Unconditional logistic model was applied to evaluate the odds ratios (ORs) and its 95% confidence intervals (CIs) of daily psychological stress or lifestyle-related factors after adjusting for age and other factors. Age was treated as continuous variables, and indicator variables were used for other factors. Ages of controls at the survey were used instead of the diagnosed age of SLE patients. To test for multiplicative interactions smoking status and daily psychological stress, we entered interaction terms reflecting the product of smoking stress into the logistic models. All statistical analyses were conducted using a statistical analysis system package (SAS Institute Inc., Cary, NC). All p-values were two-sided, with those less than 0.05 considered statistically significant.

Results

Table 2 showed the adjusted odds ratios for SLE and 95% CIs in relation to daily psychological stress and lifestyle factors for all participants. Smoking (OR = 2.68; 95% CI, 1.85–3.89), drinking (OR = 1.52; 95% CI, 1.08–2.27), walking (OR = 1.76, 95% CI: 1.20–2.58) and daily psychological stress (OR = 2.06; 95% CI, 1.26–3.67) were increased in patients with SLE after adjusting for age and region. Even after adjusting for age, region and all factors in the table, smoking (OR = 2.59; 95% CI, 1.74–3.86), walking (OR = 1.75; 95% CI, 1.81–2.56) and daily psychological stress (OR = 1.88; 95% CI, 1.14–3.10) were associated with SLE.

Even the analyses of patients treated for SLE for < 5 years (patients, $n = 77$, controls, $n = 660$) showed that odds ratios for smoking (OR, 2.99; 95% CI, 1.78–5.03) and psychological stress (OR, 1.53; 95% CI, 0.79–2.94) were increased over unity after adjusting for age, region and all factors in Table 2 (OR and CI values are not included in the tables).

As shown in Table 3, smoking (OR = 1.91; 95% CI, 1.17–3.13) increased in patients with SLE among the participants in the Kyushu region after adjusting for age. After adjusting for age and all factors in the table, smoking (OR = 1.87, 95% CI: 1.11–3.15) showed a significantly increased OR while walking (OR = 1.61, 95% CI: 0.98–2.65) and daily psychological stress (OR = 1.52, 95% CI: 0.82–2.79) showed marginally increased ORs.

Table 4 illustrated the adjusted odds ratios in patients with SLE and 95% CIs for the participants in the Hokkaido region. Smoking (OR = 4.67, 95% CI: 2.53–8.59), walking (OR = 2.03, 95% CI: 1.10–3.77) and daily psychological stress (OR = 2.91, 95% CI: 1.19–7.15) were increased in patients with SLE after adjusting for age. After controlling for age and all factors in the table, smoking (OR = 4.70, 95% CI: 2.43–9.07) showed a significantly increased OR while walking (OR = 1.90, 95% CI: 0.99–3.64) and daily psychological stress (OR = 2.46, 95% CI: 0.97–6.24) showed a marginally increased ORs.

Daily psychological stress and smoking status in all participants are shown in Table 5. This table also shows the interaction between daily psychological stress and smoking status. Interaction refers to the extent to which the joint effect to two factors differs from the independent effects of each of the factors. Two factors (smoking

Table 2. Lifestyle factors in all participants.

Variables	SLE patients $n = 160$	Controls $n = 660$	Age- and region-adjusted OR (95% CI)	Fully adjusted OR (95% CI)
Smoking (current and ex-smokers)	65 (40.6%)	138 (20.9%)	2.68 (1.85–3.89)*	2.59 (1.74–3.86)*
Drinking (once/week or more)	43 (26.9%)	129 (19.6%)	1.52 (1.08–2.27)*	1.02 (0.65–1.57)
Walk (30 min/day or more)	112 (70.0%)	389 (58.9%)	1.76 (1.20–2.58)*	1.75 (1.81–2.56)*
Sleeping (7 h/day or more)	139 (86.9%)	581 (88.0%)	0.92 (0.55–1.55)	0.97 (0.51–1.64)
Felt daily stress	139 (86.9%)	502 (76.1%)	2.06 (1.26–3.67)*	1.88 (1.14–3.10)*

CI, confidence interval; OR, odds ratio.
Fully adjusted odds ratio: adjusted for age and all factors in the table.
*$p < 0.05$.

Table 3. Lifestyle factors in participants in Kyushu Region.

Variables	SLE patients $n = 94$	Controls $n = 393$	Age-adjusted OR (95% CI)	Fully adjusted OR (95% CI)
Smoking (current and ex-smokers)	32 (34.0%)	81 (20.6%)	1.91 (1.17–3.13)*	1.87 (1.11–3.15)*
Drinking (once/week or more)	21 (22.3%)	66 (16.8%)	1.38 (0.79–2.40)	1.05 (0.58–1.91)
Walk (30 min/day or more)	65 (69.2%)	222 (56.5%)	1.59 (0.97–2.61)	1.61 (0.98–2.65)
Sleeping (7 h/day or more)	80 (85.1%)	331 (84.2%)	1.03 (0.54–1.93)	1.02 (0.54–1963)
Felt daily stress	79 (84.0%)	303 (77.1%)	1.58 (0.87–2.89)	1.52 (0.82–2.79)

CI, confidence interval; OR, odds ratio.
Fully adjusted odds ratio: adjusted for age and all factors in the table.
*$p < 0.05$.

Table 4. Lifestyle factors in participants in Hokkaido Region.

Variables	SLE patients $n = 66$	Controls $n = 267$	Age-adjusted OR (95% CI)	Fully adjusted OR (95% CI)
Smoking (current and ex-smokers)	33 (50.0%)	57 (21.4%)	4.67 (2.53–8.59)*	4.70 (2.43–9.07)*
Drinking (once/week or more)	22 (33.3%)	63 (23.6%)	1.57 (0.86–2.85)	0.86 (0.43–1.71)
Walk (30 min/day or more)	47 (71.2%)	167 (62.6%)	2.03 (1.10–3.77)*	1.90 (0.99–3.64)
Sleeping (7 h/day or more)	59 (89.4%)	250 (93.6%)	0.72 (0.28–1.86)	0.91 (0.34–2.47)
Felt daily stress	60 (90.9%)	199 (74.5%)	2.91 (1.19–7.15)*	2.46 (0.97–6.24)

CI, confidence interval; OR, odds ratio.
Fully adjusted odds ratio: adjusted for age and all factors in the table.
*$p < 0.05$.

and stress) may act independently or together thereby increasing or decreasing the effect of one another. Smokers with daily psychological stress (OR = 4.70, 95% CI = 2.53–8.77) are more prevalent in patients with SLE than nonsmokers without daily psychological stress. The multiplicative interaction measures between smoking status and daily psychological stress did not reach statistical significance.

Discussion

Emotional stress might be suggested to trigger the onset of SLE or to worsen its course in Western countries [16]. The epidemiological study by Minami et al. [12] also suggests that daily psychological factors may play roles not only in the development of SLE but also in health status in Japanese SLE patients. In the present study, daily psychological stress was recognized more frequently in patients with SLE after adjusting for age and region in all participants. The impact of stress on SLE was just as pronounced in patients treated for SLE for periods of ≤ 5 years. This result indicates that stress is positively associated with SLE. Furthermore, when we restricted the analyses to the participants in the Kyushu or the Hokkaido region, daily psychological stress showed marginally increased ORs in Kyushu regions and in Hokkaido regions. Although these findings suggest that daily psychological stress is increased in patients with SLE among Japanese females, the mechanism remains unknown. Psychological factors including stress might alter the immune system through the brain and endocrine system, and one possible explanation would be the activation of prolactin involved in the developmental course of autoimmune disorders [17].

Smoking is demonstrated as a risk factor for SLE [5–9,11] while daily psychological stress is associated with smoking [14]. In the present study, smoking was prevalent in patients with SLE even after the daily psychological stress and other factors. Even in the analyses to the patients treated for SLE less than 5 years, smoking was more prevalent. Furthermore, when we restricted the analyses to the participants in each region, smoking was associated with SLE in the Kyushu region as well as in the Hokkaido region. These findings may support the possibility that smoking might be a risk factor for SLE in Japanese females. The mechanism through which cigarette smoking increases the risk of SLE is unknown. However, cigarette smoke affects a wide range of immunological functions in humans [18,19]. Because reactive oxygen species (ROS) promote the autoimmune response, exposure to ROS through cigarette smoking may increase the risk of SLE [20]. Another possible explanation is that cigarette smoke contains several thousand chemicals, among which about 50, including aromatic amines, are proven carcinogens. Aromatic amines that contain hydrazine are an established cause of drug-induced lupus, and these compounds in cigarette smoke might explain the association between smoking and SLE [21].

Alcohol drinking is suggested to decrease the risk in some studies [6,7,10]. In contrast, drinking was increased in patients with SLE after adjusting for age and region in the present study. However, drinking failed to show any meaningful association with SLE after adjusting for smoking and other factors. These findings may

Table 5. Daily psychological stress and smoking status in all participants.

Stress and smoking status	SLE patients $n = 160$	Controls $n = 660$	Crude OR (95% CI)	Age- and region-adjusted OR (95% CI)
Nonsmokers without stress	15 (9.4%)	131 (19.8%)	1.00 (reference)	1.00 (reference)
Smokers without stress	6 (3.8%)	27 (4.1%)	1.94 (0.69–5.45)	1.95 (0.69–5.50)
Nonsmokers with stress	80 (50.0%)	391 (59.2%)	1.79 (0.99–3.21)	1.74 (0.97–3.12)
Smokers with stress	59 (36.9%)	111 (16.8%)	4.62 (2.50–8.63)*	4.70 (2.53–8.77)*
Multiplicative interaction measure			1.34 (0.44–4.05)	1.39 (0.46–4.20)

CI, confidence interval; OR, odds ratio.
Smokers: current and ex-smokers. Stress: daily psychological stress.
*$p < 0.05$.

be explained by the confounding bias [22], because smokers were more likely to drink than their counterparts [14,23]. In the present study, 86 of 203 smokers drunk once a week or more while only 86 of 617 non-smokers did so (42.4% vs. 13.9%, $p < 0.01$) (data not shown in the table).

Physical activity is reported to reduce the risk of cancer [24,25] and coronary artery disease [26]. However, there is little information about physical exercise and the risk of SLE. Nagai et al. [10] reported that neither outdoor sports nor physical activity had any meaningful association with SLE. In the present study, walking was increased in patients with SLE after adjusting for age and region. Furthermore, even after controlling other factors, walking was associated with SLE among all participants. Furthermore, when we restricted the analyses to the participants in each region, walking showed a non-significantly increased OR in the Kyushu region as well as in the Hokkaido region. Since skin sensitivity to sunlight increases the risk of SLE [9,10], walking may be a surrogate of staying outdoors under the sunlight.

Because both smoking and stress are shown to be significantly associated with SLE in the whole population, we therefore evaluated whether an interaction existed between smoking and stress (Table 5). A smoking–stress interaction was suggested, with smokers with stress conferring significantly higher association with SLE compared to nonsmokers without stress. However, no multiplicative interaction of smoking and stress with SLE was observed (the impact of stress did not differ between smokers and nonsmokers, or the impact of smoking was similar between subjects with stress and those without stress). Two reasons could account for why the multiplicative interaction measures between smoking status and daily psychological stress did not reach statistical significance. One is that no significance actually exists between them, and the other is that the number of patients with SLE was too small to reveal significance. However, the sample size in this study was sufficient to detect significant interaction. Accordingly, these results suggest that smoking status and daily psychological stress do not interact. That is, daily psychological stress would similarly influence SLE development in smokers and non-smokers. Further investigation of the smoking–stress interaction is warranted because of the paucity of smoking–stress interaction studies.

SLE is a chronic inflammatory autoimmune disease [2–4], and subjective sleep quality is reported to influence immunity [27]. These findings suggest that good sleeping habits may reduce the risk of SLE. However, there is little information about the relationship between sleep hygiene and the risk of SLE except the study by Nagai et al. [10], who reported that sleeping 8 h/day or more failed to reduce the risk of SLE. In the present study, sleeping 7 h/day or more failed to show any meaningful relation to SLE.

This study has some limitations. First, cases were not newly diagnosed SLE patients but patients treated for SLE less than 10 years. Second, controls were not randomly selected from general population. Our controls were not free from selection bias because they were not randomly selected from the general population. In Kyushu, controls were recruited from nursing college students and care workers in nursing homes. Their life styles may be different from the general population. However, the risk of smoking for SLE may be underestimated in Kyushu because high smoking prevalence of nursing students and nurses is a serious social problem [28–30]. On the other hand, in Hokkaido, controls were participants in a health check-up in a local town. They may well have had more healthy lifestyles than the general population. Third, we conducted a case–control study in Kyushu (i.e. southwestern edge of Japan with a temperate climate) as well as in Hokkaido (i.e. northern edge of Japan with a subarctic climate). Daily psychological stress was significantly increased in patients with SLE only for all participants, but daily psychological stress showed only a marginally increased OR because of the small number of participants when we restricted the analyses to the participants in each region.

On the other hand, this study has its strength as well. First, the present study demonstrated that daily psychological stress was increased in patients with SLE not only for all participants but also for participants in the different two regions of Kyushu, south-western Japan, and Hokkaido, northern Japan although the association failed to reach significance. Second, the study by Minami et al. [12] is a cross-sectional study although it suggests that daily psychological stress may be associated with an increased risk of SLE in Japanese females. As far as we know, this is the first case–control study suggesting the possibility that daily psychological stress might be associated with an increased risk of SLE in Japanese females.

In conclusion, our study demonstrated that daily psychological stress as well as smoking was associated with SLE for all participants, suggesting the possibility that daily psychological stress might be associated with an increased risk of SLE among Japanese females. However, daily psychological stress failed to show significance when we restricted the analyses to the participants in each of the Kyushu or in the Hokkaido region although it showed an increased OR. Since small number of participants may explain this failure, further studies with larger case population and control will be needed to understand the role of daily psychological stress in SLE.

Acknowledgements

The authors thank Takasu Town, Hokkaido Prefecture, and its town people for their kind cooperation.

Funding

This work was supported in part by Health and Labour Sciences Research Grants, Research on Intractable Diseases, and the Research Committee on Epidemiology of Intractable Diseases from the Ministry of Health, Labour, and Welfare, Japan (Chief: Yutaka Inaba, 2002-04 and Masaki Nagai, 2004-10).

Conflict of interest

None.

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Appendix

Members of the Kyushu Sapporo SLE (KYSS) Study Group were listed in alphabetical order for each affiliation.

Saburo Ide, Hiroko Kodama, Masakazu Washio [principal investigator] (St. Mary's College); Koichi Akashi, Mine Harada, Takahiko Horiuchi [co-principal investigator], Chikako Kiyohara [co-principal investigator], Hiroshi Tsukamoto (Graduate School of Medical Sciences, Kyushu University); Toyoko Asami, Takao Hotokebuchi, Kohei Nagasawa, Yoshifumi Tada, Osamu Ushiyama (Faculty of Medicine, Saga University); Mitsuru Mori, Asae Oura, Yasuhisa Sinomura, Hiromu Suzuki, Hiroki Takahashi, Motohisa Yamamoto (Sapporo Medical University School of Medicine); Gen Kobashi (Research Center for Charged Particle Therapy, National Institute of Radiological Science); Takashi Abe (Kushiro City General Hospital); Hisato Tanaka (Tanaka Hospital); Tatsuya Atsumi, Tetsuya Horita, Shinsuke Yasuda (Hokkaido University Graduate School of Medicine); Norihiko Nogami (Wakakusury-ouikuen Hospital); Kazushi Okamoto (Aichi Prefectural College of Nursing and Health); Naomasa Sakamoto (Hyogo College of Medicine); Satoshi Sasaki (School of Public Health, the University of Tokyo); Yoshihiro Miyake (Faculty of Medicine, Fukuoka University); Tetsuji Yokoyama (National Institute of Public Health); Masayo Oumi (Nakamura Gakuen University); Yoshio Hirota (Osaka City University); Yutaka Inaba (Juntendo University School of Medicine); Masaki Nagai (Saitama Medical School).

Rheumatol Int (2014) 34:1225–1230
DOI 10.1007/s00296-014-2951-0

SHORT COMMUNICATION

Comparative analysis of different enzyme immunoassays for assessment of phosphatidylserine-dependent antiprothrombin antibodies

Olga Amengual · Tetsuya Horita · Walter Binder · Gary L. Norman · Zakera Shums · Masaru Kato · Kotaro Otomo · Yuichiro Fujieda · Kenji Oku · Toshiyuki Bohgaki · Shinsuke Yasuda · Tatsuya Atsumi

Received: 26 November 2013 / Accepted: 22 January 2014 / Published online: 5 February 2014

Abstract Phosphatidylserine-dependent antiprothrombin antibodies (aPS/PT) were strongly correlated with the presence of lupus anticoagulant showing a high specificity for the diagnosis of antiphospholipid syndrome. However, the main criticism for the clinical applicability of aPS/PT testing is the lack of reproducibility of the results among laboratories. In this study, we measured IgG and IgM aPS/PT using our original in-house enzyme-linked immunosorbent assays (ELISA) and commercial ELISA kits to assess the assay performance and to evaluate the accuracy of aPS/PT results. The study included 111 plasma samples collected from patients and stored at our laboratory for aPS/PT assessment. Sixty-one samples were tested for IgG aPS/PT using two assays: (1) aPS/PT in-house ELISA and (2) QUANTA Lite™ aPS/PT IgG ELISA kit (INOVA Diagnostics, Inc., USA). Fifty samples were evaluated for IgM aPS/PT using two assays: (1) aPS/PT in-house ELISA and (2) QUANTA Lite™ aPS/PT IgM ELISA kit (INOVA Diagnostics). Ninety-eight percent of samples yielded concordant results for IgG aPS/PT and 82 % for IgM aPS/PT. There was an excellent agreement between the IgG aPS/PT assays (Cohen $\kappa = 0.962$) and moderate agreement between the IgM aPS/PT assays ($\kappa = 0.597$). Statistically significant correlations in the aPS/PT results were obtained from both IgG and IgM aPS/PT assays ($r = 0.749$, $r = 0.622$, $p < 0.001$, respectively). In conclusion, IgG and IgM detection by ELISA is accurate. The performance of aPS/PT is reliable, and concordant results can be obtained using different ELISA methods.

Keywords Antiphospholipid syndrome · Antiphospholipid antibodies · Thrombosis · Lupus anticoagulant

O. Amengual (✉) · T. Horita · M. Kato · K. Otomo · Y. Fujieda · K. Oku · T. Bohgaki · S. Yasuda · T. Atsumi
Division of Rheumatology, Endocrinology and Nephrology, Hokkaido University Graduate School of Medicine, N15 W7, Kita-ku, Sapporo 060-8638, Japan
e-mail: olgaam@med.hokudai.ac.jp

W. Binder · G. L. Norman · Z. Shums
INOVA Diagnostics, Inc., San Diego, CA, USA

Introduction

Antiphospholipid antibodies (aPL) are a heterogeneous group of antibodies detected in patients with antiphospholipid syndrome (APS). Lupus anticoagulant (LA), detected by clotting assays, anticardiolipin antibodies (aCL) and antiβ2glycoprotein I (β2GPI) antibodies, detected by enzyme-linked immunosorbent assay (ELISA), are the laboratory tests included in the current classification criteria for definite APS [1]. However, a number of issues regarding the laboratory criteria for the diagnosis of APS are still under debate.

Antibodies against prothrombin, one of the major antigen targets for aPL, are frequently found in patients with APS. The antiprothrombin antibody family comprises two types of antibodies: those detected by ELISA using prothrombin alone as the target antigen (aPT-A) and those directed against phosphatidylserine/prothrombin complexes, the so-called phosphatidylserine-dependent antiprothrombin antibodies (aPS/PT) [2]. Numerous studies have investigated the implications of aPT-A in the clinical manifestation of APS with controversial results [3–6]. On the other hand, several groups have reported that the presence of aPS/PT strongly correlates with that of LA and that aPS/PT were highly specific for the diagnosis of APS [7–11]. However, the clinical applicability and diagnostic utility

of aPS/PT testing have not yet been fully defined mainly as a result of the lack of a standardized procedure to test aPS/PT and the low reproducibility of the results between laboratories.

In this study, we aimed to assess the performance of two different ELISA methods, our original in-house assay [7] and commercial kits, to detect aPS/PT and to evaluate the reproducibility of the aPS/PT assay.

Materials and methods

The study comprised 111 patients' plasma samples submitted to our Department of Rheumatology for aPL assessment. Clinical/demographic data and laboratory findings were retrospectively extracted from medical records.

The study was performed in accordance with the Declaration of Helsinki and the Principles of Good Clinical Practice. Approval was obtained from the Local Ethics Committee.

Plasma samples

Venous blood was collected into tubes containing a one-tenth volume of 0.105 M sodium citrate and was centrifuged immediately at 4 °C. Plasma samples were depleted of platelets by filtration then stored at −80 °C.

Phosphatidylserine-dependent antiprothrombin antibody assays

In-house aPS/PT ELISA

For the detection of aPS/PT antibodies, an in-house ELISA was performed as previously described [7]. Briefly, non-irradiated microtiter plates (Sumilon type S, Sumitomo Bakelite, Tokyo, Japan) were coated with 30 μl of 50 μg/ml phosphatidylserine (Sigma Chemical Co., St. Louis, USA) and dried overnight at 4 °C. To avoid non-specific binding of proteins, wells were blocked with 150 μl of Tris-buffered saline (TBS) containing 1 % fatty acid-free bovine serum albumin (BSA, A-6003, Sigma) and 5 mM $CaCl_2$ (BSA–Ca). After three washes in TBS containing 0.05 % Tween 20 (Sigma) and 5 mM $CaCl_2$ (TBS–Tween–Ca), 50 μl of 10 μg/ml human prothrombin (Diagnostica Stago, Asnieres, France) in BSA–Ca was added to half of the wells in the plates and the same volume of BSA–Ca alone (as sample blank) was added to the other half. After 1 h incubation at 37 °C, plates were washed and 50 μl of serum diluted in BSA–Ca in 1:100 were added in duplicate. Plates were incubated for 1 h at room temperature, followed by alkaline phosphatase (ALP)-conjugated goat antihuman IgG or IgM. After 1 h incubation at room temperature and four washes, 100 μl/well of 1 mg/ml 4-nitrophenylphosphate disodium (Sigma) in 1 M diethanolamine buffer (pH 9.8) were added. In all the assays, the samples were run in parallel on the phosphatidylserine-/prothrombin-coated wells and in wells coated only with phosphatidylserine. Results were expressed as final optical density (OD) corresponding to OD detected in phosphatidylserine-/prothrombin-coated wells minus OD detected in phosphatidylserine-alone-coated wells. The aPS/PT titer of each sample was derived from the standard curve according to dilutions of the positive control. Normal ranges of IgG (>2.0 units) and IgM (>9.6 units) aPS/PT were previously established using the 99th percentile of the levels in non-pregnant 132 healthy controls as the cut-off values.

aPS/PT commercial ELISAs

Samples were tested using two commercial ELISA kits from INOVA Diagnostics, Inc., San Diego, CA, USA (INOVA kits). QUANTA Lite™ aPS/PT IgG ELISA was used for the detection of IgG aPS/PT and QUANTA Lite™ aPS/PT IgM ELISA for IgM aPS/PT detection. aPS/PT testing was performed according to the manufacturer's instructions. Positive cut-off values for the IgG and IgM ELISA kits were set up by the manufacturer's as >30 units.

All the aPS/PT ELISAs were performed by the same person in our laboratory.

Statistical analysis

Statistical evaluation was carried out by Fisher's exact test or χ^2 test, as appropriate. Cohen's kappa test was applied to compare the results obtained using different tests in the same sample. The diagnostic accuracy of the assays was assessed by receiver operating characteristic (ROC) curve analysis. p values <0.05 were considered significant. All statistical analyses were performed using SPSS (Chicago, IL, USA).

Results

The 111 plasma specimens belonged to 87 patients, 72 woman and 15 men with a mean age of 46 years (range 23–70 years). Forty-seven patients (54 %) were diagnosed as having APS according to the classification criteria for definite APS [1], 20 patients had primary APS and in 27 patients systemic lupus erythematous (SLE) was diagnosed in association with APS. Seventeen patients (20 %) had SLE, 20 patients (23 %) other autoimmune diseases and three patients (3 %) had positive aPL in the absence of any diseases.

Twenty-four patients (28 %) had history of arterial thrombotic events, 19 venous thrombosis (40 %) and 10 females had history of pregnancy complications (14 %).

IgG/IgM aCL, IgG/IgM antiβ2GPI antibodies and LA were found in 36 (41 %), 34 (39 %) and 61 (70 %) patients, respectively.

Sixty-one samples from 58 patients were tested for IgG aPS/PT, and 50 samples from 48 patients were assayed for IgM aPS/PT. Samples were selected to be tested in the IgG or in the IgM aPS/PT ELISA based on previous data obtained with our in-house aPS/PT ELISA. We analyzed the results for each sample at the first determination in the in-house and commercial assays. Ninety-eight percent of samples yielded concordant results for IgG aPS/PT, while 82 % samples displayed concordant IgM aPS/PT results (Table 1). One sample displayed discrepant results for IgG aPT/PT, and 9 samples were discrepant for IgM aPS/PT results (Table 2). There was an excellent agreement between the IgG aPS/PT assays ($\kappa = 0.962$) and a moderate agreement in the IgM aPS/PT assays ($\kappa = 0.597$).

There was a statistically significant correlation in the OD values and units of IgG aPS/PT, as well as IgM aPS/PT, obtained with homemade and commercial ELISAs (Pearson's correlation coefficients: $r = 0.835$, $r = 0.749$ and $r = 719$, $r = 0.622$, $p < 0.001$, for IgG aPS/PT and IgM aPS/PT, respectively) (Fig. 1).

The distribution of aPS/PT and classical aPL in 58 patients tested for IgG aPS/PT and in 48 patients tested for IgM aPS/PT is shown in Table 3.

We performed ROC analysis and evaluated the sensitivity, specificity, likelihood ratio positive and likelihood ratio negative of the aPS/PT assays for the diagnosis of APS. The area under the curve (AUC) values were 0.799 and 0.808 for in-house and INOVA IgG aPS/PT assays and 0.791 and 0.705 for in-house and INOVA IgM aPS/PT assays, respectively (Fig. 2).

The sensitivity, specificity, likelihood ratio positive and likelihood ratio for APS diagnosis were 93.6 %, 59.3 %, 2.30, 0.11, 93.6 %, 63.0 %, 2.53, 0.10, 75.0 %, 84.6 %, 2.33, 0.24 and 88.5 %, 50.0 %, 1.77, 0.23 for IgG aPS/PT in-house ELISA, IgG INOVA ELISA, IgM aPS/PT in-house ELISA and IgM INOVA ELISA, respectively.

Discussion

In this manuscript, we assessed the performance of two ELISA methods to determine aPS/PT showing that the detection of aPS/PT is accurate.

Antiprothrombin antibody family is commonly detected by ELISA-based methods. ELISAs using gamma-irradiated plates coated with prothrombin reveal aPT-A [12] and ELISAs in which prothrombin is exposed to immobilized phosphatidylserine identified aPS/PT [7]. The good correlation between aPS/PT ELISA and LA supports the use of aPS/

Table 1 Detection of aPS/PT

	Tested samples	Concordant positive results (%)	Concordant negative results (%)	Total concordance (%)
IgG aPS/PT assays[a]	61	41 (67)	19 (31)	60 (98)
IgM aPS/PT assays[b]	50	29 (58)	12 (24)	41 (82)

[a] In-house IgG aPS/PT ELISA, IgG aPS/PT INOVA ELISA kit

[b] In-house IgM aPS/PT ELISA, IgM aPS/PT INOVA ELISA kit

Table 2 Discrepant results using in-house ELISAs and commercial ELISAs

ELISA isotype	Sample	In-house ELISA		INOVA ELISA		Diagnosis	LA
		U	Results	U	Results		
IgG			(cut-off > 2 U)		(cut-off > 30 U)		
	S-1	2.9	(low +)	21.5	(−)	SLE	(−)
IgM			(cut-off > 9.6 U)		(cut-off > 30 U)		
	S-2	12.5	(low +)	5	(−)	ITP	(−)
	S-3	15.5	(low +)	14.5	(−)	PAPS	(+)
	S-4	7.2	(−)	130	(high +)	PAPS	(+)
	S-5	3.5	(−)	125	(high +)	APS/SLE	(+)
	S-6	<1.5	(−)	140	(high +)	aPL (+) only	(+)
	S-7	2.7	(−)	49	(low +)	SLE	(−)
	S-8	8.8	(−)	37	(low +)	RA	(+)
	S-9	9	(−)	72	(low +)	APS/SLE	(+)
	S-10	<1.5	(−)	32	(low +)	RA	(−)

LA lupus anticoagulant, *SLE* systemic lupus erythematosus, *ITP* idiopathic thrombocytopenic purpura, *PAPS* primary antiphospholipid syndrome, *aPL* antiphospholipid antibodies, *RA* rheumatoid arthritis, *S* sample number, *U* units, (+) positive results, (−) negative results

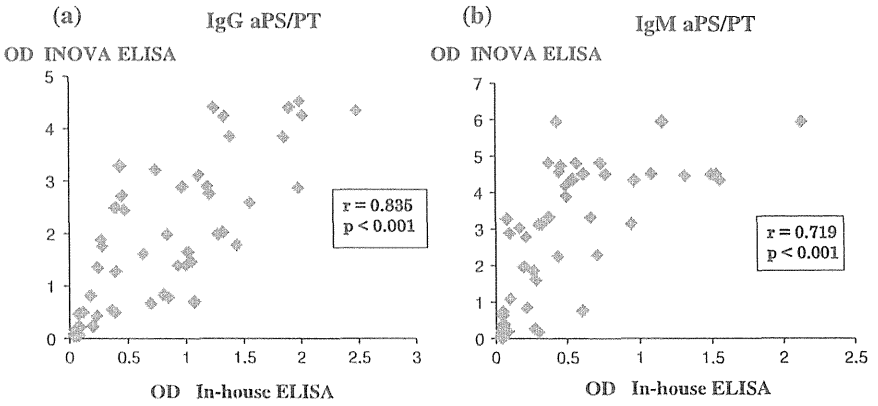

Fig. 1 Correlation of the optical density values of aPS/PT obtained with in-house ELISAs and commercial ELISAs. a Correlation of the IgG aPS/PT optical density (OD) values between INOVA ELISA kit and in-house ELISA. b Correlation of the IgM aPS/PT OD values between INOVA ELISA kit and in-house ELISA. There was a statistically significant correlation in the aPS/PT OD values obtained with both IgG and IgM ELISAs. *aPS/PT* phosphatidylserine-dependent antiprothrombin antibodies

Table 3 Profile of antiphospholipid antibodies in the population analyzed

	APS (%)	Non-APS (%)	χ^2	*p* value
aPS/PT IgG				
Tested patients $N = 58$	31 (53)	27 (47)		
aPS/PT IgG in-house	29 (50)	11 (20)	18.80	<0.001
aPS/PT IgG INOVA	29 (50)	10 (17)	20.92	<0.001
aCL (IgG/M)	14 (24)	5 (9)	4.65	n.s
Antiβ2GPI antibodies (IgG/M)	12 (21)	8 (14)	0.53	n.s
LA	30 (52)	7 (12)	31.34	<0.001
aPS/PT IgM				
Tested patients $N = 48$	26 (54)	22 (46)		
aPS/PT IgM in-house	22 (46)	8 (17)	11.84	0.001
aPS/PT IgM INOVA	23 (48)	11 (23)	8.53	0.005
aCL (IgG/M)	18 (24)	4 (8)	12.51	0.001
Antiβ2GPI antibodies (IgG/M)	14 (29)	6 (13)	3.46	n.s
LA	25 (52)	8 (17)	19.83	<0.001

APS antiphospholipid syndrome, *aCL* anticardiolipin antibodies, *β2GPI* β2Glycoprotein I, *IgG/M* IgG and/or IgM positive, *LA* lupus anticoagulant, χ^2 Pearson's χ^2 test, *n.s* no significant

PT as one of the "screening" or "confirming" assays for APS-associated LA [11, 13]. Moreover, aPS/PT are associated with the clinical manifestations of APS [11, 14, 15], and the determination of aPS/PT would potentially contribute to a better recognition of APS, especially in cases of suspected APS, but without evidence of aCL, antiβ2GPI antibodies or LA [16].

The major limitation of aPS/PT determination is the lack of standardization of the aPS/PT ELISA [17]. In this study, we aimed to evaluate the accuracy of the results using different aPS/PT ELISAs that are currently used to detect aPS/PT. We analyzed the results obtained for each samples at the first determination in the in-house and commercial ELISAs and observed a high agreement, indicating that aPS/PT results are precise.

For the IgG aPS/PT assays, we found discrepant results for only one sample. This sample was obtained from a patient with SLE and displayed low-positive IgG aPS/PT titer in the in-house ELISA but negative result in the INOVA test (21.5 units, cut-off >30). The sample was re-tested, and the low-positive result in the in-house assay was confirmed. The differences in the interpretation of this specimen are likely due to the cut-off selected for each assay. Although the INOVA result was interpreted as negative, it fell in the upper portion of the normal range.

For the IgM aPS/PT assays, nine samples displayed discrepant results. Three samples (S-2, S-9, S-10, Table 2) became negative after re-testing, implying false-positive aPS/PT results at the first determination. Therefore, true discrepancy for the IgM aPS/PT assay was detected in 6 out of 50 samples leading to 88 % concordance. The discrepant results in the IgM aPS/PT assays could be explained by the different methodologies used in each ELISA systems. In the commercial kits, plastic microtiter plate wells were coated with purified phosphatidylserine/prothrombin complexes and then stabilized. In the in-house assay, plastic microtiter plate wells were coated with phosphatidylserine and dried overnight at 4 °C. Prothrombin was then added, and samples were run in parallel on the phosphatidylserine-/prothrombin-coated wells and in wells coated only with phosphatidylserine. In the commercial ELISA, the presence or absence of aPS/PT is determined by measuring and comparing the direct color intensity that develops in the sample wells with that of

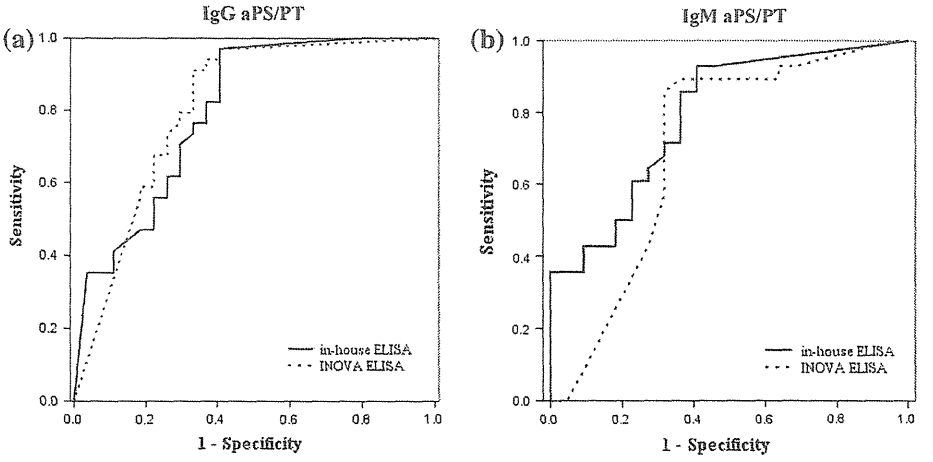

Fig. 2 Receiver operating characteristic (ROC) curves for the diagnosis of APS. a IgG aPS/PT assays. The area under the ROC curve were 0.799 and 0.808 [95 % confidence interval (95 % CI) 0.685–0.913 and 0.690–0.926] for in-house ELISA and INOVA ELISA, respectively. b IgM aPS/PT assays. The area under the ROC curve were 0.791 and 0.705 (95 % CI 0.666–0.917 and 0.545–0.866) for in-house ELISA and INOVA ELISA, respectively

a calibration curve. However, in the in-house assay, the presence of aPS/PT is determined by measuring the color intensity that developed in the sample phosphatidylserine-coated wells and subtracted from the color intensity that developed in the sample phosphatidylserine-/prothrombin-coated wells. Subtracted color intensity is compared with that of a calibration curve. Interestingly, samples with positive IgM aPS/PT results only in the commercial ELISA showed a high binding to phosphatidylserine-alone-coated wells in the in-house assay, suggesting that the positive binding might be related to IgM binding to phosphatidylserine rather than true binding to phosphatidylserine/prothrombin complexes. The aPS/PT false-positive pattern, i.e., IgM binding positive to phosphatidylserine alone but negative to aPS/PT, could be found in some cases, but the clinical significance needs to be clarified. In addition, in general, IgM ELISAs show more variation compared with IgG assays presumably due to the low-affinity characteristics of the IgM isotype.

We observed high correlations between the IgG or IgM results from both assays, implying that accurate results can be obtained using IgG and IgM aPS/PT available ELISAs.

The APS score [18, 19] and GAPSS [20] showed that multiple positivity for aPL will increase the thrombotic risk. Testing aPS/PT would be useful for the prediction of the thrombotic risk in patients with APS.

We believe that our findings add additional evidence suggesting that aPS/PT testing should be considered as a tool for the diagnosis of APS. Standardization of aPS/PT assay is a necessary step for the worldwide implementation of aPS/PT testing and will potentially lead to the inclusion of aPS/PT as one of the laboratory criteria for the APS classification and to a better identification of patients with APS.

In conclusion, our data demonstrated that the performance of the currently available aPS/PT assays is accurate and reliable.

Acknowledgments The authors thank Miki Aoto for the technical support. This work was supported by the Japanese Ministry of Health, Labour and Welfare, the Japanese Ministry of Education, Culture, Sports, Science and Technology (MTX) and the Japanese Society for the promotion of the Science (JSPS).

Conflict of interest Gary L Norman and Zakera Shums are employees of INOVA Diagnostics, Inc., San Diego, USA. Walter Binder was an employee of INOVA Diagnostics at the time of the study. The other authors declare that they have no conflict of interest.

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Lupus (2014) 23, 1124–1132
http://lup.sagepub.com

PAPER

Long-term outcome in Japanese patients with lupus nephritis

M Kono[1], S Yasuda[1], M Kato[1], Y Kanetsuka[1], T Kurita[1], Y Fujieda[1], K Otomo[1], T Horita[1], K Oba[2], M Kondo[3], M Mukai[3], M Yanai[4], Y Fukasawa[4] and T Atsumi[1]

[1]Division of Rheumatology, Endocrinology and Nephrology, Hokkaido University Graduate School of Medicine, Sapporo, Japan; [2]Translational Research and Clinical Trial Center, Hokkaido University Hospital, Sapporo, Japan; [3]Department of Rheumatology, Sapporo City General Hospital, Sapporo, Japan; and [4]Department of Pathology, Sapporo City General Hospital, Sapporo, Japan

The objective of this study was to clarify the long-term outcome in patients with lupus nephritis (LN) according to the International Society of Nephrology and Renal Pathology Society classification. This retrospective analysis comprised 186 Japanese patients given a diagnosis of LN by renal specimen with a mean observation period of 12 years. Primary end point was defined as death or end-stage renal disease, and standardized mortality ratios were calculated. Five patients presented with histopathological class I, 62 with II, 21 with III or III+V, 73 with IV or IV+V and 25 with V. Fourteen deaths occurred, corresponding to an overall standardized mortality ratio of 3.59 (95% confidence interval 2.02–5.81, $p < 0.0001$). Kaplan-Meier analysis revealed a 10-year overall survival of 95.7%. Nephrotic proteinuria (≥3.5 g/day) at baseline was identified as an independent poor prognostic factor for overall survival in Cox regression analysis. Kaplan-Meier analysis revealed a 10-year renal survival as 94.3%. Male gender and nephrotic proteinuria at baseline were identified as independent poor prognostic factors for renal survival in Cox regression analysis. In conclusion, LN was associated with a 3.59-fold increase in mortality compared with the general population. Male gender and nephrotic proteinuria were predictive for poor renal outcome. *Lupus* (2014) **23**, 1124–1132.

Key words: Nephritis; renal lupus; systemic lupus erythematosus

Introduction

Lupus nephritis (LN) is one of the most important manifestations in patients with systemic lupus erythematosus (SLE). Renal histopathological classification of LN is essential to guide treatment and predict prognosis of patients complicated with LN. Since the first classification of LN issued by the World Health Organization (WHO) in 1974,[1] revision was made in 1982[2] and 1995.[3] The 2003 International Society of Nephrology and Renal Pathology Society (ISN/RPS) classification[4] is currently used for a number of clinical trials. Aggressive immunosuppressive therapy has improved the prognosis of the patients with LN. However, 5% to 20% of LN patients progress to end-stage renal disease (ESRD) within 10 years after the diagnosis of LN.[5–8]

There are ethnic or regional variations in the prevalence and prognosis of LN. The cumulative incidence of LN is higher in people of Asian (55%), African (51%) and Hispanic (43%) ancestry compared with Caucasians (14%).[7] Some studies have reported black and Hispanic patients with LN are more likely to have a poorer prognosis than other racial groups.[9–16] Many reports showed there were ethnic or regional variations in the prognosis of LN; however, there were ethnic or regional variations in the expected number of deaths, too. Therefore, standardized mortality ratios (SMRs) are a more accurate method of survival evaluation than survival rates. Few studies, however, have investigated a long-term outcome and SMRs in patients with LN.[17–19] In fact, there is no study of calculated SMRs and long-term sustained renal remission rate, with mean observation period over 10 years, using the ISN/RPS classification.

In the present study, we examined the mortality and occurrence of ESRD in more than 180 Japanese patients with histologically proven LN with a mean duration of approximately 12 years.

Correspondence to: Shinsuke Yasuda, Division of Rheumatology, Endocrinology and Nephrology, Hokkaido University Graduate School of Medicine, N15 W7, Kita-ku, Sapporo, Japan.
Email: syasuda@med.hokudai.ac.jp
Received 9 September 2013; accepted 24 April 2014

10.1177/0961203314536246

SMRs in our cohort against that in the general Japanese population were calculated. We also identified risk factors for mortality and ESRD among clinical parameters and histological findings according to the ISN/RPS classification, renal response and sustained renal remission.

Patients and methods

The present study was a retrospective cohort study of Japanese patients with LN, conducted in a single center at Hokkaido University Hospital in Sapporo. Our study comprised consecutive 187 Japanese patients given a diagnosis of LN by histopathological analyses of renal specimens between January 1984 and March 2010. All of these LN patients fulfilled the 1997 revised SLE classification criteria of the American College of Rheumatology.[20] Only one patient was excluded from the current analysis at entry for insufficient clinical data.

The following data were evaluated at the time of biopsy: sex, age, time at onset of SLE, serum creatinine level, serum albumin level, C3, C4, CH50, serum anti-DNA antibodies, proteinuria and Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2 K).[21] Serum creatinine levels, serum albumin levels, proteinuria, urinary sediments and casts were evaluated for the analyses of renal response and renal flare during the observation period. Data for renal response and renal flare during follow-up were available for 174 patients as 12 patients were excluded for insufficient clinical data. Data on the first induction therapy and maintenance therapy used for the treatment of LN were available for 168 patients; six patients were excluded because of missing data.

Definition of renal outcome

The primary end point was defined as death or ESRD. ESRD was defined when the patient was introduced with permanent hemodialysis and/or renal transplantation. Complete renal response (CR) was defined as proteinuria <0.5 g/gCr and normal or near normal (within 10% of normal glomerular filtration rate (GFR) if previously abnormal) GFR in the first year after the first biopsy.[22] Partial renal response (PR) was defined as ≥50% reduction in proteinuria to subnephrotic levels (<3.5 g/gCr) and normal or near-normal GFR. Renal flare was defined as nephritic or proteinuric flares following response. Nephritic flares included reproducible increase of serum creatinine by ≥30% (or decrease in GFR by ≥10%) and active urine sediment with increase in glomerular hematuria by ≥10 red blood cells per high-power field, irrespective of changes in proteinuria. Proteinuric flares included reproducible doubling of proteinuria to >1.0 g/gCr after CR or reproducible doubling of proteinuria to >2.0 g/gCr after PR.[22] Sustained renal remission was defined as no renal flare after renal response during the period of this study. Hypertension was defined as a systolic blood pressure of ≥140 mm Hg, a diastolic blood pressure of ≥90 mm Hg or antihypertensive medication use except use only to decrease proteinuria at baseline. Diabetes was defined as the use of antidiabetic medication or an increased level of hemoglobin A1c ≥6.5% at baseline.

Renal histopathological analyses

Renal biopsy samples were histologically re-classified according to the ISN/RPS criteria by experienced pathologists blinded to clinical information. If patients underwent renal biopsy more than once, classifications at the first biopsy were used. Activity and chronicity index were calculated using the scoring systems of the United States National Institutes of Health (NIH).[23]

Statistical analyses

Follow-up began at the date of the first renal biopsy, continued until ESRD (in analyses of renal outcome) or death, if they experienced death or ESRD, whichever came first, and censored at their final visits or March 31, 2011.

The expected number of deaths in our cohort was calculated on the basis of national age-, sex- and calendar-year period-specific mortality rates provided by the Japanese Ministry of Health, Labor and Welfare. Multiplication of person-years under observation for the patients by the appropriate national sex-specific mortality rates in five-year age groups and five-year calendar-year periods yielded the expected number of deaths in this cohort. SMRs were calculated as the ratio between the observed and the expected number of deaths. Corresponding 95% confidence intervals (95% CIs) were calculated based on the assumption that the observed number of deaths followed a Poisson distribution.

Cumulative incidences of death and ESRD were determined using the Kaplan-Meier method. The log-rank tests were used to evaluate the prognostic importance on survival or renal survival of baseline factors that were available in this study and were previously reported to be of prognostic importance

in LN, including age, gender, baseline serum creatinine level, serum albumin level (≤3.0 mg/dl versus >3.0 mg/dl), CH50, proteinuria (<3.5 g/day versus ≥3.5 g/day) and SLEDAI-2K, ISN/RPS classification (ISN/RPS class IV or IV + V versus the others), activity index, chronicity index and hypertension. Non-CR was added to these factors as a factor to evaluate the prognostic importance on sustained remission. The following factors were classified into two categories by median (serum creatinine level <0.8 mg/dl versus ≥0.8 mg/dl, CH50 <22 U/ml versus ≥22 U/ml, SLEDAI-2K ≤14 versus >14, and activity index ≤one versus >one). Chronicity index was classified into two categories (<one versus ≥one) because the median of the chronicity index was 0 and the mean was 0.49. Previously known prognostic factors for overall survival or renal survival in LN (age, gender and serum creatinine level) were included in all multivariate Cox proportional hazard models regardless of their significance in the log-rank tests. Because the number of patients and events in our study was limited, we decided to select the covariates to analyze properly. Factors other than these known prognostic factors were included in multivariate models if the p value was <0.2 in the log-rank test. Factors that were strongly associated with other prognostic factors were excluded from the multivariate models. The Mann-Whitney rank sum test was used for comparison of continuous data, while the Fisher's exact test was used for comparison of categorical variables. In all analyses, p values of less than 0.05 defined statistical significance. The SMR calculations were carried out using the PROC GENMOD procedure in SAS, version 9.3 (SAS Institute). All other analyses were performed using SPSS, version 18.0 (SPSS).

Results

Patient characteristics

Clinical features and laboratory and histological findings at baseline are summarized in Table 1. All 186 patients were Japanese, of them 150 were women. Median age at the time of first renal biopsy was 31 (range 9–65) years old. The mean and median observation periods after the first biopsy were 12.2 ± 7.2, and 12.0 (0.2–26.9) years, respectively. Data at biopsy and histopathological findings according to the ISN/RPS classification are included in Table 1. Forty-one patients had their first biopsy between 1984 and 1989, 83 between 1990 and 1999, and 62 between 2000 and 2010. Ten patients had diabetes mellitus and 41 patients had hypertension at baseline.

Table 1 Baseline data of 186 Japanese patients with lupus nephritis at renal biopsy

	Value
Gender, female, *n* (%)	150 (80.1)
Age at SLE diagnosis, years	27 (9–65)
Age at the time of biopsy, years	31 (12–65)
Observation period, years	12.2 ± 7.2
Serum albumin, mg/dl	3.23 ± 0.80
Serum creatinine, mg/dl	0.75 ± 0.45
C3, mg/dl	48.0 ± 22.9
C4, mg/dl	10.7 ± 8.4
CH50, U/ml	22.2 ± 16.0
anti-DNA, IU	34.5 (0–3732)
Proteinuria, g/day	1.2 (0–14.9)
SLEDAI-2K	15.9 ± 7.4
ISN/RPS classification	
Class I, *n* (%)	5 (2.7)
Class II, *n* (%)	62 (33.3)
Class III, *n* (%)	16 (8.6)
Class III+V, *n* (%)	5 (2.7)
Class IVS, *n* (%)	6 (3.2)
Class IVG, *n* (%)	54 (29.0)
Class IV+V, *n* (%)	13 (7.0)
Class V, *n* (%)	25 (13.4)
Activity index score	1 (0–15)
Chronicity index score	0 (0–6)

Data are expressed as median (minimum–maximum), mean ± S.D. or percentage. Normal ranges of C3, C4, and CH50 are 86–160, 17–45, and 31.5–48.4, respectively. SLE: systemic lupus erythematosus; SLEDAI-2K: Systemic Lupus Erythematosus Disease Activity Index 2000; ISN/RPS: International Society of Nephrology/Renal Pathology Society.

We administrated intravenous cyclophosphamide (IVCY) in combination with corticosteroids for induction of remission in most of the patients with class III, III+V, IV or IV+V after the later 1980s. Fifty-one patients were treated with IVCY and corticosteroids for induction, two patients with daily oral cyclophosphamide and corticosteroids, and one patient with mycophenolate mofetil and corticosteroids. Only four patients didn't receive either high-dose corticosteroids monotherapy or immunosuppressants in combination with corticosteroids. Eleven patients received IVCY during the period of study after induction therapy for renal flare or other lupus flare, and two received daily oral cyclophosphamide. Sixty-nine patients received immunosuppressants for maintenance therapy. The total number of patients who can be accommodated was 42 of tacrolimus, 21 of azathioprine, 20 of cyclosporine, and 18 of mizoribin. Some patients received several

immunosuppressants during other periods, or a combination of two immunosuppressants.

Mortality and risk factors

Fourteen deaths occurred during the observation period, corresponding to an overall SMR of 3.59 (95% confidence interval (95% CI) 2.02–5.81, $p < 0.0001$). The SMR estimates were 4.10 (95% CI 2.13–7.03, $p < 0.001$) and 2.45 (95% CI 0.61–6.37, $p = 0.120$) for male patients and female, respectively. Kaplan-Meier analysis revealed five- and 10-year survival rates of 97.2% and 95.7%, respectively (Figure 1(a)). One of the dead patients presented with class I, four with class II, one with class III+V, six with class IV, one with class IV+V and one with class V (Table 2). The causes of death are illustrated in Table 2. Infection corresponded to 50% of the cause of deaths, suicide to 14%, acute myocardial infarction to 14% and stroke to 7%. As a result of the log-rank test, proteinuria ≥3.5 g/day ($p = 0.004$) and serum albumin level ≤3.0 mg/dl ($p = 0.005$) were identified as risk factors for poor renal prognosis. SLEDAI-2 K > 14 ($p = 0.16$), class IV or IV+V ($p = 0.10$), activity index > one ($p = 0.29$), chronicity index ≥one ($p = 0.16$) and the other factors were not identified as risk factors for poor renal prognosis. Since there was a strong association between serum albumin level and proteinuria (Pearson's test, $|r| = 0.51$, $p < 0.0001$) and between SLEDAI-2 K and ISN/RPS classification (Mann-Whitney rank sum test, ISN/RPS class IV or IV+V versus the others, 20.2 versus 12.9, $p < 0.0001$), we chose proteinuria and ISN/RPS classification as covariates in the multivariate model. After the selection of variables, nephrotic proteinuria (≥3.5 g/day) at baseline was identified as an independent poor prognostic factor for survival in the multivariate Cox regression analysis (Table 3).

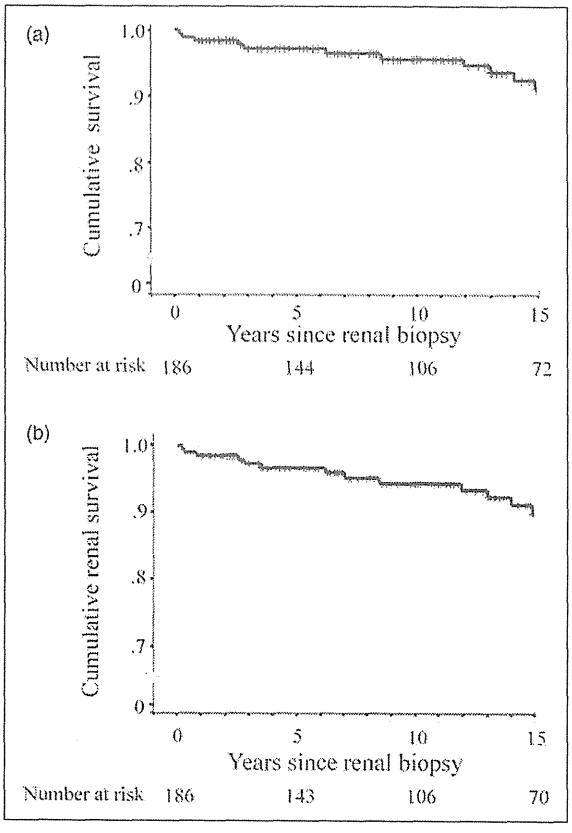

Figure 1 Cumulative survival and renal survival in a cohort of 186 patients with lupus nephritis.
(a) Kaplan-Meier survival estimates in patients with lupus nephritis. The survival estimates at five and 10 years were, respectively, 97.2% and 95.7%. (b) Kaplan-Meier survival estimates in patients with lupus nephritis. The renal survival estimates at five and 10 years were, respectively, 96.6% and 94.3%.

Renal outcomes and risk factors for ESRD

Nine patients had ESRD at their final visit. Kaplan-Meier analysis revealed a five- and 10-year renal survival rate of 96.6% and 94.3%, respectively (Figure 1(b)). All ESRD patients needed maintenance hemodialysis, and no patients received renal transplantation. Five out of nine ESRD patients died during the observation period. Four out of nine ESRD patients were male. One ESRD patient presented with class I, one with class II, six with class IV, and one with class V. The ESRD patient of class I at baseline presented with class V nephritis later at the second biopsy, and the patients of class II developed class IV nephritis later. Six out of nine ESRD patients received IVCY during the period of this study. As a result of the log-rank test, proteinuria ≥3.5 g/day ($p = 0.0003$), male gender ($p = 0.01$) and class IV or IV+V ($p = 0.02$) were identified as risk factors for poor renal prognosis (Figure 2(a)–(c)). Serum albumin level ≤3.0 mg/dl ($p = 0.0003$), serum creatinine level ≥0.8 mg/dl ($p = 0.02$) and SLEDAI-2 K >14 ($p = 0.03$) were also identified as risk factors for poor renal prognosis. In terms of long-term survival and renal outcome, there was no significant difference between the diffuse global (IVG) and diffuse segmental (IVS) group as a result of the log-rank test ($p = 0.28$). The activity ($p = 0.056$)/chronicity index ($p = 0.10$, Figure 2(d)) on the first renal biopsy at baseline were not identified as independent poor prognostic factors for renal outcome. Hypertension at baseline was not

Table 2 The cases of death and/or hemodialysis patients

No.	*Sex*	*Age at time of biopsy, years*	*Follow-up period, years*	*ISN/RPS classification*	*Rebiopsy Class*	*Endpoints*	*Cause of death*
1	F	38	15.3	II	–	Death	Pneumonia and renal cell carcinoma
2	F	38	15.2	II	–	Death	Subarachnoid hemorrhage
3	F	27	0.3	II	–	Death	Suicide
4	F	48	2.6	II	–	Death	Unknown
5	F	26	14.0	III (A/C)+V	–	Death	Iliopsoas abscess and cirrhosis
6	F	26	0.2	IVG (A)	–	Death	Suicide
7	M	23	8.5	IVG (A/C)	–	Death	Sudden death
8	M	44	0.8	IVG (A/C)	–	Death	*Pneumocystis jiroveci* pneumonia
9	F	39	11.9	IVS (A)+V	–	Death	Acute myocardial infarction
10	F	35	18.0	V	IVG (A)+V	Death, HD	Acute myocardial infarction
11	F	17	13.0	I	V	Death, HD	Sepsis and pneumonia
12	F	30	14.9	IVG (A)	IVG (A)+V	Death, HD	Sepsis and uterine cervical cancer
13	F	51	6.2	IVG (A)	–	Death, HD	Pneumonia and alveolar hemorrhage
14	M	26	2.8	IVG (A/C)	–	Death, HD	Sepsis
15	F	21	17.2	II	IVG (A/C)	HD	–
16	M	22	3.5	IV(A)	IVG (C)+V	HD	–
17	M	15	17.0	IVG (A)	IVG (A/C)	HD	–
18	M	64	7.0	IVG (A/C)	–	HD	–

F: female; M: male; ISN/RPS: International Society of Nephrology/Renal Pathology Society; HD: hemodialysis; IVG: diffuse global lupus nephritis; IVS: diffuse segmental lupus nephritis.

Table 3 Result of Cox regression analysis relating risk against survival, renal survival, and sustained renal remission to variables

	Survival (n = 186)		*Renal survival (n = 186)*		*Sustained remission (n = 152)*	
Variable	*HR (95% CI)*	p	*HR (95% CI)*	p	*HR (95% CI)*	p
Male sex	1.57 (0.41–5.97)	0.51	3.33 (1.14–9.73)	0.03	2.18 (0.82–5.76)	0.12
Age at the time of biopsy	1.02 (0.97–1.07)	0.46	1.02 (0.98–1.06)	0.44	0.96 (0.93–0.99)	<0.01
Serum creatinine level ≥0.8 mg/dl	1.03 (0.30–3.56)	0.96	1.52 (0.51–4.53)	0.45	0.90 (0.40–2.05)	0.81
Proteinuria ≥3.5 g/day	3.59 (1.01–12.8)	0.049	3.71 (1.15–12.0)	0.03	1.21 (0.50–2.91)	0.68
ISN/RPS class IV or IV+V	1.26 (0.36–4.24)	0.72	1.54 (0.47–5.03)	0.47	1.96 (0.88–4.36)	0.10
Chronicity index ≥1	1.49 (0.45–4.91)	0.52	1.37 (0.44–4.24)	0.59	1.40 (0.68–2.85)	0.36
Non-complete renal response	–	–	–	–	6.71 (2.24–20.1)	<0.01

CI: confidence interval; ISN/RPS: International Society of Nephrology/Renal Pathology Society; HR: hazard ratio; *p*: *p* value.

identified as independent poor prognostic factors for renal outcome in the log-rank test ($p = 0.29$). There is no statistical difference in survival or renal survival between the calendar-year periods (1984–1989, 1990–1999 and 2000–2010). The activity index showed a high association with ISN/RPS classification (Mann-Whitney rank sum test, ISN/RPS class IV or IV+V versus the others, 4.59 versus 0.56, $p < 0.0001$). We did not include serum albumin level, SLEDAI-2 K or activity index in the multivariate models for renal outcome because of the strong associations with other factors. After the selection of variables, male gender and nephrotic proteinuria at baseline were identified as independent poor prognostic factors for renal outcome in the multivariate Cox regression analysis (Table 3).

Forty-seven out of 186 patients had nephrotic proteinuria at baseline. We analyzed the risk factors of poor renal prognosis in the patients with nephrotic proteinuria as a sub-analysis. Male gender ($p = 0.023$) and failure of partial response ($p = 0.0495$) were identified as poor prognostic factors by Kaplan-Meier analysis (Figure 3(a) and (b)). Class IV or IV+V ($p = 0.42$) was not a significantly worse prognosis factor. We compared patient characteristics between females and males at baseline. Serum creatinine levels and estimated GFRs were higher in male patients than in females.

Renal response and sustained renal remission

Of 174 patients, 145 (83.3%) achieved CR: five of five (100%) with class I, 54/56 (96.4%) with class

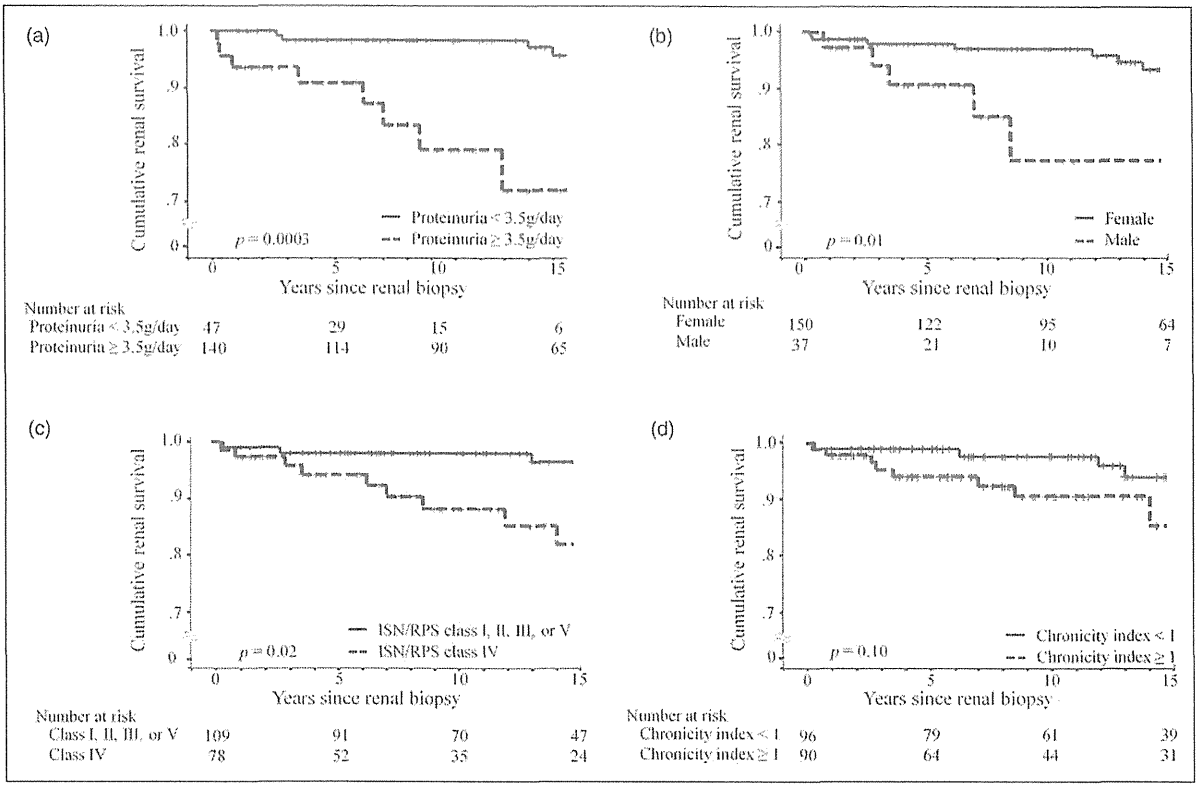

Figure 2 Cumulative renal survival by individual variables.
(a) Kaplan-Meier renal survival estimates in 186 patients with lupus nephritis according to urine protein more than 3.5 g/day. (b) Kaplan-Meier renal survival estimates according to gender. (c) Kaplan-Meier renal survival estimates according to International Society of Nephrology/Renal Pathology Society (ISN/RPS) classification. (d) Kaplan-Meier renal survival estimates according to chronicity index. Urine protein ≥3.5 g/day ($p = 0.0003$), male gender ($p = 0.01$) and class IV or IV+V ($p = 0.02$) were identified as risk factors for poor renal prognosis.

II, 13/14 (92.9%) with class III, four of five (80.0%) with class III+V, five of six (83.3%) with class IVS, 39/53 (73.6%) with class IVG, 11/13 (84.6%) with class IV+V and 14/22 (63.6%) with class V. Of 174 patients, 152 (87.4%) achieved CR or PR: five of five (100%) with class I, 54/56 (96.4%) with class II, 13/14 (92.9%) with class III, four of five (80.0%) with class III+V, five of six (83.3%) with class IVS, 42/53 (77.4%) with class IVG, 13/13 (100%) with class IV+V and 16/22 (72.7%) with class V. Of 152 patients who achieved CR or PR, Kaplan-Meier analysis revealed five- and 10-year sustained renal remission rates of 91.5% and 77.3% (100% and 80.0% of class I, 98.2% and 86.4% of class II, 90.9% and 90.9% of class III, 100% and 100% of class III+V, 95.2% and 64.7% of class IV, 40.5% and 40.5% of class IV+V and 93.8% and 93.8% of class V, respectively. As a result of the log-rank test, proteinuria ≥3.5 g/day ($p = 0.003$), class IV or IV+V ($p = 0.007$), chronicity index ($p = 0.04$) and non-CR ($p < 0.0001$) were identified as risk factors for poor renal prognosis. Male gender ($p = 0.08$), serum albumin level ≤3.0 mg/dl ($p = 0.11$) and serum creatinine level ≥0.8 mg/dl ($p = 0.09$) were not identified as independent poor prognostic factors. Serum albumin level was excluded from multivariate models as it correlated with proteinuria. After the selection of variables, younger age at the time of biopsy and non-CR were identified as independent poor prognostic factors for renal flare in the multivariate Cox regression analysis (Table 3).

Discussion

To our knowledge, this study is the first report to provide SMRs with a mean observation period over 10 years for patients with LN according to the ISN/RPS classification. We could not evaluate the SMR of each ISN/RPS class because dividing each ISN/RPS class into subgroups makes patient numbers in each group too small for statistical

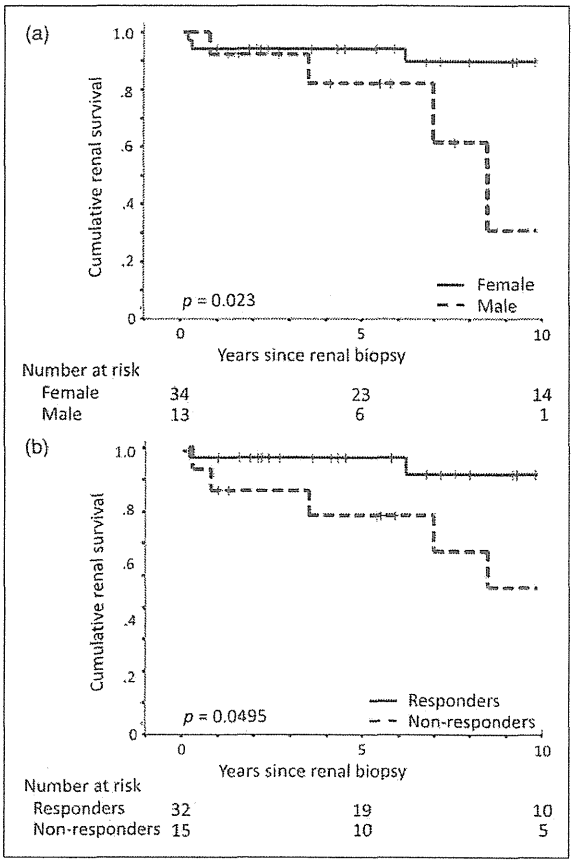

Figure 3 Cumulative renal survival by individual values in patients with urine protein more than 3.5 g/day.
Forty-seven out of 186 patients had nephrotic proteinuria at baseline. We analyzed the risk factors of poor renal prognosis in the patients with nephrotic proteinuria as a sub-analysis. (a) Kaplan-Meier renal survival estimates in 47 patients with urine protein ≥3.5 g/day according to gender. (b) Kaplan-Meier renal survival estimates according to responders or non-responders (failure of partial response). Male gender ($p = 0.023$) and failure of partial response ($p = 0.0495$) were identified as poor prognostic factors by Kaplan-Meier analysis.

analyses of SMR. For patients with LN according to the WHO classification, a Danish report was the first one, which showed an overall SMR was 6.8.[17] For patients with LN according to the ISN/RPS classification, a Chinese report showed an overall SMR was 9.0, although only 77% of the patients with LN had undergone renal biopsy.[18] Another Chinese report showed an overall SMR was 5.9, although 90% of the patients with LN had undergone renal biopsy according to the ISN/RPS classification.[19] In the present study, an overall SMR in patients with LN was 3.59. The 10-year survival rate was 95.7%. For comparison, a recent report on Japanese lupus patients, 46% of whom had LN, showed a 10-year survival rate of 92%.[24] Korbet et al. reported LN patient survival rate at 10-year was 81%, 59%, 73% in whites, in blacks and in other ethnic groups, respectively.[15] The Euro-Lupus Nephritis Trial revealed the 10-year survival rate in biopsy-proven LN patients was 92%,[8] and another showed 77% in Saudi LN patients.[25] Taiwanese reports showed the six-year survival rate was 93.8%.[26] In our cohort, survival and renal survival rates in patients with LN were relatively high compared with other reports. There would be ethnic or regional variations in the prognosis of LN. Some studies have reported black and Hispanic patients with LN are more likely to have a worse prognosis than other ethnic groups.[9–16] Whether Japanese patients with LN have good outcomes remains unclear, but a review pointed out the characteristic of Asian patients with LN. Asian patients have higher rates of LN-associated autoantibodies and more active glomerulonephritis than white patients.[27] This review arrived at a conclusion that the difference in mortality between Asian patients and other ethnicities in different geographical regions was found to vary depending on socioeconomic factors such as access to health care.

In terms of causes of death, infections (50%), suicides (14%), acute myocardial infarctions (14%) and stroke (7%) were found in our study. In Saudi LN patients, the main causes of death were renal failure (50%) and infections (44%).[28] In the Danish report, the causes of death were cardiovascular diseases (31%), SLE activity (19%), infections (14%) and cerebrovascular diseases (14%).[17] Another report showed main causes of death were infections (47%), renal failure (16%), and cardiovascular diseases (16%) as main causes of death in white LN patients.[29] In the Korean reports, the main causes of death were SLE activity (43%) and infections (39%), and in the Chinese reports, they were infection (50.0%), and cardiovascular disease (20.8%).[19] The causes of death in our study was similar to other Asian reports; however, there was a difference from Western reports in cardiovascular events. Generally in Japan, the prevalence of cardiovascular events is less than that in Western countries,[30] presumably contributing to better survival rates in our cohort. Higher survival and renal survival rates in our cohort may due to a combination of difference in genetics and socioeconomic environment.

Of 174 patients, 145 (83.3%) achieved CR, and Kaplan-Meier analysis revealed the five- and 10-year sustained renal remission rate of 91.5% and 77.3% in our study. Patients with class V nephritis showed the lowest renal response rate; however, they showed high sustained renal remission

and renal survival rates. In contrast, the patients with class IV or IV+V showed low 10-year sustained renal remission rates. Younger age at the time of biopsy and failure of complete response were identified as independent poor prognostic factors for renal flare in the Cox regression analysis. There have been no other reports that analyzed long-term sustained renal remission rate and poor prognostic factors for renal flare.

According to our analyses, nephrotic proteinuria and male gender were identified as independent risk factors for poor renal prognosis. Among patients with nephrotic proteinuria, male gender and failure of partial remission were poor prognostic factors. Serum creatinine levels were higher accompanied by lower estimated GFRs in male patients than in females. Lower renal functions in males at baseline may contribute to poor renal outcome.

Previous reports focused a number of poor prognostic factors such as older age at onset,[28] hypertension,[17,28] high serum creatinine level at baseline,[17,28,29,31] diagnostic delay,[17] class III, IV, III+V or IV+V,[28,32] and chronicity in renal histopathological analyses;[33] however, each study reported different poor prognostic factors. Our data were fundamentally in accordance with those previous findings as classical risk factors of ESRD, except for male gender.

Some studies showed renal involvement is more prevalent in male lupus patients than female[34–36] and suggested that male gender is associated with a more aggressive disease, including a higher incidence of proliferative glomerulonephritis and renal failure.[34,35] To the best of our knowledge, however, only three studies were able to demonstrate an increase in the ESRD in male subjects.[26,37–39] These reports were studies of American, Danish and Taiwanese patients with LN. In Asian patients, Hsu et al. also reported male gender was a poor prognostic factor in Taiwanese patients, although the end point of this study was chronic renal insufficiency (doubling of serum creatinine lasting for at least six months with a value of at least 2 mg/dl).[40] We also showed that male gender is a poor prognostic factor of ESRD in Japanese patients with LN. There are no compelling data or a satisfactory hypothesis about reasons for differences between females and males, although a sex hormone hypothesis,[41–44] a sex chromosome hypothesis,[42,45–50] and an intrauterine selection hypothesis[51–53] have been proposed.

Our present study certainly includes some limitations; this is a single-center-based retrospective observational study and the patient number is somewhat underpowered. In addition, some of the most severe patients were not included in this study since they did not receive renal biopsies. On the other hand, patients with milder renal disease in whom renal biopsy was not performed were not included. However, long-term observation based on detailed clinical records was achievable only in a single-center cohort. We also believe re-evaluation of renal specimens using ISN/RPS classification in relation to renal prognosis is quite meaningful for the choice of future treatment for LN. In conclusion, our report showed LN was associated with a 3.59-fold increase in mortality compared with the general population, and male gender and nephrotic proteinuria at baseline were predictive of poor renal outcome.

Funding

The work was supported by a grant from the Japanese Ministry of Health, Labor and Welfare and by the Japanese Ministry of Education, Culture, Sports, Science, and Technology.

Conflict of interest statement

The authors have no conflicts of interest to declare.

Acknowledgments

We would like to thank all the members of the Rheumatology and Nephrology Division and laboratory assistants.

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