Vertebral Fracture and Bone Mineral Density in Women Receiving High Dose Glucocorticoids for Treatment of Autoimmune Diseases

SHUNICHI KUMAGAI, SEIJI KAWANO, TATSUYA ATSUMI, SHIGEKO INOKUMA, YOSUKE OKADA, YOSHIKI KANAI, JUNICHI KABURAKI, HIDETO KAMEDA, AKIRA SUWA, HIROYUKI HAGIYAMA, SHUNSEI HIROHATA, HIROFUMI MAKINO, and HIROSHI HASHIMOTO

ABSTRACT. ***Objective.*** To evaluate the factors influencing the occurrence of vertebral fracture in patients receiving high dose glucocorticoids (GC).

Methods. A cross-sectional study was performed on women who had received at least 0.5 mg/kg of oral glucocorticoid for the treatment of autoimmune diseases for more than 1 month between 1998 and 2003. Logistic regression analysis and chi-square test were used to examine the effects of glucocorticoid dose and other factors on vertebral fractures. Receiver-operating characteristics curve (ROC) analysis was used to determine the bone mineral density (BMD) cutoff value for the risk of vertebral fracture.

Results. The study population comprised 160 women, including 35 with vertebral fractures. In ROC analysis, the BMD threshold of the risk of fracture for postmenopausal women (0.787 g/cm^2, T score –2.1) was lower than that for premenopausal women (0.843 g/cm^2, T score –1.7). Among patients with fractures, 7 of 16 premenopausal patients had normal BMD values (T score > –1), whereas only one of 19 postmenopausal patients showed a comparable level of BMD. Additionally, vertebral fracture was more frequent for patients with high total cholesterol values (> 280 mg/dl) than for those with normal total cholesterol values (< 220 mg/dl). Moreover, patients with high total cholesterol values had lower BMD values than those with normal total cholesterol values.

Conclusion. The fact that vertebral fracture frequently occurred in premenopausal patients with normal BMD and evidence that hyperlipidemia correlated with fracture suggest the pathology of vertebral fracture secondary to high dose glucocorticoid therapy is multifactorial and possibly involves lipid metabolism. (J Rheumatol 2005;32:863–9)

Key Indexing Terms:

OSTEOPOROSIS	VERTEBRAL FRACTURE	GLUCOCORTICOID
MENOPAUSE	BONE MINERAL DENSITY	HYPERLIPIDEMIA

Glucocorticoids are widely used for the treatment of a variety of autoimmune diseases. Even now, when various novel drugs for the treatment of these diseases are being introduced, glucocorticoids remain the main drugs of choice. However, it has been well established that the use of glucocorticoids can lead to rapid loss of bone mineral density

From the Department of Clinical Pathology and Immunology, Kobe University Graduate School of Medicine, Kobe; Department of Medicine II, Hokkaido University Graduate School of Medicine, Sapporo; Department of Internal Medicine, Tokyo Metropolitan Komagome Hospital, Tokyo; First Department of Internal Medicine, University of Occupational and Environmental Health, School of Medicine, Fukuoka; Department of Internal Medicine and Rheumatology, Juntendo University School of Medicine, Tokyo; Department of Internal Medicine, Tokyo Electric Power Company Hospital, Tokyo; Second Department of Internal Medicine, Saitama Medical Center, Saitama; Department of Internal Medicine, Keio University School of Medicine, Tokyo; Department of Medicine and Rheumatology, Graduate School, Tokyo Medical and Dental University, Tokyo; Department of Internal Medicine, Teikyo University School of Medicine, Tokyo; and Department of Medicine and Clinical Science, Okayama University Graduate School of Medicine and Dentistry, Okayama, Japan.

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S. Kumagai, MD, PhD; S. Kawano, MD, PhD, Department of Clinical Pathology and Immunology, Kobe University Graduate School of Medicine; T. Atsumi, MD, PhD, Department of Medicine II, Hokkaido University Graduate School of Medicine; S. Inokuma, MD, PhD, Department of Internal Medicine, Tokyo Metropolitan Komagome Hospital; Y. Okada, MD, PhD, First Department of Internal Medicine, University of Occupational and Environmental Health, School of Medicine, Fukuoka; Y. Kanai, MD, PhD; H. Hashimoto, MD, PhD, Department of Internal Medicine and Rheumatology, Juntendo University School of Medicine; J. Kaburaki, MD, PhD, Department of Internal Medicine, Tokyo Electric Power Company Hospital; H. Kameda, MD, PhD, Second Department of Internal Medicine, Saitama Medical Center; A. Suwa, MD, PhD, Department of Internal Medicine, Keio University School of Medicine; H. Hagiyama, MD, PhD, Department of Medicine and Rheumatology, Graduate School, Tokyo Medical and Dental University; S. Hirohata, MD, PhD, Department of Internal Medicine, Teikyo University School of Medicine; H. Makino, MD, PhD, Department of Medicine and Clinical Science, Okayama University Graduate School of Medicine and Dentistry.

Address reprint requests to Prof. S. Kumagai, Department of Clinical Pathology and Immunology, Kobe University Graduate School of Medicine, Kusunoki-Cho 7-5-2, Chuo-Ku, Kobe, Hyogo 650-17, Japan. E-mail: kumagais@kobe-u.ac.jp

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(BMD) and to an increased risk of fracture[1]. Several epidemiologic studies have reported a doubling of the risk of hip fracture for users of glucocorticoids[2-4], while large-scale studies have demonstrated a rapid increase in fracture risk following the start of glucocorticoid therapy and a strong correlation of risk with daily glucocorticoid dose[4,5]. Other smaller studies have shown that the cumulative dose, rather than the daily dose, was the more reliable and accurate predictor of fracture[6,7]. When high dose glucocorticoids are used, the loss of bone such as vertebrae can be rapid and lead to vertebral compression fractures within a few months.

Glucocorticoids are also known to affect bone through various pathways, affecting mainly bone formation and, to a lesser extent, bone resorption[8,9]. Findings have been accumulating about the possible role of micro-architectural changes in glucocorticoid induced fracture, although fracture in glucocorticoid users may also occur simply as a result of bone loss. A recent hypothesis is that osteocyte apoptosis is an important factor in deterioration of bone quality and the concomitant rapid increase in the risk of fracture[10]. In addition, there is a report that glucocorticoid users with fracture had considerably higher BMD than patients with fracture due to primary osteoporosis[11]. These reports support the notion that a non-BMD-related mechanism may also be responsible for inducing fracture in users of glucocorticoids[12].

We conducted a multicenter, cross-sectional analysis, specifically investigating high dose glucocorticoid users treated for autoimmune diseases, to determine the BMD cutoff value for the risk of vertebral fracture, and to examine the correlation between glucocorticoid induced vertebral fracture or loss of BMD and multiple factors including menopause, glucocorticoid dose, and other glucocorticoid induced secondary complications.

MATERIALS AND METHODS

Study population of glucocorticoid users. Data on 160 Japanese women, aged 16–85 years and treated with glucocorticoids for autoimmune diseases, were collected from the rheumatology departments of 11 institutions that joined the Research Committee for Glucocorticoid-Induced Osteoporosis organized by the Japanese Ministry of Health, Labor and Welfare. This study was limited to patients who had been receiving oral glucocorticoid therapy (mean daily dose 0.5 mg/kg prednisone or equivalent) for at least 1 month between April 1998 and March 2003. The basic clinical data including risk factors and dose and duration of glucocorticoid therapy were collected retrospectively by treating physicians in reference to medical records from each institution, and the collected data were reviewed by the central committee for selecting eligible patients. As for treatment or prevention of osteoporosis, there were no restrictions for enrollment of patients based on protocols for the use of bisphosphonates, calcium, vitamin D, or other antiresorptive drugs. Diseases they were treated for included systemic lupus erythematosus (SLE; 79 cases), Sjögren's syndrome (15 cases), polymyositis (13 cases), mixed connective tissue disease (12 cases), adult onset Still's disease (8 cases), polymyalgia rheumatica (7 cases), dermatomyositis (6 cases), systemic sclerosis (5 cases), and others (15 cases). Patients with rheumatoid arthritis were excluded from this study.

BMD of the patients was assessed for the lumbar spine (L2–L4), femoral neck, and radial head by means of dual-energy x-ray absorptiometry (DEXA). Since the DEXA machines used for the measurement of BMD differed from hospital to hospital, the raw BMD values were converted to comparable values for the QDR-2000 (Hologic Inc., Waltham, MA, USA) as described[13]. High dose glucocorticoid therapy was defined as a mean daily dose > 0.5 mg/kg of prednisone or equivalent dose of other glucocorticoids for at least 1 month.

Vertebral fracture was confirmed radiologically by lateral radiographs of the thoracolumbar spine with the method established by Orimo, *et al*[14]; the presence of vertebral fracture was semiquantitatively confirmed if either the ratio of middle/anterior or middle/posterior height of a vertebral body was < 0.8, or the ratio of anterior/posterior height of a vertebral body was < 0.75. The judgment of fracture was double-checked by 2 examiners in each institution. If BMD was measured more than once in the same patient, the last BMD value was adopted for patients without vertebral fracture, and for patients with fracture, the BMD measured at the timepoint nearest the radiological confirmation of initial vertebral fracture was used.

The daily, cumulative, and maximum glucocorticoid doses, and the total duration (in days) of prior glucocorticoid therapy were also entered into the analysis. Clinical factors that may affect the occurrence of vertebral fracture, comprising age, body mass index (BMI), menopause, BMD (T scores), hypertension, total cholesterol, and HbA1c were evaluated. Diagnoses for hypertension and diabetes mellitus were determined according to American Heart Association[15] and American Diabetes Association[16] guidelines, respectively. Hyperlipidemia was diagnosed according to the criteria of the Japanese Atherosclerosis Society[17], in which total cholesterol level > 220 mg/dl is regarded as hyperlipidemia.

Statistical analysis. Logistic regression analysis was used to calculate the influence of various variables on vertebral fracture including age, BMI, menopause, BMD, and glucocorticoid related parameters. For determination of BMD cutoff values to identify women with vertebral fracture, sensitivity, specificity, and BMD cutoff values were calculated using receiver-operating characteristics curve (ROC) analysis. As for patients with vertebral fracture, the chi-square test was used to determine the difference in BMD between premenopausal and postmenopausal glucocorticoid users. P values < 0.05 were deemed to be statistically significant. The MedCalc statistical analysis software package (MedCalc Software, Mariakerke, Belgium) was used for statistical analyses.

RESULTS

Variables affecting vertebral fracture in high dose glucocorticoid users. For this study, 160 patients were assessed. The baseline information of enrolled patients is shown in Table 1. BMD values of this group negatively correlated with patients' age ($p < 0.001$, $r = -0.366$). A logistic regression analysis of patients with vertebral fracture (fracture group) and those without vertebral fracture (non-fracture group) is presented in Table 2. The respective mean BMD values of the fracture group (35 cases; 19 postmenopausal, 16 premenopausal) and the non-fracture group (125 cases) were 0.781 and 0.871 g/cm^2 ($p = 0.004$). There was a significant difference between the 2 groups in BMI and BMD, but no difference in age, ratio of menopause, and total glucocorticoid dose, as shown in Table 2. The logistic regression analyses including the other glucocorticoid related variables such as cumulative days of glucocorticoid use, mean glucocorticoid dose (daily), cumulative glucocorticoid dose, and maximal glucocorticoid dose showed no significant difference between the 2 groups (data not shown). The mean daily glucocorticoid dose for premenopausal women (age 34.9 ± 9.4 yrs) was 16.4 ± 16.5 mg/day and for postmenopausal

Table 1. Baseline characteristics of 160 patients in the study.

	Premenopausal	Postmenopausal	Total	p
Age, yrs, mean ± SD	34.9 ± 9.4	62.6 ± 9.9	47.9 ± 16.9	< 0.05
BMI, kg/m^2	21.7 ± 14.1	22.0 ± 3.5	21.9 ± 3.6	NS
BMD, g/cm^2	0.926 ± 0.149	0.767 ± 0.149	0.852 ± 0.168	< 0.05
Daily prednisolone dose*, mg/day	16.4 ± 16.5	10.7 ± 9.9	13.7 ± 14.1	< 0.05
Cumulative dose of prednisolone*, g	17.1 ± 31.3	8.2 ± 10.4	12.8 ± 24.0	NS
Duration of glucocorticoid treatment, days	1993.1 ± 2091.9	2069.9 ± 2317.4	2027.8 ± 2189.4	NS

* Adjusted to the dose equivalent to prednisolone. NS: not significant.

Table 2. Logistic regression analysis of treatment related variables and vertebral fracture in high dose user of glucocorticoid.

	Vertebral Fracture			
	Yes	No	Z	p
Age, yrs, mean ± SD	50.7 ± 3.2*	47.1 ± 1.4	0.5925	0.554
Menopause (%)	19/35 (54.3)	56/125 (44.8)	0.270	0.787
BMI	22.4 ± 0.8	21.8 ± 0.3	1.961	< 0.05
BMD, L2–4, g/cm^2	0.781 ± 0.033	0.871 ± 0.014	2.218	< 0.03
Total glucocorticoid dose*, g	24.3 ± 6.6	22.2 ± 4.4	0.789	0.430

* Adjusted to the dose equivalent to prednisolone.

women (age 62.6 ± 9.9 yrs) 10.7 ± 9.9 mg/day ($p < 0.05$). Compared to postmenopausal glucocorticoid users, premenopausal glucocorticoid users had significantly higher average BMD (L2–L4) in the lumbar spine, femoral neck, and radial head (data not shown).

For postmenopausal women, the mean BMD value of the fracture group was significantly lower than that of the non-fracture group ($p < 0.01$), as shown in Figure 1. In contrast, there was no significant difference in BMD values between the fracture group and non-fracture group among premenopausal women. Of special interest is that 7 of the 16 premenopausal patients (43.7%) in the fracture group showed normal values (T score > –1), whereas only one of the 19 postmenopausal patients (5.3%) did ($p < 0.01$). There was no statistically significant difference between the fracture group and non-fracture group for maximum glucocorti-

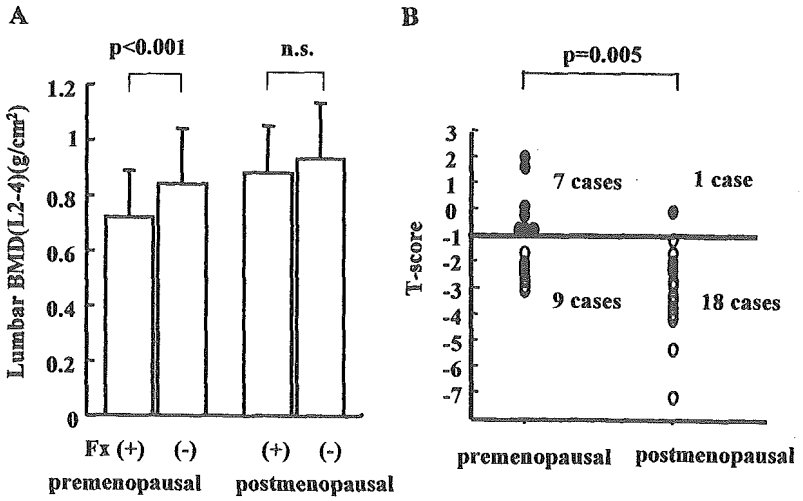

Figure 1. (A) Lumbar BMD from fracture (Fx) and non-fracture patient groups taking high dose glucocorticoids. There were significant differences in lumbar BMD between fracture and non-fracture groups in premenopausal women ($p < 0.001$), whereas no difference was detected between the 2 groups in postmenopausal women. ns: not significant. (B) T scores from premenopausal or postmenopausal women with vertebral fracture. Premenopausal glucocorticoid users frequently incurred vertebral fracture even when BMD was not reduced (T > –1) compared with postmenopausal women ($p = 0.005$). ●: fracture patients whose T scores were not reduced.

coid dose, mean daily glucocorticoid dose, disease background, and history of methylprednisolone pulse therapy in premenopausal women (data not shown).

BMD cutoff values for vertebral fracture in glucocorticoid users assessed by ROC analysis. ROC analysis was used to determine the BMD cutoff level for vertebral fracture in high dose glucocorticoid users. The cutoff values were defined as the values that proved to be effective for the sensitive and specific differentiation of subjects with and without vertebral fracture. As shown in Figure 2, the cutoff values for the risk of vertebral fracture for premenopausal, postmenopausal, and total patients were 0.843, 0.787, and 0.787 g/cm^2, respectively.

Hyperlipidemia correlates with BMD value and vertebral fracture. The influence of common glucocorticoid induced complications such as hyperlipidemia, diabetes mellitus, and hypertension on vertebral fracture were not entered into the logistic regression analysis, since those variables are not recognized as independent to glucocorticoid dose-related variables. Table 3 shows that hyperlipidemia has negative correlation with BMD, while HbA1c level did not correlate with BMD values. Nor did hypertension correlate with the level of BMD (data not shown). Then we compared patients with normal total cholesterol (< 220 mg/dl) value to those with above-normal values for further analysis. The peak value of total cholesterol after initiation of glucocorticoid therapy was used for the analysis in each patient. When we raised the comparative total cholesterol level to > 280 mg/dl, patients with high total cholesterol (> 280 mg/dl) value had lower BMD ($p = 0.016$) and higher risk of vertebral fracture (relative risk 3.1, $p = 0.032$) than those with normal total cholesterol level (Figure 3). These results suggest that hyperlipidemia following high dose glucocorticoid therapy may contribute to the risk for BMD reduction and vertebral fracture.

DISCUSSION

High dose glucocorticoid therapy is often the first choice for patients with autoimmune diseases, such as SLE, that frequently affect premenopausal women. Although the efficacy of bisphosphonate has recently been reported in high dose glucocorticoid users[18], there is only limited knowledge of the clinical risk factors for secondary osteoporosis occurring in high dose glucocorticoid users. This is the first extensive study focusing on the relationship of vertebral fracture and BMD in patients with high dose glucocorticoid therapy. We observed unique effects of high dose glucocorticoid therapy: First, the BMD cutoff value for the risk of vertebral fracture applicable to premenopausal glucocorticoid users was higher than that applicable to postmenopausal glucocorticoid users. Second, premenopausal glucocorticoid users, even with normal BMD values, were found to frequently incur vertebral fracture. Third, hyperlipidemia significantly correlated with vertebral fracture and low BMD.

ROC analysis showed that the BMD cutoff value for the risk of vertebral fracture for premenopausal women was 0.843 (T score = –1.7) and for postmenopausal women 0.787 (T score = –2.1). These cutoff values lie between 70% (T score = –2.6) and 80% (T score = –1.7) of the young adult

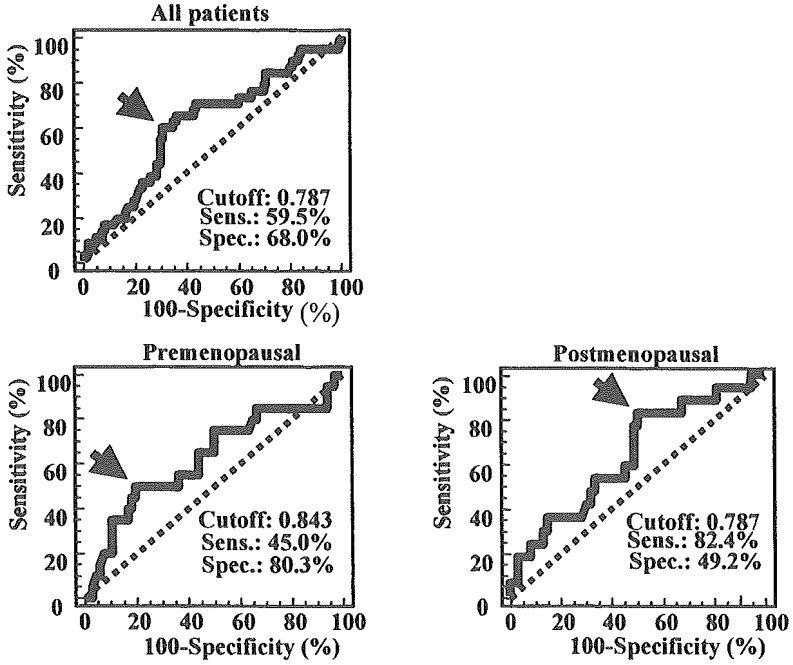

Figure 2. ROC analysis of lumbar BMD values for all patients, premenopausal and postmenopausal patients with vertebral fracture treated with high dose glucocorticoid. Arrows indicate cutoff points. Sens: sensitivity; Spec: specificity.

Table 3. The relationship between other glucocorticoid related complications and BMD or vertebral fracture in high dose glucocorticoid users (chi-square test).

Vertebral Fracture	Yes	No	p
Diabetes mellitus	26	134	
HbA1c, mg/dl*	7.68 ± 1.93	5.15 ± 0.66	< 0.01
BMD, g/cm^2	0.858 ± 0.149	0.850 ± 0.17	NS
Vertebral fracture, yes/no (%)	5/21 (19.2)	29/105 (21.6)	NS
Hyperlipidemia (cases)	95	65	
Total cholesterol, mg/dl*	283.2 ± 54.8	207.8 ± 23.0	< 0.01
BMD, g/cm^2	0.834 ± 0.176	0.876 ± 0.173	0.03
Vertebral fracture, yes/no (%)	23/72 (24.2)	11/54 (16.9)	NS

* Peak values after glucocorticoid therapy are shown. Patients whose value was > 220 mg/dl was defined to have hyperlipidemia. NS: not significant.

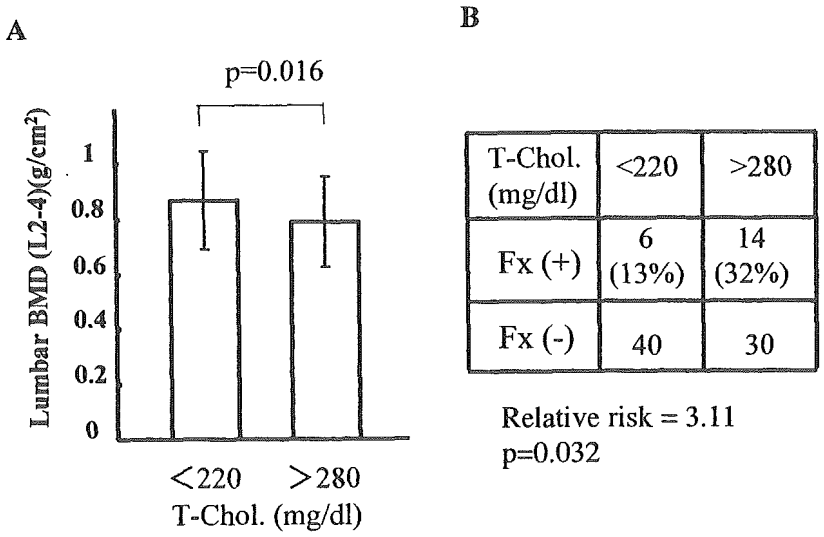

Figure 3. Influence of hyperlipidemia on lumbar BMD and vertebral fracture (Fx) in high dose glucocorticoid users. (A) Comparison of lumbar BMD between patients with high (> 280 mg/dl) and with normal (< 220 mg/dl) total cholesterol (T-Chol) values. (B) Comparison of the ratio of vertebral fracture between patients with high (> 280 mg/dl) and with normal (< 220 mg/dl) total cholesterol values. Chi-square analysis revealed that vertebral fracture was more frequent in patients with high total cholesterol level than in those with normal level (relative risk = 3.11, p = 0.032).

mean value of a large-scale Japanese study of primary osteoporosis by Orimo, *et al*, in which the cutoff value for osteoporosis was determined to be 70% of young adult mean[14]. There have been arguments about the difference of BMD threshold for fractures between postmenopausal users of glucocorticoids and nonusers. There are reports showing the BMD distribution of patients with vertebral fractures was similar for glucocorticoid users and nonusers[19,20]. On the other hand, other studies found that postmenopausal women taking glucocorticoids had a higher risk of fracture compared with nonusers, even at comparable levels of BMD[11,21]. Although our study was not designed to address this controversy, the relatively high BMD cutoff value, 80% of the young adult mean, for premenopausal women established in our study suggests that BMD alone may not be sufficient for predicting the risk of vertebral fracture for premenopausal users of glucocorticoids.

This notion is supported by our finding that premenopausal glucocorticoid users frequently experienced complications of vertebral fracture even when they registered normal BMD values. Vertebral fracture was seen in as many as 43% of premenopausal glucocorticoid users even when their BMD values were not particularly low (T score > −1). Recent guidelines from Europe and North America have been developed to establish intervention thresholds for glucocorticoid induced osteoporosis in patients with high BMD levels[22,23] or regardless of BMD level[24]. The recent guidelines of the American College of Rheumatology advocate intervention for all patients whose therapy calls for use of > 5 mg/day glucocorticoid for at least 3 months, and for

patients on a longterm glucocorticoid regimen with a BMD below a T score of −1.0[22]. Guidelines from the UK advocate an intervention threshold at a T score of −1.5 for patients who are scheduled to be given > 7.5 mg/day glucocorticoid for at least 6 months[23]. Our results suggest the need for developing a new therapeutic approach to prevent glucocorticoid induced osteoporosis in addition to starting antiresorptive therapy at high BMD thresholds.

Accumulating findings indicate that BMD is not the only factor that affects the risk of vertebral fracture[1,12,25]. One mechanism for the rapid onset of fracture risk could be osteocyte apoptosis, which leads to a deterioration of bone quality and a rapid increase in fracture risk[10]. Osteocyte apoptosis is prevalent in glucocorticoid induced osteoporosis[26]. The network of osteocytes is thought to detect microdamage to bone and be involved in bone repair remodeling. Therefore, osteocyte apoptosis together with glucocorticoid induced suppression of osteoblast generation could lead to growing micro-damage and a resultant increase in bone fragility. Thus, it is important to develop a new method to estimate bone fragility besides BMD measurement.

Another candidate factor that may contribute to the risk of osteoporosis from our study is hyperlipidemia. Our results showed that high total cholesterol (> 280 mg/dl) may be a risk factor for low BMD and vertebral fracture. There are reports of *in vitro* studies suggesting that low density lipoprotein oxidation products could promote osteoporosis by inhibiting osteoblast differentiation and by directing progenitor marrow stroma cells to undergo adipogenic instead of osteogenic differentiation[27,28]. Although these *in vitro* studies imply the possible involvement of lipid metabolism in the process of osteoporosis, there has been no report confirming the relationship of hyperlipidemia and glucocorticoid induced osteoporosis, and many clinical trials examining the efficacy of HMG-CoA reductase in preventing osteoporosis have had negative results. Therefore, further investigation is needed to establish a therapeutic strategy for preventing glucocorticoid induced osteoporosis in patients with hyperlipidemia.

Some reports stress the importance of daily glucocorticoid dose (mean) over cumulative glucocorticoid dose as an effective predictor of fracture[4,5,11], while others stress cumulative rather than daily glucocorticoid dose[6,7]. We detected no statistically significant difference between the occurrence of fracture and the mean daily glucocorticoid dose ($p = 0.483$) or cumulative glucocorticoid dose ($p = 0.794$), probably because of the limitation of our cross-sectional study and the limited numbers of patients with fracture. An important factor affecting our results may be differences in the use of antiresorptive drugs, especially bisphosphonates. This may be due partly to the Japanese legislative environment, since prophylactic use of drugs has not been allowed yet in the Japanese health insurance system. As this is a cross-sectional study, there are some limitations to interpreting our results. The onset of vertebral fracture is not predictable in prevalent fracture cases, and in these cases the influence of BMD may be different from that in incident fracture cases. To address these questions, we are now conducting a randomized cohort trial on patients who start glucocorticoid administration at a high dose, > 0.5 mg/kg.

Our findings support the hypothesis that treatment with glucocorticoids influences the occurrence of vertebral fracture by means of a mechanism independent of BMD. Moreover, it will be necessary to develop a new approach to assess and reduce the risk of vertebral fracture in premenopausal users of glucocorticoids.

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ORIGINAL ARTICLE

**Binbin Zhong · Michiko Tajima · Hidenari Takahara
Hiromi Nochi · Koichi Tamoto · Naoto Tamura
Shigeto Kobayashi · Yoshifumi Tamura · Makoto Ikeda
Tomohiro Akimoto · Shinichi Yoshino
Hiroshi Hashimoto**

Inhibitory effect of mizoribine on matrix metalloproteinase-1 production in synovial fibroblasts and THP-1 macrophages

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Abstract To investigate the mechanism of antirheumatic action of mizoribine (MZR), we examined the expression of matrix metalloproteinase-1 (MMP-1) and MMP-3 utilizing THP-1 derived macrophage-like cells (THP-1 macrophages) and human synovial fibroblasts (SFs). The cells were respectively stimulated with lipopolysaccharide (LPS) and interleukin-1β in the presence or absence of MZR in vitro. The concentrations of MMP-1 and MMP-3 in the supernatant were measured by enzyme-linked immunosorbent assay. The secretion of MMP-1 from SFs, as well as THP-1 macrophages, was inhibited by MZR in a dose-dependent manner. Furthermore, a quantitative real-time polymerase chain reaction revealed that MZR decreased the expression of MMP-1 messenger RNA. These findings may be an explanation for the clinical effect of MZR in patients with rheumatoid arthritis.

Key words Matrix metalloproteinase (MMP) · Mizoribine · Rheumatoid arthritis (RA) · Synovial fibroblast · THP-1 derived macrophage-like cells

B. Zhong · M. Tajima · N. Tamura (✉) · S. Kobayashi · M. Ikeda · T. Akimoto · H. Hashimoto
Department of Rheumatology and Internal Medicine, Juntendo University School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan
Tel. +81-3-5802-1067; Fax +81-3-5800-4893
e-mail: tnaoto@med.juntendo.ac.jp

H. Takahara
Laboratory of Molecular Biology and Biochemistry, School of Agriculture, Ibaraki University, Inashiki, Ibaraki, Japan

H. Nochi · K. Tamoto
Department of Immunology and Microbiology, Faculty of Pharmaceutical Sciences, Health Sciences, University of Hokkaido, Ishikari-Toubetsu, Hokkaido, Japan

Y. Tamura
Department of Medicine, Metabolism and Endocrinology, Juntendo University School of Medicine, Tokyo, Japan

S. Yoshino
Department of Joint Disease and Rheumatism, Nippon Medical School, Tokyo, Japan

Introduction

Rheumatoid arthritis (RA) is an autoimmune disease characterized by synovial inflammation and the destruction of cartilage and bone. Fibroblast-like synoviocytes and macrophages are predominantly observed in RA synovial tissue containing a cartilage–pannus junction,[1] and matrix metalloproteinases (MMPs) secreted from these cells are generally believed to play a critical role in joint destruction.[2] Among the known MMPs, MMP-1 and MMP-3 were initially reported to be expressed in the synovial membranes of RA patients.[3] Recently, other MMPs, such as MMP-9, MMP-12, and MMP-13, have also been found in the synovial membranes of RA patients.[4–6] Furthermore, the serum levels of MMP-1 and MMP-3 are correlated with generalized clinical disease activity,[7] whereas synovial fluid MMP-1, MMP-3, and TIMP-1 activities are correlated with local joint inflammation.[8] Although the mechanisms of the antirheumatic actions of disease-modifying antirheumatic drugs (DMARDs) are not fully understood, inhibiting the production of MMPs has been postulated to contribute to the prevention of joint destruction.[9,10]

Mizoribine (4-carbamoyl-1-β-D-ribofuranosylimidazolium-5-olate, MZR) is an immunosuppressive drug that has been used to treat patients with renal transplants and lupus nephritis since the 1980s.[11] Mizoribine suppresses the proliferation of synovial fibroblasts[12] and offers clinical advantages to patients with RA. However, the mechanism by which MZR benefits RA patients remains uncertain. To examine the antirheumatic action of MZR, we focused on MMP and tested whether MZR decreased the production of MMP-1 and MMP-3 in human synovial fibroblasts (SFs) and macrophage-like cells matured from the THP-1 cell line.

Materials and methods

Cell culture and measurement of MMP-1 and MMP-3 in the supernatant

THP-1, a human acute myelomonocytic leukemia cell line, was cultured in RPMI supplemented with 10% fetal calf serum (FCS) containing 100 ng/ml of phorbol myristate acetate (PMA; Sigma, Tokyo, Japan) at a concentration of 1×10^5 cells/well in a 24-well plate for 48 h, allowing the cells to mature into macrophage-like cells (THP-1 macrophages). The cells were washed once with the medium and stimulated with 1 μg/ml of lipopolysaccharide (LPS; Sigma) for 48 h. The cells were untreated or treated with 1, 3, 10 and 30 μg/ml of MZR (kindly donated by Asahikasei Pharma, Tokyo, Japan) during the stimulation. In another experiment, the same amount of MZR was added 24 h prior to LPS for pretreatment and coincubated during the stimulation. Human SFs were derived from the synovial tissues of healthy volunteers (CS-ABI-479 cells, purchased from Dainippon Pharmaceutical, Osaka, Japan), cultured using a CS-C complete medium kit (Dainippon), and stimulated with 0.1 ng/ml of interleukin (IL)-1β (R&D Systems, Minneapolis, MN, USA) for 48 h. Mizoribine, at concentrations of 1 and 10 μg/ml, was added 24 h prior to IL-1β or simultaneously, and coincubated during the stimulation. The viability of cells after the stimulation was examined with 0.4% trypan blue staining. The supernatants were stored at −80°C, and the concentrations of MMP-1 and MMP-3 were measured using enzyme-linked immunosorbent assay (ELISA) kits (Fuji Chemical Industries, Toyama, Japan).

Quantitative real-time polymerase chain reaction

mRNA was isolated from the SFs using an RNA extraction kit (Qiagen, Hilden, Germany) and quantified using a spectrophotometer. One microgram of total RNA was reverse transcribed into cDNA for use as a polymerase chain reaction (PCR) template. The RNA samples were then denatured at 65°C for 5 min and reverse transcribed at 42°C for 60 min. The PCR products were analyzed using 2% agarose gel electrophoresis. Quantitative real-time PCR was performed using an ABI Prism 7700 sequence detection system (Biosystems, Foster City, CA, USA), according to the manufacturer's instructions. The β-actin gene was used to control the amount of template in each sample. The PCR amplifications were conducted in 25-μl reactions using 40 cycles with 1 μM of the appropriate primers (forward 5′-CTGAAGGTGATGAAGCAGCC-3′ and reverse 5′-AGTCCAAGAGAATGGCCGAG-3′ for MMP-1,[13] forward 5′-ATGAAGAGTCTTCCAATCCTACTGT-3′ and reverse 5′-CATTATATCAGCCTCTCCTTCATAC-3′ for MMP-3,[14] and forward 5′-GGTCTCAAACATGATCTGGG-3′ and reverse 5′-GGGTGAGAAGGATTCCTATG-3′ for β-actin) and 12.5 μl of SYBR Green Master Mix (ABI). Each cDNA sample was tested in triplicate. The PCR conditions for MMP-1 consisted of a 5-min hot start at 95°C, followed by 40 cycles for 1 min at 95°C and 1 min at 55°C,[13] while those for MMP-3 consisted of a 5-min hot start at 95°C, followed by 40 cycles at 95°C for 45 s and 45 s at 55°C.[14] The results of the real-time PCR were analyzed using the ABI Prism 7700HT sequence detection system.

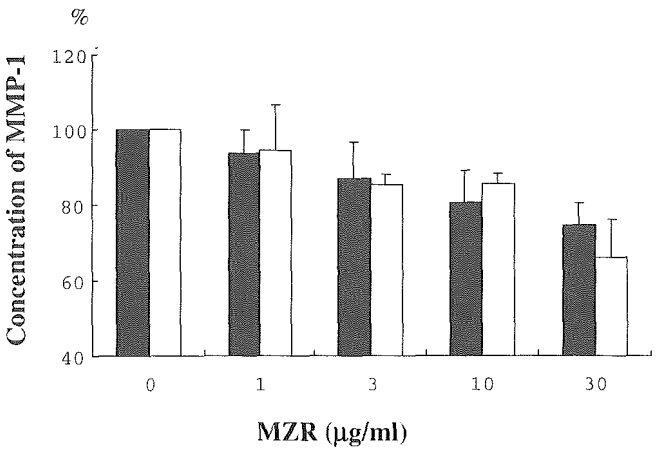

Fig. 1. Effect of mizoribine (*MZR*) on the secretion of matrix metalloproteinase-1 (*MMP-1*) from THP-1 macrophages. Macrophages derived from 1×10^5 THP-1 cells were stimulated with lipopolysaccharide (LPS) for 48 h. The indicated concentrations of MZR were added 24 h prior to LPS (*black columns*) or simultaneously (*white columns*), and coincubated during the stimulation. The concentrations of MMP-1 in the supernatant were measured using an enzyme-linked immunosorbent assay (ELISA). Results were calculated into percentages based on the amount of MMP-1 in the sample without MZR. *Bars* show the mean ± standard deviation (SD) of three independent experiments

Results

Effect of MZR on the secretion of MMP-1 and MMP-3 from stimulated THP-1 macrophages

To examine whether MZR decreases the release of MMP-1 and/or MMP-3 from THP-1 macrophages, we stimulated the cells with LPS in the absence or presence of various concentrations of MZR and measured the concentrations of MMP-1 and MMP-3 in the supernatant using an ELISA. When the MZR and LPS were added simultaneously, the release of MMP-1 was inhibited by approximately 20%–30% in a dose-dependent manner (Fig. 1). The inhibitory effect was also equally observed when the cells were pretreated with MZR prior to LPS stimulation. On the other hand, the release of MMP-3 from the THP-1 macrophages was inhibited much weakly and not dose dependently (Fig. 2). The addition of MZR did not affect the cell viability and form of the cells even at 30 μg/ml, the maximum concentration used in this study.

Effect of MZR on release and mRNA expression of MMP-1 and MMP-3 in stimulated SF

Next, we tested the inhibitory effect of MZR on MMP-1 and MMP-3 secretion from SFs, thought to be a major

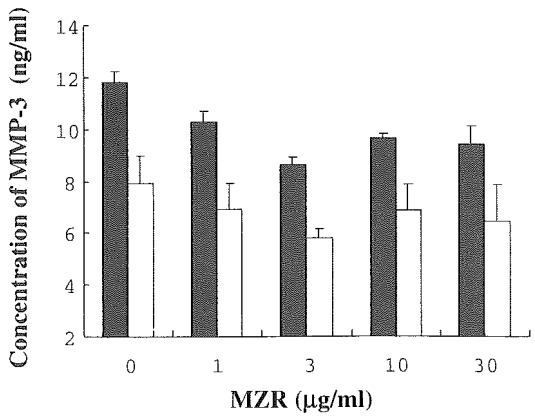

Fig. 2. Effect of mizoribine (*MZR*) on the secretion of matrix metalloproteinase-3 (*MMP-3*) from THP-1 macrophages. THP-1 macrophages were stimulated with LPS for 48h. The indicated concentrations of MZR were added 24h prior to LPS (*black columns*) or simultaneously (*white columns*), and coincubated during the stimulation. The concentrations of MMP-3 in the supernatant were determined using an ELISA. *Bars* express the mean ± SD of three independent experiments

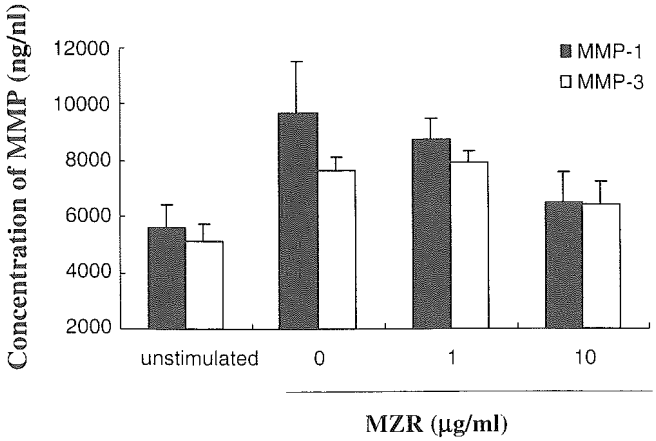

Fig. 3. Effect of mizoribine (*MZR*) on the secretion of matrix metalloproteinase (*MMP*)-1 and MMP-3 from synovial fibroblasts (SFs). The SFs were stimulated with 0.1 ng/ml of interleukin-1β (IL-1β) and cultured for 48h. The indicated concentrations of MZR were added 24h prior to IL-1β and coincubated during the stimulation. The concentrations of MMP-1 and MMP-3 were determined using an ELISA. *Bars* express the mean ± SD of triplicate wells

source of the serum MMPs found in patients with RA. The cells were pretreated with MZR for 24h and stimulated with IL-1β for another 48h. The concentrations of MMP-1 and MMP-3 in the supernatant were then measured using the ELISA. As shown in Fig. 3, MMP-1 secretion from the SFs was inhibited by approximately 60% by the addition of 10μg/ml of MZR. In contrast, the secretion of MMP-3 was not significantly decreased by an equivalent dose of MZR. The viability and form of the cells did not change by addition of MZR.

To further investigate the inhibitory effect of MZR on MMP-1 secretion in stimulated SFs, the expression of MMP-1 mRNA was quantified using real-time PCR. Both protein secretion and the expression of MMP-1 mRNA

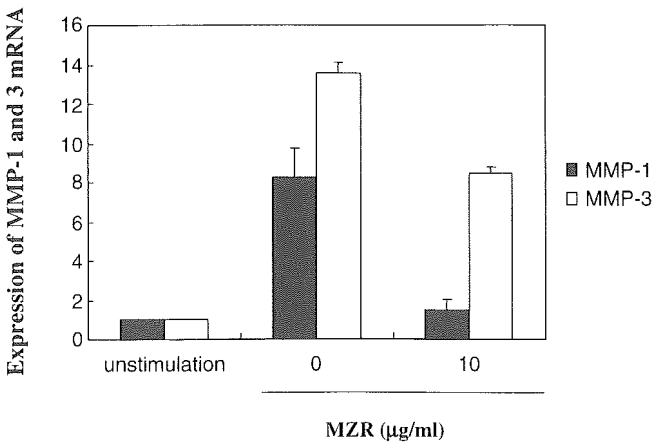

Fig. 4. Effect of mizoribine (*MZR*) on the expression of matrix metalloproteinase (*MMP*)-1 mRNA in SFs. RNA was extracted from SFs treated as described in Fig. 3. The mRNA of MMP-1 and MMP-3 were quantified using real-time polymerase chain reaction. The results were calculated as -fold based on the amount of unstimulated samples. *Bars* indicate mean ± SD in triplicate

were decreased in the presence of 10μg/ml of MZR (Fig. 4), whereas the inhibition of MMP-3 mRNA was weaker.

Discussion

In the inflamed synovial tissue of patients with RA, fibroblast-like synoviocytes and macrophages are the dominant cell populations in areas adjacent to the cartilage–pannus junction,[1] and the secretion of proteolytic enzymes from these cells plays a critical role for cellular invasion and the degradation of cartilage and subchondral bone.[15,16] Among the proteases, the abundant production of MMP-1, which can digest collagen types I, II, III, VI, and X, and gelatins, was first demonstrated at the sites of joint erosion;[17] later, MMP-3 was also reported to be involved in the degradation of articular cartilage and synovium.[18–20] The expression of these protease genes has been observed in tissues obtained only a few weeks after the onset of symptoms,[21] emphasizing the very early potential for joint destruction, and indicating the importance of MMP inhibition in the treatment of RA.

Mizoribine is an imidazole nucleoside isolated from the culture medium of the mold *Eupenicillum brefeldianum* M-2166, found in the soil of Hachijo island, Tokyo, Japan in 1974.[11] Mizoribine was approved in Japan for the clinical treatment of RA in 1992 after the marked amelioration of adjuvant arthritis via the suppression of T-cell function was demonstrated in rats[22] and a low incidence of adverse clinical effects was reported.[23] Moreover, MZR treatment was reported to improve bone lesions in the hind legs of animals with adjuvant arthritis.[24] Here, we examined whether MZR could inhibit MMP-1 and MMP-3 production in stimulated SFs and macrophage-like cells. The concentration of MZR in body fluids has been reported to reach 5–10 μM (approximately equivalent to 2.0–4.0μg/ml) at its peak,[25] and MZR has been shown to partially inhibit MMP-1 production in

vitro at approximately this concentration in THP-1 macrophages and at 3–5-fold higher concentration in SFs. We expected MZR pretreatment to increase the reduction of MMP-1 production on macrophages due to cell growth inhibition; however, such an effect was not recognized. Others have reported that mepacrine, an antimalarial drug, inhibited the release of MMP-1 dose dependently in stimulated human fibroblast-like synoviocytes.[13] Methotrexate also inhibited the synthesis of MMP-1, but not of MMP-3, in SFs.[26] More recently, leflunomide was reported to inhibit the production of MMP-1, MMP-3, and MMP-13 secretion from stimulated SFs, and this effect was suggested to be induced by the suppression of the mitogen-activated protein kinase signaling pathway.[10,27,28] In fact, methotrexate and leflunomide have been reported to be capable of preventing joint destruction in patients with RA.[29-31]

Although the mechanisms responsible for the inhibitory effects of MZR are unclear, nuclear factor-κB (NF-κB)-mediated transcription in synovial macrophages and activator protein 1 (AP-1), prominent in SFs, were reported to induce MMPs synthesis in the synovia of patients with RA,[32-36] suggesting that MZR may inhibit part of the signal transduction pathway conducted to NF-κB and/or AP-1. Several existing drugs, include glucocorticosteroids, gold thiolates, and D-penicillamine, have actions that directly or indirectly inhibit NF-κB and/or AP-1.[37] Moreover, in RA synovial fibroblasts, IL-6 and MMP-1 are regulated by the cyclin-dependent kinase inhibitor p21, and alterations in p21 expression may activate AP-1 leading to enhanced proinflammatory cytokine and MMP production.[38] It was reported that MZR could inhibit IL-6 production in RA synovial fibroblasts,[39] suggesting that MZR may inhibit IL-6 and MMP-1 production in the same signal transduction pathway.

Safety of MZR was assessed between two dosage groups, 150 mg/day and 300 mg/day, of a report from post-marketing surveillance, and no statistical difference was observed (1.3% and 7.1%, respectively).[40] Furthermore, administration of 25 mg/kg per day of MZR induced only mild reduction of splenic lymphocytes, and even 100 mg/kg per day of MZR did not decrease the number of bone marrow cells in mice.[41] In our study, the dose-dependent inhibitory effect of MZR to MMP-1 production was recognized without affecting the cell forms and number of dead cells. These findings indicate that a higher dose of MZR may be tolerated and required to obtain a sufficient clinical effect.

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ORIGINAL ARTICLE

Yoshiaki Tokano · Shinji Morimoto · Hirofumi Amano
Toshiaki Kawanishi · Tetsuro Yano · Masayuki Tomyo
Masahiro Sugawara · Shigeto Kobayashi · Hiroshi Tsuda
Yoshinari Takasaki · Hiroshi Hashimoto

The relationship between initial clinical manifestation and long-term prognosis of patients with systemic lupus erythematosus

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Abstract The relationship between clinical manifestations and prognosis was examined and evaluated among systemic lupus erythematosus (SLE) patients. A total of 542 patients with SLE were selected and divided into nine groups according to their main clinical manifestation at the time of initial diagnosis. The relationship between these clinical manifestations and long-term prognosis was evaluated in respect to the survival, remission, relapse rates, the development of a new clinical manifestation, and/or damage index. Patients with neuropsychiatric SLE (NPSLE), accompanied with acute confusional state/seizure disorder, cerebral vascular disease, or pneumonitis had poor survival rates with cause of death related to their major organ involvement. Patients with nephropathy or leukopenia had lower remission rates, and an increase in relapse rates was frequently recognized in patients with pneumonitis. Body damage (damage index) was higher in patients with lupus psychosis, pneumonitis, and/or arthritis. The translation of the main manifestations after diagnosis was confirmed in 64 patients (11.8%), and often observed in patients with autoimmune hemolytic anemia and arthritis. The majority of these manifestations were nephropathy, NPSLE, thrombocytopenia, and pneumonitis, and the prognosis of patients with nephropathy and thrombocytopenia as a new main manifestation had a poor outcome. The results of long-term prognosis in SLE greatly differed with respect to the initial clinical manifestation at the time of diagnosis.

Key words Clinical manifestation · Damage index · Relapse · Survival · Systemic lupus erythematosus (SLE)

Y. Tokano · S. Morimoto (✉) · H. Amano · T. Kawanishi · T. Yano · M. Tomyo · M. Sugawara · S. Kobayashi · H. Tsuda · Y. Takasaki · H. Hashimoto
Department of Rheumatology and Internal Medicine, Juntendo University School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan
Tel. +81-3-3813-3111 (ext. 3315); Fax +81-3-5800-4893
e-mail: morimoto@med.juntendo.ac.jp

Introduction

The prognosis for systemic lupus erythematosus (SLE) has greatly improved in the last two decades.[1–11] Some studies have reported of more than 80% survival rate of 10 years for SLE.[9,11] These improvements in prognosis were related to various factors. For example, the development of therapy[12,13] revealed by the use of steroid or immune suppressive agents, the increase in the number of patients with mild disease,[2,11] having only leukopenia or skin involvement without organ involvement, are considered. Also, these improvements have altered the cause of death. For example, SLE-related cause of death has decreased, while SLE-unrelated cause of death has increased.[11,14] Thus, a number of factors are related to the change in the outcome of prognosis.

Some studies have discussed these factors related to prognosis, for example, the relation to clinical manifestations,[1,3,8,9,15–24] age,[5,25] race,[7,17] and socioeconomic status.[25–28] Concerning clinical manifestations, nephritis or central nervous involvement (so-called neuropsychiatric SLE: NPSLE) have been known to have a poor prognosis, and many studies concerning lupus nephritis have been specifically reported,[29–34] while other studies have reported on the relationship with other involvements including thrombocytopenia, hemolytic anemia, serositis, and antiphospholipid syndrome.[3,9,18–21,24] These studies revealed the relationship with the prognosis possessed by each factor involved, including patients with relapse or the translation of the main manifestation after diagnosis. However, since patients with SLE have multiple organ involvement, the therapy administered is based according to the most severe manifestation. Also, there was a difference in prognosis between new-onset cases and cases with relapse or the translation of the main manifestation. Therefore, when long-term prognosis is discussed, the major problems of importance are how the manifestation at onset or initial diagnosis is related to the prognosis or cause of death, and how many patients have had a translation of their main manifestations after diagnosis. Since previous reports did

not consider these points, we cannot simply estimate their results in heterogeneous SLE patients. Furthermore, recent prognosis of SLE has also been assessed by factors other than the survival rate, for example, the rate of remission or relapse and body damage,[35] and the importance of these factors have been pointed out.

In this study, we evaluated the long-term prognosis of SLE patients divided according to organ involvement at the time of diagnosis, and discussed their relationship with the cause of death, translation of the main manifestation after diagnosis, or relapse and body damage.

Patients and methods

Patients

Patients in this study were examined and evaluated either as an outpatient or as an inpatient at Juntendo Hospital between 1970 and 1996. The medical records at initial diagnosis of patients who fulfilled the criteria of the American College of Rheumatology (ACR)[36] were retrospectively reviewed, and patients with a follow-up period of more than 2 years and all deceased patients were included. Regarding the results, a total of 542 patients were enrolled in this study. Two hundred patients between 1970 and 1979, 277 patients between 1980 and 1989, and 65 patients between 1990 and 1996 were enrolled. For analysis, these patients were divided into nine groups according to their major clinical manifestation confirmed with the ACR criteria at the time of diagnosis, as shown in Table 1. Generally, the major clinical manifestation was defined as the most severe manifestation, and patients with antiphospholipid antibody were excluded, since this antibody has a specific influence on prognosis. Also, patients with thrombotic thorombocytopenic purpura (TTP) and hemophagocytic syndrome (HPS) were excluded, since the number of such patients is extremely limited and the clinical manifestation and prognosis is unique. Although some patients with severe organ involvement (groups I–V) also had other manifestations, the most important involvement for steroid treatment was defined as the major clinical manifestation. Patients without organ involvement (groups VII–IX) were further divided into three groups by their main clinical manifestation, according to their order of importance in selection of treatment, as shown in Table 1. The profile of the clinical manifestation in these nine groups (groups I–IX) is summarized in Table 1. Patients with NPSLE (group I) were further divided into two groups: patients with neurological disorder (24 cases) and those with the so-called lupus psychosis (10 cases). The former was further subdivided into: patients with acute confusional state/seizure disorder (so-called organic brain syndrome) (13 cases), cerebral vascular disease (CVD) (4 cases), cranial nerve disturbance (1 case), peripheral neuropathy (3 cases), and so-called lupus headache (3 cases). Patients with thrombocytopenia (group III) had less than 50000/mm^3 platelet count and received high-dose steroid treatment. Among patients with serositis

Table 1. Definition and frequency of each group

Manifestations	Group								
	I	II	III	IV	V	VI	VII	VIII	IX
(With severe organ involvement)									
NPSLE (I)	○	×	×	×	×	×	×	×	×
Thrombocytopenia (II)†	△ (14.7)††	○	×	×	×	×	×	×	×
Pneumonitis (III)	△	△	○	×	×	×	×	×	×
Serositis (IV)	△	△	△ (18.2)	○	×	×	×	×	×
AIHA (V)	△ (2.9)††	△ (12.5)	△	△	○	×	×	×	×
Nephropathy (VI)	△ (91)†††	△ (62.5)	△ (27.5)	△ (47.8)	△ (5.9)	○	×	×	×
(Without organ involvement)									
Leukopenia (VII)	△ (55.8)	△ (79.1)	△ (63.6)	△ (73.9)	△ (70.5)	△ (61.3)	○	×	×
Skin involvement (VIII)	△ (73.5)	△ (33.3)	△ (27.2)	△ (43.2)	△ (29.4)	△ (68.3)	△ (58.6)	○	×
Joint (IX)	△ (64.7)	△ (70.8)	△ (45.6)	△ (73.9)	△ (70.5)	△ (80.3)	△ (74.1)	△ (74.7)	○
No. of patients	34	22	11	23	17	259	58	91	27
% (total patients)	6.3	4.1	2.0	4.2	3.1	47.8	10.7	16.8	5.0

NPSLE, neuropsychiatric systemic lupus erythematosus; AIHA, autoimmune hemolytic anemia
○, present in all patients and main manifestation; △, present in some patients (%); ×, not present
† Patients with this manifestation with a platelet count of less than 50 000/mm^3 were required to receive high dose steroid therapy
†† Patients with NPSLE and thrombocytopenia or AIHA demonstrated acute confusional state or lupus psychosis
††† Patients with mild NPSLE (cranial nerve disturbance, peripheral neuropathy and lupus headache) had no nephropathy or mild nephropathy (intermittent proteinuria)

(group IV), 5 had pericarditis and 1 had peritonitis. Patients with only renal involvement (group VI) were divided into two groups: patients who underwent renal biopsy examination and those who did not. The former were further divided by the classification of renal pathology for lupus nephritis proposed by the World Health Organization[37]: type I, 6 cases; type II, 35 cases; type III, 12 cases; type IV, 39 cases; and type V, 14 cases. The latter were further divided into three groups: patients with intermittent proteinuria, persistent proteinuria (<3.5 g/day), or nephritic syndrome (≥3.5 g/day). Other organ involvements in groups I–V are tabulated in Table 1. The patients with a mild type of NPSLE (group I) had no renal involvement (data not shown). Although some patients with thrombocytopenia had nephropathy, not all patients underwent renal biopsy due to bleeding tendency. The pathology of patients with serositis (group IV) who underwent renal biopsy were of WHO type III (1 case) or type IV (3 cases), and that of patients with pneumonitis (group III) were of WHO type III (1 case) or type IV (2 cases). One patient in group IV had pancreatitis.

Clinical manifestations presented at any time during the follow-up period for each patient were recorded. Although these manifestations were generally defined according to the SLE classification criteria,[36] some specific examinations were also performed. For example, electroencephalography, cerebral spinal fluid, magnetic resonance imaging, and single photo emission computer tomography were performed for the diagnosis of NPSLE, and pulmonary function test, chest computer tomography, ultra cardioechography were utilized for the diagnosis of serositis or pneumonitis.

The treatment generally administered was high-dose steroid therapy (more than 1 mg/kg per day of prednisolone) for patients with severe organ involvement (NPSLE with acute confusional state/seizure disorder or cerebral vascular disease, thrombocytopenia, autoimmune hemolytic anemia [AIHA], or pneumonitis), and severe nephropathy (WHO type III or IV nephritis or nephritic syndrome), medium-dose steroid therapy (0.5–1 mg/kg per day of prednisolone) for moderate organ involvement (WHO type V nephritis, lupus psychosis, serositis), and low-dose steroid therapy (less than 0.5 mg/kg per day of prednisolone) for mild organ involvement (leukopenia, skin involvement, joint involvement). Some patients with steroid resistance, severe adverse reaction, or difficulty in tapering of steroid administration received immunosuppressive agents (azathioprine, oral or intravenous cyclophosphamide).

Definition of remission, relapse, and the development of new clinical manifestation after diagnosis

"Remission" was determined within 6 months after the diagnosis, and defined as follows. In patients with nephropathy, the criteria described in our previous report[13] were utilized. In patients with NPSLE, the improvements in patient symptoms and findings of cerebral spinal fluid or electroencephalography were used. In patients with thrombocytopenia, AIHA, and leukopenia, improvement of laboratory data was used (platelet > 100000/mm^3, white blood cell > 4000/mm^3, Hb > 10.0 g/dl). In patients with serositis or pneumonitis, improvements seen on chest X-ray, blood oxygen level, echocardiography, or computer tomography were used. In skin or joint involvement, the improvement of symptoms was used.

"Relapse" was defined by the reproduction of the initial manifestations which once improved and the increase in the dose of steroid administered. Relapse was recorded throughout the course if this definition was fulfilled. "The translation of a main manifestation after diagnosis" was defined as the appearance of a new manifestation after more than 6 months, and other involvement that appeared within 6 months from onset was classified as an initial manifestation.

Body damage

Body damage was estimated by the damage index described in the previous report.[35] Body damage was determined by the maximum damage index throughout the course.

Serological data

CH_{50} was determined using Mayer's method. Anti-DNA antibody was determined with Farr's assay, and anti-U1 RNP and Sm antibody were determined with the double immune diffusion method. The thrombocytic antibody count was determined with the positive reactivity to platelet or the increase of platelet adhered IgG (PAIgG).

Statistical analysis

Analysis of the survival rate was determined with the life table method, and differences among groups were compared using the generalized Wilcoxon test. The differences in rate of remission, relapse, and the development of new clinical manifestations were determined with the χ^2 test. The difference in the damage index was determined with the rank-sum test.

Results

Survival rate for each manifestation

Eighty-six of 542 patients (15.9%) died, and 61 patients (11.3%) died due to SLE-related causes. Twenty-five patients (4.6%) died due to SLE-unrelated causes, and the cause of death in 11 patients (2.0%) was related to secondary complications associated with treatment (infection and atherosclerosis). Sixteen patients with NPSLE (group I), 38 patients with nephropathy (group VI), and 32 patients with other manifestations (groups II–V, VII–IX) died. The survival rates in these groups were evaluated at 2, 5, 10, 15, and 20 years.

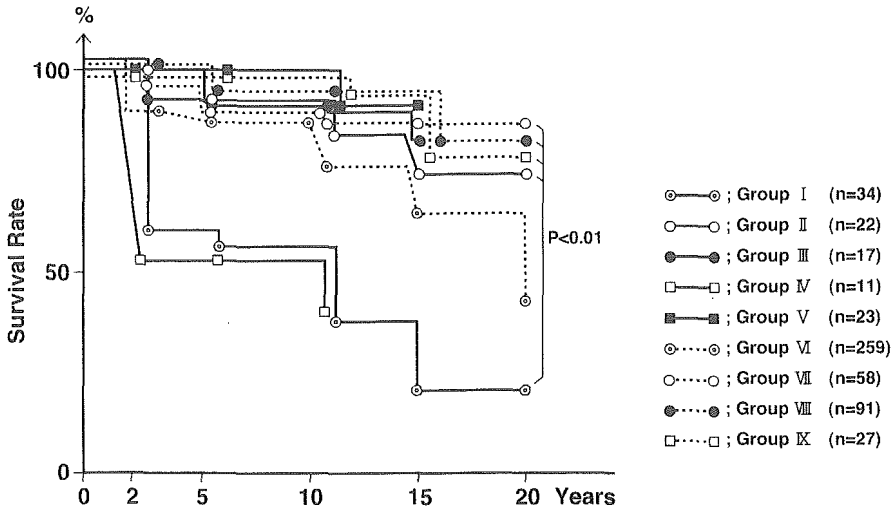

Fig. 1. Survival of patients in the nine groups described. The survival rate of patients with NPSLE (group I) and pneumonitis (group IV) was significantly lower than the other groups ($P < 0.01$)

The survival rates of each manifestation are shown in Fig. 1. Patients with NPSLE (group I) or pneumonitis (group III) had a poor prognosis, and the survival rate significantly decreased as compared with other groups (groups I, III versus groups II, IV, V, VI, VII, VIII, IX: $P < 0.01$). Patients with thrombocytopenia (group II), serositis (group IV), and AIHA (group V) generally had a good prognosis, similar to those with leukopenia (group VII), skin involvement (group VIII), or joint involvement (group IX). Among some groups, we evaluated the prognosis chronologically (1970–1979, 1980–1989, 1990–1996). However, no differences were revealed.

On the other hand, among patients with NPSLE (group I), patients with acute confusional state/seizure disorder and CVD had a poor prognosis, and the survival rates significantly decreased when compared to cranial nerve disturbance, peripheral neuropathy, or headache ($P < 0.05$: data not shown). Also, patients with psychosis had a moderately poor prognosis, although there was no significance. Although the survival rate of patients with nephropathy showed no significant difference in comparison with other groups, the survival rate of patients with WHO type III or nephritic syndrome significantly decreased when compared with other groups ($P < 0.01$: data not shown). However, when patients after 1980 were evaluated, the significance disappeared.

Cause of death for each manifestation

Next, the causes of death for each manifestation were evaluated. In patients with NPSLE, the most common cause of death in cases with acute confusional state/seizure disorder (10 cases) or CVD (2 cases) was convulsion or brain hemorrhage (11 cases) during the early stage of the disease course (less than 3 months), while that of patients with other manifestations (4 cases) was uremia (1 case) during the early stage, pneumonitis (1 case) as a development of a new manifestation, or SLE-unrelated cause of death (acute myocardial infarction [1 case], suicide [1 case]). The causes of death in patients with thrombocytopenia (group II; 4 cases) were bleeding (brain hemorrhage [1 case], gastrointestinal (GI) bleeding [1 case]), NPSLE (1 case), or pneumonitis (1 case) as the translation of the main manifestation. Among patients with pneumonitis (group III; 5 cases), the causes of death were pneumonitis (3 cases) or infections (2 cases), which mainly consisted of Carini pneumonia. Among patients with serositis (group IV; 2 cases), the causes of death were uremia (1 case) or pneumonitis (1 cases) as a translation of manifestation. Among patients with AIHA (group V; 2 cases), the causes of death were NPSLE (1 case) as a translation of a main manifestation, or infection (1 case). Among patients with nephropathy, the cause of death in 16 patients with WHO type III, type IV, persistent proteinuria, or nephritic syndrome was mainly uremia (14 cases), while only a few patients with intermittent proteinuria (1 case) and no patients with WHO type II or type V died due to uremia. The majority of the deaths caused by uremia occurred within 2 years after initial diagnosis, and were recorded in patients prior to 1980. Causes other than uremia were various. SLE-related causes were mainly identified in patients with WHO type III or type IV, or those without renal biopsy, and included pulmonary hypertension (PH), acute pneumonitis, pulmonary hemorrhage, and NPSLE. Most of these events occurred as the translation of a main manifestation. On the other hand, SLE-unrelated causes were mainly identified in WHO type I, type II, or type V, and included heart failure, infections, CVD, GI bleeding, malignancy, or suicide.

Patients without organ involvement (groups VII–IX; 19 cases) died due to various lesions associated with translation of the main manifestation (uremia [5 cases], NPSLE [1 case], PH–lung infarction [2 cases], vasculitis [1 case], intestinal perforation [2 cases]), or SLE-unrelated cause of death (infections [4 cases], heart failure [1 case], CVD [1 case], asthma [1 case]).

Remission, relapse, and body damage for each manifestation

The rates of remission within 2 years and those of relapse during 20 years of follow-up observation were evaluated for each manifestation, with the exception of patients who had

Table 2. Rate of remission, relapse, and damage index with each manifestation

Group[†]	Remission cases[a]	Relapse cases[b]	Damage index[b]
I (n = 19)	16 (84.2%)	2 (10.5%)	0.19 ± 0.4[e]
II (n = 20)	15 (75.0)	1 (5.0)	0.07 ± 0.26
III (n = 5)	3 (60.0)	2 (40.0)[d]	0.6 ± 0.54[e]
IV (n = 16)	15 (93.4)	0 (0)	0.04 ± 0.2
V (n = 13)	11 (84.6)	1 (7.7)	0.05 ± 0.22
VI (n = 233)	112 (48.1)[c]	25 (10.7)	0.11 ± 0.43
VII (n = 44)	21 (48.8)[c]	0 (0)	0.02 ± 0.13
VIII (n = 51)	33 (64.7)	0 (0)	0.08 ± 0.38
IX (n = 22)	16 (72.7)	3 (13.6)	0.18 ± 0.62[e]

[†] 119 patients who died within 2 years after onset were excluded, and the remaining 423 patients were included in these figures. The numbers indicate enrolled patients in each group
[a] The rate was calculated after 2 years
[b] The rate was calculated at the end point of the 20-year follow-up
[c] Significantly lower rate (VI/VII versus I/IV/V: $P < 0.01$)
[d] Significantly higher rate (III versus IV/VII/VIII: $P < 0.01$, II: $P < 0.05$)
[e] Significantly higher level (I versus IV/V/VII: $P < 0.001$; III versus II/IV/V/VII: $P < 0.001$; III versus VI/VIII: $P < 0.01$; IX versus VII: $P < 0.05$)

Table 3. Translation of a main manifestation in each group

New manifestation	Group (total number)									
	I (34)	II (22)	III (11)	IV (23)	V (17)	VI (259)	VII (58)	VIII (91)	IX (27)	Total (%)[a]
NPSLE		2			2	6	1	2	2	15 (30.6%)[d]
Thrombocytopenia				1		5			4	10 (31.3%)[d]
Pneumonitis	1	1				3				5 (31.3%)[d]
Serositis						1	1	1		3 (11.5%)
Nephropathy				4	3		6	10	3	26 (9.1%)
Others[b]						1				1
Total (%)	1 (2.9)	3 (13.6)	0 (0)	5 (21.7)	5[c] (29.4)	16 (7.3)	8 (13.7)	13 (14.3)	9 (33.3)	64 (11.8)

[a] The percentages indicate the number of new manifestations/this number + the number of this involvement at initial diagnosis
[b] Ulcerative colitis
[c] Significantly high ratio (V/IX versus I/VI: $P < 0.05$)
[d] Significantly high ratio (NPSLE, thrombocytopenia, and pneumonitis versus nephropathy: $P < 0.001$)

died within 2 years after onset. As shown in Table 2, group VI (nephropathy) and group VII (leukopenia) had significantly low rates of remission as compared to the other groups. In group VI, patients with WHO type V, intermittent proteinuria, and persistent proteinuria had low rates (data not shown). The low rate in group VII (leukopenia) was related to the fact that the leukocyte count as a parameter did not change significantly.

On the other hand, the rate of relapse was most significant in group III (pneumonitis) (Table 2). Although some patients in group VI (nephropathy) had relapse, these relapses were most frequently identified in patients with WHO type V.

Body damage was evaluated according to the damage index. As shown in Table 2, the patients in groups I (NPSLE), III (pneumonitis), and IX (arthritis) had a significantly higher damage index than the other groups. In group I, patients with psychosis had higher averages, and psychotic complications were related to these high averages. In group III, respiratory failure was related to the high average. Also, patients who received hemodialysis (HD) had higher averages in group VI. On the other hand, some patients had body damage related to adverse effects or complications (cataracts, angina, osteoporosis, avascular necrosis, osteomyelitis, premature gonadal failure, diabetes, and malignancy), and the majority of these patients were elderly and received high-dose steroid therapy. There was no relationship revealed between these adverse effects or complications and each group.

Translation of the main manifestation

The translation of the main manifestation was identified in 64 patients (11.8%), and most frequently recognized in groups IV (AIHA) and IX (arthritis), as shown in Table 3. The frequency of a new manifestation was significantly greater in NPSLE, thrombocytopenia, and pneumonitis, and the number was greatest in nephropathy (Table 3).

We also evaluated the prognosis of these patients, and the result was compared with those of patients with the same manifestation at initial diagnosis. As shown in Fig. 2A, patients with nephropathy as the translation of the main manifestation had a significantly poor prognosis ($P < 0.01$), and the majority of these patients originally belonged to

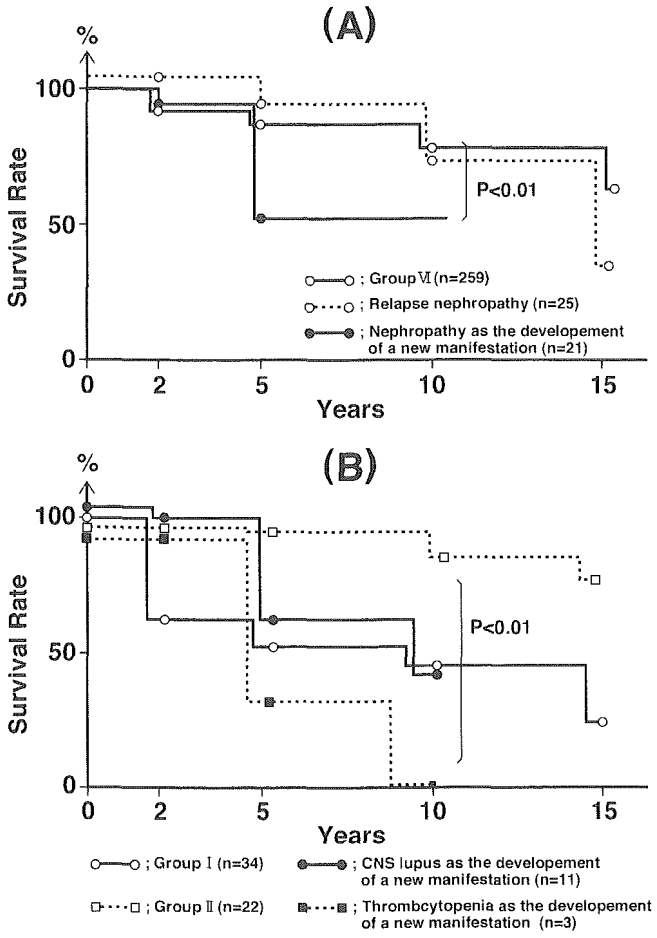

Fig. 2A,B. Survival rate of patients with translation of main manifestations. Patients followed for more than 2 years were selected in this comparison. **A** The prognosis of patients with nephropathy as the translation of a main manifestation. Twenty-five patients in group VI who had a relapse in the course were selected as relapse nephropathy for control. Patients with nephropathy as a new manifestation had a significantly worse prognosis than those with nephropathy at initial diagnosis (group VI) ($P < 0.01$). **B** The prognosis of patients with neuropsychiatric systemic lupus erythematosus (NPSLE) or thrombocytopenia as the translation of a main manifestation. Patients with thrombocytopenia as a new manifestation had a significantly worse prognosis than patients who initially had thrombocytopenia (group II) ($P < 0.01$), while in patients with NPSLE as a new manifestation, a similar prognosis to those with initial NPSLE was observed

groups VII (leukopenia), VIII (skin involvement), and IX (joint involvement). Concerning NPSLE, the difference in prognosis with translation of main manifestation was not recognized (Fig. 2B). With thrombocytopenia, patients with translation of the main manifestation had a significantly poor prognosis ($P < 0.01$) (Fig. 2B).

Factors related to translation of the main manifestation

Finally, we evaluated the factors related to the translation of main manifestation. First, we focused on the clinical data within 2 years from onset. As shown in Table 4, patients with translation of the main manifestation tended to have higher levels of anti-DNA antibodies, although there was no significance. There was also no significance in anti-U1 RNP, Sm antibody, and CH_{50} (Table 4). Although there was no significance in remission, some patients had the worst symptoms concerned with the original manifestation prior to the translation of a main manifestation.

Discussion

This single-center study is important from the standpoint that the majority of the patients received a uniform, standard form of treatment. The first characteristic of this study is the fact that the prognosis was estimated according to the clinical manifestation at the time of initial diagnosis. Previous studies enrolled patients with various disease courses or manifestations. However, we believe that the relationship between prognosis and organ involvement should be discussed as the main clinical manifestation at the time of initial diagnosis, since it is possible that some patients might have multiple organ involvement, and that the most critical manifestation among these involvements is related to the prognosis. We therefore divided these patients according to the main clinical manifestation for the investigative purpose of this study. As to the results, we were able to reveal that the prognosis and the cause of death greatly differed among these subclassification groups. For example, in patients with NPSLE, especially those with acute confusional state/seizure disorder or CVD type, had a poor prognosis and the death was caused by NPSLE-related factors. These results support the view that patients with acute confusional state/seizure disorder or CVD type require intensive therapy, as previously reported.[38] However, the prognosis of patients with recent onset of acute confusional state/seizure disorder or CVD type did not reveal any improvement, although steroid pulse therapy for these cases had been established. Thus, the management for acute confusional state/seizure disorder or CVD type of NPSLE still remain as an important issue, although some reports[22,23] neglect the relationship between the prognosis and NPSLE. Furthermore, patients with pneumonitis also had a worse prognosis, resulting in death due to this manifestation. This result suggests that the management of pneumonitis also remains as an important issue. On the other hand, patients with nephropathy did not have a relatively poor prognosis. Certainly, some patients with WHO type III or nephritic syndrome had a poor prognosis, resulting in death due to uremia. However, the majority of the deceased patients was evaluated within the last 20 years, and the deaths due to uremia have markedly decreased with the development of HD. Although previous reports have stressed nephritis as the risk factor of survival,[1,3,4] at least death due to uremia has not been a major problem in recent times. Rather, interestingly, some patients with WHO type III or type IV, or those without renal biopsy had nonrenal causative factors of death with the translation of the main manifestation, while most patients with WHO type I or type II died due to SLE-unrelated causes. This result suggests that care for other

Table 4. Clinical data of patients with or without the translation of a main manifestation

	With translation (n = 23)	Without translation (n = 60)	Significance
CH_{50} (U)			
Initial diagnosis	30.8 ± 8.5	29.3 ± 8.7	n.s.
After 2 years	27.4 ± 7.2	28.5 ± 7.1	n.s.
Anti-DNA antibody (IU/ml)			
Initial diagnosis	50.1 ± 83.6	20.5 ± 23.4	n.s.
After 2 years	47.1 ± 52.5	32.9 ± 79.6	n.s.
Anti-Sm antibody (+)	16.7%	14.6%	n.s.
Anti-U1-RNP antibody (+)	38.5%	39.0%	n.s.
Remission rate (after 2 years)	43.5%	39.3%	n.s.

severe organ involvement is required during the disease course of patients with severe nephropathy, and that SLE-unrelated cause of death is an important risk in those with mild nephropathy, even though the nephritic condition may have improved. Even if the patient suffered from renal failure and received HD, some patients may die due to nonrenal factors (infection, etc.), so specific care is required. Furthermore, concerning other manifestations the prognosis of patients with thrombocytopenia, AIHA, and serositis had a better prognosis, although some previous reports have pointed out the importance of thrombocytopenia.[18,19,22,23,35] This discrepancy may be related to the fact that patients with antiphospholipid antibody, TTP, and HPS were included in these previous studies. Indeed, our study reveals that these patients have specific results concerning the survival rate, remission, relapse, and body damage (data not shown). Therefore, we believe that patients with thrombocytopenia had a good prognosis except those with antiphospholipid antibody, TTP, and HPS. Concerning the cause of death, most patients with thrombocytopenia died due to bleeding tendency. Although patients with severe organ involvement had other involvements, the prognosis tended to be influenced by the main clinical manifestation, and this manifestation, not unexpectedly, is of major importance.

The second characteristic of this study is the fact that we also focused on remission, relapse, body damage, and the translation of the main manifestation, and related them to the prognosis. Although low rates of remission and high rates of relapse were recognized in patients with WHO type V nephropathy or pneumonitis, other manifestations were revealed to have generally excellent results, suggesting that the present therapy generally induces efficient remission. However, the problem concerning the translation of main manifestation cannot be ignored. In this study, the translation of main manifestation was recognized in 11.8% of the patients, and was frequently recognized in patients with AIHA, serositis, and leukopenia as the main manifestation at the time of initial diagnosis. Also, the prognosis of patients with translation was generally worse when compared with that of patients with the same manifestation at the time of initial diagnosis. These results suggest the importance of management for cases with translation. Although prediction for having the translation is difficult, worsening of symptoms and the change in the anti-DNA antibody level may be an important clue. Moreover, we cannot ignore the possibility that the high frequency in patients with mild involvement at initial diagnosis is related to the low dose of steroid therapy. However, we do not believe that this possibility requires intensive therapy, since most of these patients were cured by low-dose steroid therapy. On the other hand, differences were also identified concerning body damage among these groups. For example, patients with lupus psychosis or pneumonitis have a higher level of body damage. This result suggests that management of body damage and quality of life is also required for these manifestations. Since the type of damage is related to each manifestation, a variety of management protocols may be required for body damage of each manifestation. Most parameters of disease activity, for example, SLEDAI (Systemic Lupus Erythematosus Disease Activity Index), tend to emphasize only a limited lesion, while our damage index covers various lesions. Therefore, this index was useful in this study. However, the problem is that this index contains items concerning adverse effects. Indeed, we ignored this influence, even though the result concerning the damage index did not change when we excluded this item concerning the adverse effect.

The prognoses of our SLE patients were generally favorable, and further, intravenous cyclophosphamide[39] or cyclosporine A[40] have recently contributed to the improvement of prognosis in treatment-resistant nephropathy or pneumonitis cases. Home oxygen therapy has also contributed to the improvement in quality of life in patients with pneumonitis. However, some patients with these manifestations still have poor outcomes, resistance to treatment, and poor quality of life, and treatment and management of these patients are important issues to consider in the future. Although the rate of death was not so high, the management for adverse reactions to treatment is also an important issue. In addition, it is also important that some patients had a poor prognosis due to lack of comprehension of the disease or treatment, and some previous studies pointed out the importance of socioeconomic status in the prognosis of SLE.[25-28] This problem is very difficult and may remain as a topic of concern for a long time in the future. Lastly, we would like to stress that the establishment of a concrete diagnosis of the clinical manifestation and the selection of treatment for this manifestation are important issues for the management of SLE.

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