Table 1. Background characteristics of the patients with CTDs complicated by PCP

Patient No.	Sex/Age (years)	Underlying CTDs			
		Disease	Duration (years)	Organ involvement	Treatment
1	M/52	SLE	3	NP lupus	Daily PSL 20 mg, CsA 200 mg/day
2	F/37	SLE	11	NP lupus, NS	Daily PSL 1.2 mg/kg bw, mPSL pulse[a] × 3
3	F/69	SjS, MPA	0.3	MM, IP (inactive)	Daily betamethasone 8.5 mg, betamethasone pulse[b] × 1
4	M/69	RP	0.1	Chondritis	Daily PSL 1.2 mg/kg bw
5	F/68	SjS, MPA	13	IP (inactive), GN	Daily PSL 1.2 mg/kg bw, mPSL pulse[a] × 3
6	M/72	RA	1	Pleuritis	Daily PSL 1.2 mg/kg bw, mPSL pulse[a] × 3, CPA pulse[c]
7	F/61	DM	3	IP (active)	Daily PSL 1.2 mg/kg bw, mPSL pulse[a] × 3, CPA pulse[c]
8	F/53	SLE, RA, SjS	14	Protein-losing enteropathy	Daily PSL 1.2 mg/kg bw, mPSL pulse[a] × 3
9	F/33	SLE, SjS	4	Arthritis, amyloidosis	Daily PSL 6 mg, weekly MTX 4 mg

CTDs, connective tissue diseases; PCP, *Pneumocystis carinii* pneumonia; M, male; F, female; SLE, systemic lupus erythematosus; SjS, Sjögren's syndrome; MPA, microscopic polyangiitis; RP, relapsing polychondritis; RA, rheumatoid arthritis; DM, dermatomyositis; NP, neuropsychiatric; NS, nephrotic syndrome; MM, mononeuritis multiplex; IP, interstitial pneumonitis; GN, glomerulonephritis; PSL, prednisolone; CsA, cyclosporine; mPSL, methylprednisolone; bw, body weight; CPA, cyclophosphamide; MTX, methotrexate

[a] Pulsed methylprednisolone (mPSL) of 1 g/day for three days

[b] Pulsed betamethasone of a dose equivalent to 1 g of mPSL for three days

[c] Pulsed cyclophosphamide of 500 mg/body weight

Patients and methods

Patients

Patients with CTDs admitted to our department from 1978 to 2003 were enrolled in this study. The diagnoses of CTDs were based on the criteria listed in several references.[18–23] PCP was confirmed when Grocott-Gomori methenamine-silver staining (PC-specific immunostaining) revealed the presence of PC in the bronchoalveolar lavage fluid (BALF) or transbronchial lung biopsy. PCP was presumed when a patient with respiratory distress and an increased serum β-D-glucan level without any other systemic fungal infection had some of the following features highly indicative of PCP: positivity for PC in the sputum or BALF determined by polymerase chain reaction (PCR) analysis, geographical ground-glass opacities revealed by chest computed tomography (CT), and a good response to PCP treatment. Patients with a confirmed or presumed diagnosis of PCP were examined in this study.

We examined the background characteristics; physical, radiological, and laboratory findings of the patients at the time of the PCP onset; the treatment courses and outcomes of PCP; and data on whether the patients received primary or secondary prophylaxis for PCP. Other bacterial, mycobacterial, viral, or fungal infections that developed during the pre-PCP to post-PCP courses were also documented.

Statistical analysis

Peripheral blood lymphocyte count, serum immunoglobulin G (IgG) level, and serum albumin level at PCP onset were compared with those at the start of high-dose steroid therapy or those during the maintenance therapy before the PCP onset. The analyses were performed using the paired *t*-test. Statistical analysis was performed using the standard software package Statview version 5.0 for Windows (Statview, Berkeley, CA, USA). $P < 0.05$ was considered significant.

Results

Background characteristics of patients with CTDs

Of 1042 admitted patients with CTDs, 9 (6 women, 3 men; mean age ± SD: 57.1 ± 14.4 years) who contracted PCP were examined in this study (Table 1). The patients neither received an organ transplant nor had HIV infection. The underlying CTDs are listed in Table 1. The disease durations of CTDs ranged from 0.1 to 14.0 years. The estimated incidences of PCP among patients with CTDs hospitalized in our department from 1978 to 2003 were as follows: 1 of 4 patients (25.0%) with relapsing polychondritis, 2 of 65 patients (3.1%) with vasculitic syndrome, 4 of 220 patients (1.8%) with SLE, 1 of 65 patients (1.5%) with myositis, and 1 of 362 patients (0.2%) with RA. Four patients with PCP had secondary Sjögren's syndrome; no patient with primary Sjögren's syndrome ($n = 130$) contracted PCP. The organs involved by the underlying CTDs are listed in Table 1. Among them, four patients had respiratory involvement: inactive interstitial pneumonitis was seen in two and active interstitial pneumonitis or pleuritis in one each. PCP was diagnosed in patient 1 during maintenance steroid and cyclosporine therapy and in patient 9 during maintenance steroid and weekly low-dose methotrexate therapy. Patients 2–8 received high-dose steroid (prednisolone) therapy equivalent to 1.2 mg/kg body weight daily; except for patient 4, they also were given intravenous pulsed steroid therapy. In patients 2–8, PCP was diagnosed after 6–16 weeks (9.7 ± 3.4 weeks) of high-dose steroid therapy. Among them, two of them had additional pulsed cyclophosphamide therapy. Pulsed intravenous steroid and cyclophosphamide therapies were repeated biweekly.

Table 2. Diagnosis, onset, and clinical characteristics of PCP in patients with CTDs

Patient No.	PCP diagnosis	Onset after high-dose steroid (weeks)	Clinical manifestations	Preexisting lung disease	Finding on auscultation	Interval from onset to diagnosis (days)
1	PCR (sputum, BALF)	During maintenance therapy	Fever, breathlessness	None	Crackle	3
2	Clinical diagnosis	8	Fever	None	Normal	7
3	Clinical diagnosis	6	General fatigue	IP	Crackle	2
4	Clinical diagnosis	8	Fever, breathlessness	None	Crackle	1
5	Cytology (BALF)	10	Symptomless	IP	Crackle	14
6	PCR (sputum)	16	Fever, breathlessness, dry cough	Pleuritis	Crackle	3
7	PCR (BALF)	12	Fever, breathlessness, dry cough	IP	Crackle	10
8	PCR (BALF)	8	Fever, breathlessness	None	Normal	12
9	Cytology (BALF, TBLB), PCR (BALF)	During weekly low-dose MTX therapy	Fever, breathlessness, dry cough	None	Normal	7

PCP, *Pneumocystis carinii* pneumonia; CTDs, connective tissue diseases; PCR, polymerase chain reaction; BALF, bronchoalveolar lavage fluid; TBLB, transbronchial lung biopsy; MTX, methotrexate; IP, interstitial pneumonitis

Table 3. Laboratory data at the PCP onset

Patient No.	WBC (/μl) [3700–9000]	LDH (IU/l) [120–220]	β-D-glucan (ng/l) [<20]	KL-6 (U/ml) [<550]	SP-D (ng/l) [<110]	CRP (mg/dl) [<0.3]	ESR (mm/h) [<20]	Pa_{O2} (room air) (mmHg) [85–90]
1	2000	1538	471	NA	NA	8.8	80	61
2	7800	370	149	3015	545	14.6	92	64
3	14500	774	21030	1588	781	0.5	25	27
4	7800	328	126	1487	193	14.8	84	55
5	7700	392	857	1528	136	1.2	59	80
6	12900	666	201	978	NA	19.0	120	56
7	6500	315	178	2667	265	0.2	24	64
8	9700	516	114	459	257	6.0	76	78
9	7400	540	1330	3766	256	13.2	76	60

PCP, *Pneumocystis carinii* pneumonia; WBC, white blood cell; LDH, lactate dehydrogenase; KL-6, Krebs von den Lungen-6; NA, not available; SP-D, surfactant protein D; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; Pa_{O2}, arterial oxygen partial pressure
Numbers in brackets are the normal range

None of patients 1–6 or patient 9 had primary prophylaxis for PCP; patients 7 and 8 started receiving daily doses of 80mg of trimethoprim (TMP) and 400mg of sulfamethoxazole (SMX) after 4 weeks of high-dose steroid therapy.

Diagnosis and onset of PCP

Patients 5 and 9 had a confirmed diagnosis of PCP on the basis of the cytological test, and the remaining seven patients had a presumed diagnosis of PCP (Table 2). In four of the seven patients with the presumed PCP diagnosis, PC was detected by PCR analysis. In patients 2 to 4, the test for PC in the sputum or BALF could not be completely performed owing to serious respiratory distress. In patients 2–5, PCP developed between January and June 2000 and in patients 6–8 between February and June 2001. None of these patients was hospitalized in the same room simultaneously with one another.

Symptoms and signs

At the PCP onset, seven of the patients had fever, six had breathlessness, and three had a cough (Table 2). Patient 3 had only general fatigue, and patient 5 was completely asymptomatic. Crackles were audible on auscultation in three of the six patients without preexisting interstitial pneumonitis. PCP was diagnosed 1–14 days (mean ± SD: 6.6 ± 4.7 days) after the onset of symptoms or signs.

Laboratory data

Laboratory data at the PCP onset are listed in Table 3. The LDH and β-D-glucan levels increased in all patients, and the KL-6 and surfactant protein D (SP-D) levels also increased in all but one patient for whom data were available. The changes in β-D-glucan, KL-6, and SP-D levels are shown in Table 4 for the patients with available data. In the patients with inactive interstitial pneumonitis or without interstitial pneumonitis, the β-D-glucan, KL-6, and SP-D levels increased after the PCP onset compared with those before PCP onset. The CRP level and erythrocyte sedimentation

Table 4. Changes in β-D-glucan and KL-6 or SP-D levels

Patient No./Underlying IP	Before PCP development	At PCP diagnosis	After PCP diagnosis[a]
	β-D-glucan, KL-6, SP-D (ng/l, U/ml, ng/l)	β-D-glucan, KL-6, SP-D (ng/l, U/ml, ng/l)	β-D-glucan, KL-6, SP-D (ng/l, U/ml, ng/l)
3/Inactive	7.4, 281, 57.9	21030, 1588, 781	NA, NA, NA
4/None	4.3, 643, 19.4	126, 1487, 193	23, 2231, 110
5/Inactive	NA, 402, 17.2	857, 1528, 136	91, 1950, 38
6/None	19, 414, NA	201, 978, NA	537, 5170, NA
7/Active	18, 4719, 129	178, 2667, 265	82, 3672, 157
9/None	10.1, NA, NA	1330, 3766, 256	234, 1509, NA

IP, interstitial pneumonitis; PCP, *Pneumocystis carinii* pneumonia; NA, not available
[a]Representative data from one to four weeks after PCP diagnosis are shown

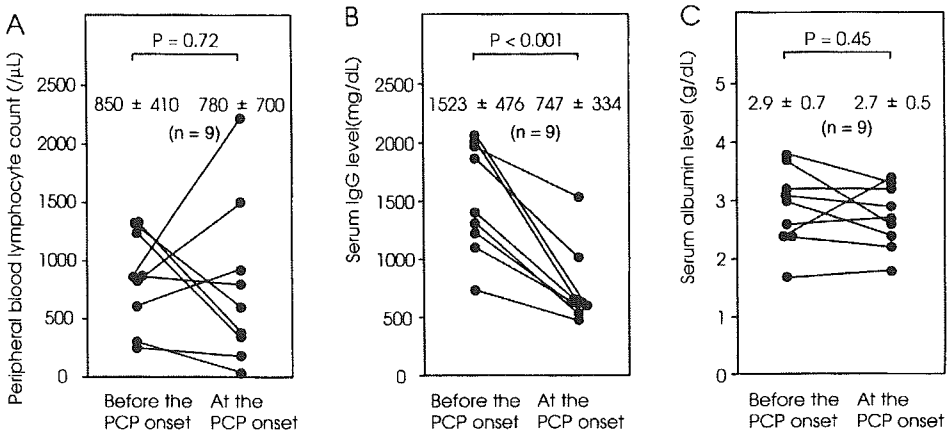

Fig. 1. Comparison of peripheral blood lymphocyte count (**A**), serum immunoglobulin G (IgG) level (**B**), and serum albumin level (**C**) before and at the onset of *Pneumocystis carinii* pneumonia (PCP). The serum IgG level decreased significantly ($P < 0.001$), but there were no significant changes in the peripheral blood lymphocyte count or the serum albumin level ($P = 0.72$ and 0.45, respectively). Values represent the mean ± standard deviation

rate was almost normal in two patients, but they increased during treatment for PCP. The partial pressures of arterial blood oxygen ranged from 27 to 80mmHg (median 64mmHg). Lymphocytopenia with a count lower than 1000/μl was observed in seven patients, a decreased serum IgG level in seven, and a decreased serum albumin level in nine at the time of PCP onset; the two youngest patients (in their thirties) had the lowest lymphocyte counts (39 and 190/μl, respectively). When we compared the above factors with those at the start of the high-dose steroid therapy or those during the maintenance therapy before the PCP onset, the serum IgG level had decreased significantly, although the peripheral blood lymphocyte count and serum albumin level did not show a significant change (Fig. 1).

Radiological findings

Eight patients, except patient 7, had newly developed diffuse ground-glass opacities with geographical or mosaic patterns, as revealed by chest CT. Thickened septal lines and reticular shadows were also revealed in four patients, linear shadows in two, and a nodular shadow, consolidation on air bronchograms, and pleural effusion in one. Ground-glass opacities were predominantly observed in the lower lung fields in five patients and were unilaterally distributed in two.

Treatment course and outcome

The treatment courses and outcomes are summarized in Table 5. Initially, TMP 20mg/kg/day and SMX 100mg/kg/day were administered orally or intravenously to all the patients. However, the antibiotics were replaced with intravenous pentamidine isethionate (PI) 4mg/kg body weight administered daily to all but one patient because of hyperkalemia, nephropathy, rash, nausea, or cytopenia. Three of these eight patients also had adverse reactions to PI that included nephropathy, rash, cytopenia, nausea, and hypo- or hyperglycosemia. Because patient 7 had severe nausea as an adverse reaction to both oral TMP-SMX and intravenous PI, she received a daily inhalation of 300mg of PI. Either TMP-SMX or PI was administered for at least 2–3 weeks in all the patients. Adjunctive steroid was administered in five patients.

Seven of the nine patients responded to the PCP treatment initially, but two patients had progressive respiratory failure despite the TMP-SMX or PI treatment. Six patients died eventually, all of whom had complicated bacterial infections. Two had cytomegalovirus antigenemia, and

Table 5. Treatment and outcomes of patients with CTDs complicated by PCP

Patient No.	Treatment	Mechanical ventilation	Secondary infection	Response to the PCP treatment and eventual outcome	Interval from the PCP onset to death (days)
1	TMP-SMX, mPSL pulse	+	Aspergillosis, sepsis (MRSA) CMV antigenemia	Responded, but died of disseminated aspergillosis	20
2	TMP-SMX, PI	+	Cellulitis, cystitis (*Klebsiella oxytoca*)	Responded, but died of respiratory failure with reincrease in β-D-glucan level	38
3	TMP-SMX, PI	+	Bacterial pneumonitis, sepsis (*Enterococcus* sp.)	Did not respond, died of progressive respiratory failure	10
4	TMP-SMX, PI, mPSL pulse	−	Bacterial pneumonitis (MRSA, *Pseudomonas aeruginosa*)	Responded, but died of bacterial pneumonia	59
5	TMP-SMX, PI, mPSL pulse	+	Bacterial pneumonitis, parotiditis, stomatitis (*Candida albicans*)	Responded, but died of respiratory failure with reincrease in β-D-glucan level	38
6	TMP-SMX, PI, mPSL pulse	+	CMV antigenemia, bacterial pneumonitis	Did not respond, died of DIC and gastrointestinal bleeding	20
7	TMP-SMX, PI, inhaled PI	−	None	Responded and survived after receiving secondary prophylaxis	−
8	TMP-SMX, PI	−	None	Responded and survived after receiving secondary prophylaxis	−
9	TMP-SMX, PI, daily mPSL 0.8 mg-kg bw	−	Herpes labialis	Responded and survived after receiving secondary prophylaxis	−

PCP, *Pneumocystis carinii* pneumonia; CTDs, connective tissue diseases; TMP, trimethoprim; SMX, sulfamethoxazole; mPSL, methylprednisolone; PI, pentamidine isethionate; bw, body weight; MRSA, methicillin-resistant *Staphylococcus aureus*; CMV, cytomegalovirus; DIC, disseminated intravascular coagulation

one had disseminated aspergillosis. Two patients died of respiratory failure not responding to PCP treatment, two had recurrence of respiratory failure associated with a reincreased β-D-glucan level after the discontinuation of PCP treatment, one had bacterial pneumonia, and one had disseminated aspergillosis.

Among the six patients, five required mechanical ventilation during the PCP treatment. The durations from the PCP onset to death ranged from 10 to 59 days (mean 30.8 days). Patients 7–9 received secondary prophylaxis with 80 mg of TMP and 400 mg of SMX daily, or they were given inhalation therapy of 300 mg of PI monthly following the initial treatment dose. They had neither a relapse nor severe secondary infections and recovered completely.

Discussion

In the present study, high-dose steroids, immunosuppressants, and hypogammaglobulinemia appeared to be the risk factors for PCP in patients with CTDs. PCP developed after 6–16 weeks of high-dose steroid therapy, suggesting that the patients became sufficiently immunocompromised to be susceptible to PCP within this period. In addition, a dose of 1 g of pulsed methylprednisolone may also be responsible for induction of the immunocompromised state of the hosts.[24]

Lymphocytopenia has been described as the most important risk factor for PCP.[11,15,16] However, in our series, the lymphocyte count at the onset of PCP did not significantly differ from the counts before the onset of PCP, although two patients in their thirties had severe lymphocytopenia (lymphocyte count <200/μl) and five patients had a lymphocyte count <1000/μl at the onset of PCP. Conversely, the lymphocyte counts increased at the PCP onset in three of the nine patients. This increase was probably due to the improvement of the underlying CTDs. In these patients, the functional impairment of lymphocytes due to immunosuppressive treatment, including impaired cellular responses and interferon-γ production in response to PC may be associated with PCP development.[25] Whether a low lymphocyte count itself is a risk factor for PCP and lymphocyte function is impaired in CTD patients who contracted PCP should be further examined with a larger number of patients.

In contrast to the increased lymphocyte count, the serum IgG level decreased significantly. The decrease may be a consequence of high-dose steroid or immunosuppressant therapy. Consistent with our results, Saito et al.[26] reported that patients with PCP associated with CTDs have significantly lower IgG levels than do patients without PCP. In HIV-infected patients, the antibody level to PC decreases at the onset of PCP and returns to normal coinciding with treatment and clinical recovery from the PC infection.[27] Moreover, PCP has been documented among children with hypogammaglobulinemia.[28] These findings suggest that hypogammaglobulinemia is not merely a coincidental event of immunosuppressive therapy but is a risk factor for PCP.

Most patients had increased levels of both β-D-glucan and KL-6 or SP-D. An increased β-D-glucan level is a diag-

nostic indicator of fungal infections such as *Aspergillus*, *Candida*, and PC infections.[29] An increase in the KL-6 or SP-D level has been reported in PCP patients[30,31] but not in patients with other fungal infections. At present, PC appears to be the only pathogenic fungus that causes an increase in both β-D-glucan and KL-6 or SP-D levels. An increase in the KL-6 or SP-D level may reflect damaged alveolar epithelium in patients with PCP. We suggest that a simultaneous increase in β-D-glucan and KL-6 or SP-D levels is a diagnostic indicator of PCP in patients with inactive interstitial pneumonitis or without interstitial pneumonitis, and that it may be useful in the differential diagnosis of exacerbation of interstitial pneumonitis due to the underlying CTDs.

Primary prophylaxis for PCP using TMP-SMX has been successful in HIV patients with a CD4 lymphocyte count <200/μl[9] and in CTD patients receiving steroid therapy who had interstitial pneumonitis or lymphocytopenia.[17] Routine primary prophylaxis was not introduced until February 2001 in our department because the incidence of PCP had been extremely low. Since the latter half of 2001, however, we have prescribed 80mg of TMP and 400mg of SMX daily for PCP prophylaxis to patients older than 50 years at the start of high-dose steroid or immunosuppressant therapy. As none of them have had PCP to date (October 2004), it appears that primary prophylaxis for PCP is effective in patients with CTDs receiving high-dose steroid therapy.

Our observation of two clusters of PCP cases suggests exogenous transmission rather than reactivation of the latent PC infection. In such cases, PC was probably transmitted via environmental sources or the medical staff because none of our patients had direct contact with each other. Gerrard described nosocomial transmission from HIV patients to patients without HIV infection.[32] A PC genome analysis can elucidate the mode of such transmission.[33] The acute PCP onset in the present patients characteristically differs from the insidious onset in HIV patients, which takes place over several months.[34] On the other hand, the symptoms, signs, and chest CT findings in this study are all similar to those observed in HIV patients.[35]

Seven of the nine patients responded to PCP treatment initially, although six of the seven patients died eventually. The mortality rate was 67%. Ewig et al.[36] reported that a significantly higher mortality rate was associated with PCP in the non-HIV group, particularly for patients with malignancy or CTDs, than that for the HIV group. The deaths in their study were due to treatment failure and bacterial or cytomegalovirus infections. In our study, the causes associated with death were as follows: severe respiratory failure not responding to PCP treatment in two patients, respiratory failure associated with a reincreased β-D-glucan level after discontinuation of PCP treatment in two, and severe secondary infections in two. In the patients with respiratory failure associated with a reincreased β-D-glucan level, PCP recurrence was suspected because they had no findings of any fungal infections other than PC. These patients did not receive secondary prophylaxis, whereas the patients who did survive had received secondary prophylaxis. A poor prognosis is forecast for patients with respiratory failure requiring mechanical ventilation, a severe secondary infection(s), and no secondary prophylaxis. Although its standard regimen and duration, as well as its validity, have not been confirmed, we recommend secondary prophylaxis with careful monitoring of adverse reactions to TMP-SMX or PI.

Conclusions

High-dose steroids, immunosuppressants, and hypogammaglobulinemia are risk factors for PCP in patients with CTDs. The serum IgG level decreased significantly at the onset of PCP. The lymphocyte count at the onset was low, but its decrease was not significant. The acute onset was characteristic of PCP patients with CTDs, whereas other features were almost consistent with those of PCP in HIV patients. A simultaneous increase in both β-D-glucan and KL-6 or SP-D levels is a diagnostic indicator of PCP. Respiratory failure requiring mechanical ventilation, severe secondary infections, and no secondary prophylaxis are poor prognostic factors for PCP. Secondary prophylaxis should be administered to all these patients.

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Study of Plasma Levels of Soluble CD40 Ligand in Systemic Lupus Erythematosus Patients Who Have Undergone Plasmapheresis

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Abstract: We studied whether soluble CD40 ligands (sCD40L) are removed by means of double filtration plasmapheresis (DFPP), and the removal may help decrease activity of systemic lupus erythematosus (SLE). We studied 10 female patients with active SLE. Double filtration plasmapheresis was conducted one or two times per week. Plasma sCD40L levels were measured before and after each round of DFPP and throughout the treatment course. The plasma sCD40L level of SLE patients was significantly higher (14.09 ± 18.88 ng/mL) than that of healthy individuals (0.19 ± 0.20 ng/mL; $P < 0.0001$). In the SLE patients, plasma sCD40L levels were significantly lower following DFPP ($P = 0.0251$). The plasma waste from DFPP of an SLE patient was subjected to gel filtration, and the sCD40L concentration in each fraction was measured. We observed a peak in the fraction corresponding to ≥60 kDa. These results indicate that trimers and higher order complexes of sCD40L are removed during DFPP. Plasma sCD40L level and SLE disease activity index (SLEDAI) were decreased following the treatment course (mean 9.3 months). sCD40L exists as both a monomer and trimer in the plasma of SLE patients. The trimer as well as higher-order compounds can be removed via DFPP. It was thought that removal of sCD40L via DFPP may be useful for improving the overall condition of SLE.
Key Words: Antibody removal, CD40 ligand, Double filtration plasmapheresis, Plasmapheresis, Systemic lupus erythematosus.

Systemic lupus erythematosus (SLE) is an autoimmune disease of unknown origin. The serum of SLE patients contains a variety of autoantibodies, including anti-DNA antibodies, which form immune complexes that precipitate in the tissues and cause various tissue disorders. While drug therapy is currently the standard treatment for SLE, concomitant plasmapheresis has been found to be effective for reducing the incidence of certain aspects of the disease, such as lupus nephritis and neuropsychiatric lupus.

CD40 ligand (CD40L) is a 39-kDa type-II glycoprotein of the tumor necrosis factor (TNF) family. CD40L primarily appears transiently on CD4-positive cells, the recipients of antigen presentation, and plays a central role in the maintenance of humoral immunity. CD40L exists in a soluble form (sCD40L) that is produced by microsomal stimulus-dependent cleavage of the membrane-bound CD40L. sCD40L is capable of inducing B-cell proliferation via CD40 present on B-cells, class switching of immunoglobulins, and production of autoantibodies. CD40 is known to be widely expressed on antigen-presenting cells such as monocytes and dendritic cells as well as on intravascular endothelial cells. By sending signals through these cells, it enhances the expression of costimulatory molecules, induces production of inflammatory cytokines, and participates in the pathogenesis of various chronic inflammatory diseases (1,2).

The ratio of CD40L-presenting T-cells is increased in the peripheral blood of SLE patients (3), and if these T-cells are stimulated in vitro, CD40L is reportedly expressed on the cell surface at higher levels and for a longer duration than in T-cells from healthy individuals (4). It has been reported that the sCD40L level in the plasma of SLE patients is high, and it

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TABLE 1. *Summary of Study Subjects*

Patient	Age	Sex	Duration of SLE	Complications	Treatment for SLE	SLEDAI	Anti-DNA Ab (IU/mL)	CH50 (u/mL)
1	32	F	1 month	APS	PSL 70 mg, mPSL 500 mg × 3 days	17	2920.9	7.5
2	19	F	3 years		PSL 30 mg	18	174.0	25.0
3	26	F	14 years	APS	PSL 15 mg	10	3.8	42.4
4	26	F	6 years	SjS	PSL 15 mg	6	56.3	29.4
5	18	F	7 months		PSL 60 mg, mPSL 500 mg × 3 days IVCY	23	1205	15.9
6	66	F	34 years	APS	PSL 15 mg, mPSL 500 mg × 3 days	14	61.5	16.3
7	12	F	3 month		PSL 50 mg, mPSL 500 mg × 3 days	14	14.8	7.0
8	32	F	1 month		PSL 40 mg	18	3.2	7.0
9	34	F	10 years		PSL 50 mg	20	163.4	17.1
10	35	F	20 years	APS	PSL 30 mg	10	8090.0	7.0

APS, antiphospholipid antibody syndrome; IVCY, intravenous cyclophosphamide; mPSL, methylprednisolone; PSL, prednisolone; SjS, Sjögren's syndrome; SLEDAI, SLE activity index.

correlates with disease activity and the level of anti-double-stranded (antiDS) DNA antibodies. Furthermore, sCD40L in the plasma of SLE patients enhances the expression of CD44 and CD95 on B-cells (5,6), and sCD40L itself is believed to have biological activity.

In light of the observations described above, we hypothesized that removal of sCD40L via plasmapheresis may help decrease disease activity. In this study, we measured the time-dependent changes in the plasma sCD40L levels in SLE patients who had undergone double filtration plasmapheresis.

MATERIALS

The subjects included 10 female patients ranging in age from 12 to 66 years (mean 30 years) who fulfilled the 1982 American College of Rheumatology (ACR) revised criteria for diagnosis of SLE. The morbidity duration ranged from 1 month to 34 years (mean 105.6 months). Elevated antiDS-DNA antibodies were detected in all subjects, and a decrease in complement was detected in seven patients. The SLE disease activity index (SLEDAI) ranged between 6 and 20 points (mean 15 points). All subjects received steroid treatment, and one subject was concomitantly treated with intravenous cyclophosphamide therapy (Table 1).

METHODS

Double filtration plasmapheresis (DFPP) was conducted one or two times per week. A secondary membrane filter with a pore size of 0.03 μm was used for DFPP. A 5% albumin solution was used as a substitution solution. For anticoagulation, 2000 U of heparin were initially administered followed by continuous administration of 2000 U/h. The plasma throughput per DFPP was 2000 mL.

Blood sample collection was conducted before and after DFPP. Whole blood was stored at 4°C and centrifuged within 30 min, and plasma was stored at –20°C.

Plasma sCD40L levels were measured before and after each round of DFPP and throughout the treatment course.

The concentration of sCD40L in plasma was determined by sandwich enzyme-linked immunosorbent assay (ELISA) using two non-cross-blocking antihuman CD40L monoclonal antibodies (Mab). Briefly, each well of a 96-well polystyrene ELISA plate (Corning Costar Corp., Cambridge, MA, USA) was coated with 5 μg/mL antihuman CD40L Mab (TRAP-1; Pharmingen, San Diego, CA, USA) in phosphate buffered saline (PBS). After washing with PBS, the plate was treated with a blocking buffer consisting of 1% bovine serum albumin in PBS at room temperature for 2 h. The plates were washed four times with 0.05% Tween-20 (SIGMA, Tokyo, Japan) in PBS (washing buffer) and incubated in washing buffer at 4°C overnight. After washing, the plates were incubated with 2 μg/mL biotinylated antihuman CD40L Mab (bio-M90, Genzyme, Cambridge, MA, USA) in washing buffer. Avidin and biotinylated horseradish peroxidase (Elite Vectastain, Vector Laboratories, Burlingame, CA, USA) were then added, and following incubation and washing, 3,3′,5,5′-tetrametylbenzidine peroxidase (Kierkegaard & Perry Laboratories Inc., Gaithersburg, MD, USA), which served as a substrate for detection, was added. Absorbance at 450 nm was determined using a micro-ELISA reader. Serial dilutions of recombinant human sCD40L (Bender Medical Systems, Vienna, Austria) were used to construct the standard curve. In the control ELISA, isotype-matched mouse IgG (mopc-21, Sigma, St. Louis, MO, USA) was used as a coating antibody, and biotinylated mouse IgG (Ancell, Bayport, MN, USA) was used for detection.

Plasma from each SLE patient and the corresponding DFPP drainage were subjected to gel filtration. 0.05 M potassium phosphate containing 0.1 M NaCl (pH 7.5) was used as an elution buffer, and a 1 × 30 cm column filled with Sephadex G-100 (Pharmacia, Uppsala, Sweden) was used as a gel filtration column.

The plasma sCD40L levels of healthy individuals and SLE patients were compared using the Mann–Whitney non-paired non-parametric test. The plasma sCD40L levels of each SLE patient before and after DFPP were compared using the paired Student's *t*-test and the SLEDAI and plasma sCD40L levels before and after treatment were compared using the Wilcoxon signed-ranks test.

RESULTS

The plasma sCD40L levels of 15 healthy individuals and 10 SLE patients were measured. The plasma sCD40L level of SLE patients was significantly higher (14.09 ± 18.88 ng/mL) than that of healthy individuals (0.19 ± 0.20 ng/mL; $P < 0.0001$) (Fig. 1). In the SLE patients, plasma sCD40L levels were significantly lower following DFPP ($P = 0.0251$) (Fig. 2). In one of the ten SLE patients (Patient no. 5), increased sCD40L levels were observed following DFPP, but the sCD40L levels tended to decrease gradually over the course of the treatment, suggesting that the post-DFPP increase was transient. During DFPP, thrombocytes receive an activation stimulus as a result of the contact between the blood and the membrane. When thrombocytes are activated, molecules such as CD40L appear on the thrombocyte surface (7). Additionally, when thrombocytes are stimulated by thrombin, CD40L is expressed on the cell surface, and within a short time, its soluble form (sCD40L) is released (8). It seems possible therefore that a transient post-DFPP increase in sCD40L levels may be caused by thrombocyte activation.

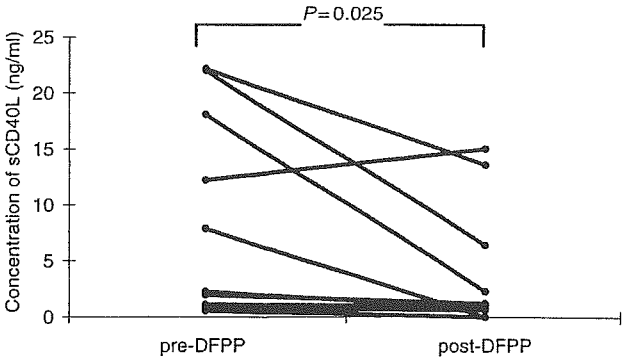

FIG. 2. Plasma sCD40L levels before and after a single round of double filtration plasmapheresis (DFPP) in systemic lupus erythematosus patients. Plasma sCD40L levels were significantly decreased following DFPP (Paired *t*-test, $P = 0.0251$).

The mean rate of sCD40L removal from the plasma of SLE patients (50.71%) during a single round of DFPP was much higher than the mean removal rates of either IgG (25.06%) or albumin (14.09%) (Fig. 3), suggesting that the molecular weight of the sCD40L removed during DFPP is higher than that of either albumin or IgG. To determine the molecular weight of the sCD40L present in the plasma of SLE patients and in the plasma waste from DFPP, corresponding samples were subjected to gel filtration, and the sCD40L concentration in each fraction was measured using ELISA. In the plasma, peaks corresponding to the expected molecular weights of sCD40L monomers (~20 kDa) and trimers (~60 kDa) were observed. In the DFPP drainage fluid, a peak was observed in the fraction corresponding to ≥60 kDa (Fig. 4). These results indicate that trimers and higher-order complexes of

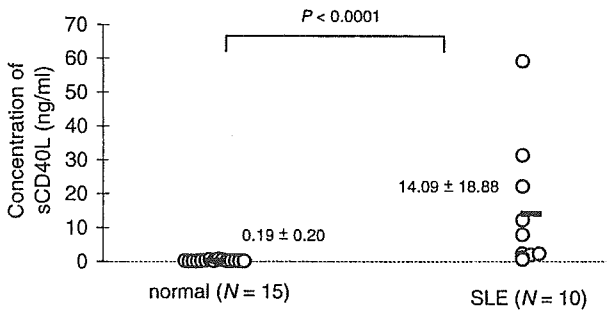

FIG. 1. Plasma sCD40L levels (ng/mL) of 15 healthy individuals and 10 systemic lupus erythematosus (SLE) patients. Mean plasma sCD40L levels were significantly higher in SLE patients(14.09 ± 18.88 ng/mL) compared to healthy individuals(0.19 ± 0.20 ng/mL) (Mann–Whitney's non-parametric non-paired test, $P < 0.0001$).

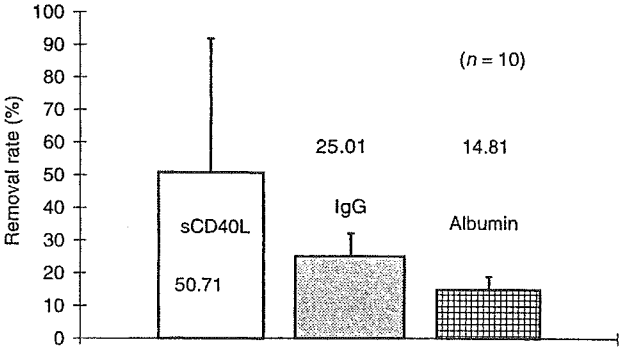

FIG. 3. The removal rates of sCD40L, IgG, and albumin from plasma of SLE patients during a single round of DFPP. The removal rates were 50.71 ± 41.09% for sCD40L, 25.01 ± 7.02% for IgG, and 14.81 ± 3.52% for albumin. The high removal rate of sCD40L suggests that it has the highest molecular weight.

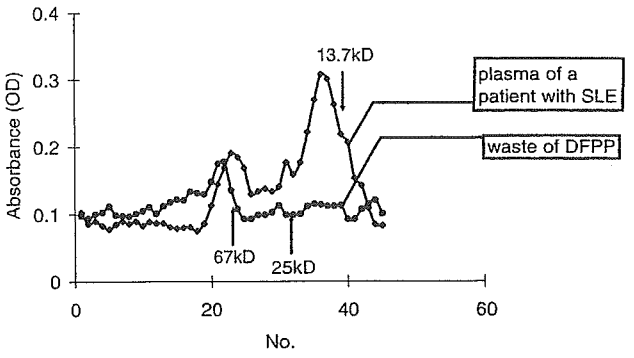

FIG. 4. Plasma from each systemic lupus erythematosus (SLE) patient and the corresponding double filtration plasmapheresis (DFPP) drainage fluid were subjected to gel filtration, and the sCD40L concentration in each fraction was measured. The concentration is shown as absorbance. (◆) indicates SLE patient plasma and (●) indicates DFPP waste fluid. In the plasma of SLE patients, peaks were observed at ~20 kDa and ~60 kDa. In the DFPP waste fluid, one peak = 60 kDa was observed. The ~20 kDa peak is thought to correspond to the expected molecular weight of the sCD40L monomer, and the ~60 kDa peak is thought to correspond to its trimer.

sCD40L are removed during DFPP. Follow-up analyses conducted in seven out of the 10 SLE patients revealed a change in plasma sCD40L level following the treatment course. The mean treatment duration was 9.3 months, and a mean of 19.2 rounds of DFPP were performed. The steroid doses remained constant or were decreased in all patients during the treatment course. The plasma sCD40L level decreased following treatment in all patients. The SLEDAI score was significantly lower following treatment, suggesting that the sCD40L level decreased with the decrease in the SLE activity (Fig. 5).

DISCUSSION

Double filtration plasmapheresis (DFPP) has been concomitantly used with drug therapy to treat intractable SLE patients. Treatment effect of DFPP is based on the non-specific removal of the medium to large molecules including immunocomplexes, antiDS-DNA antibodies, and γ-globulin from the plasma protein fraction of the treated patients. DFPP has been found to be effective for the treatment of many of the clinical conditions associated with SLE including active lupus nephritis, central nervous system (CNS) lupus, dermatological manifestations, vasculitis, and thrombocytopenia (9).

The results of this study confirm that the plasma sCD40L levels in SLE patients are significantly higher than those of the healthy individuals. Early et al. reported that in model mice of SLE, antiDS-DNA antibody production could be supported by treatment with antimouse CD40L antibody in vivo (10). It is also known that the CD40–CD40L interaction plays a significant role in SLE pathogenesis. In the recent clinical studies to treat lupus nephritis patients with humanized monoclonal antibody specific for CD40L, reduction in antiDS-DNA antibody titers, proteinuria, hematuria, and SLEDAI score were reported (11–13). In light of these findings, it was thought that the removal of sCD40L by using plasmapheresis might be useful for improving the overall condition of SLE patients.

There were, however, two questions on the removal of sCD40L by using DFPP. The first question was whether sCD40L could be removed by DFPP. A previous report has indicated that the molecular weight of sCD40L is 18–20 kDa. sCD40L exists in the body as a trimer form, and it may be biologically active by itself (14). In our study, to determine if sCD40L can be removed by DFPP, we examined the molecular weight of sCD40L by using a gel filtration method. Our results suggest that sCD40L exists as both monomer and trimer forms in the plasma of SLE patients. The trimer as well as higher-ordered compounds considered primary biological active compounds, and they can be removed by DFPP. The

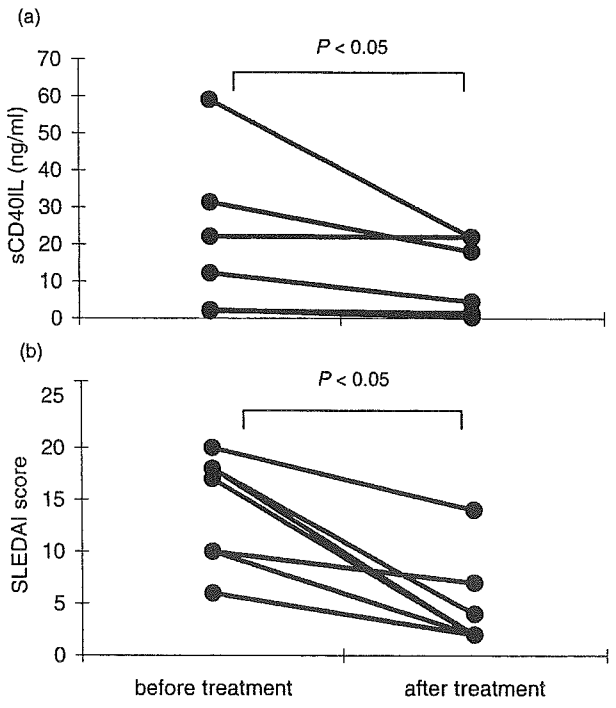

FIG. 5. Follow-up of seven patients (Patients nos. 1, 2, 3, 4, 8, 9 and 10) following the treatment course. Significant decreases in the (a) plasma sCD40L level and (b) SLEDAI score were observed following treatment in all patients (Wilcoxon sign-ranked test, $P < 0.05$).

second question was whether pathogenic substances other than sCD40L could be removed by DFPP. Because DFPP removes non-specific medium to large molecules, certain substances involved in the pathogenesis of SLE other than sCD40L can be removed. More studies should be done in order to answer this question.

It has been hoped that development of a more selective treatment against CD40-CD40L will happen in the future. It is known that compared to drug therapy, plasmapheresis is safer treatment with fewer adverse effects. In the future, we intended to study more about DFPP and the possibility of the development of selective removal of sCD40L by immunoadsorption plasmapheresis technique.

In our study, all of the seven patients we followed were taking steroid as the major drug. The degree to which DFPP contributed the overall treatment effects among the various treatments including steroid is not clear. However, it appears that the removal of sCD40L by DFPP might be one of the factors for the improvement of the symptoms we have observed.

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Combination Therapy with Corticosteroids, Cyclosporin A, and Intravenous Pulse Cyclophosphamide for Acute/Subacute Interstitial Pneumonia in Patients with Dermatomyositis

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ABSTRACT. ***Objective.*** Acute/subacute interstitial pneumonia (A/SIP) in patients with polymyositis/dermatomyositis (PM/DM) is frequently fatal within months despite high dose prednisolone (PSL) therapy. Our objective was to improve the survival rate of patients with A/SIP associated with PM/DM; and to characterize patients with PM/DM who are at high risk of developing A/SIP.

Methods. We conducted a pilot trial of combined immunosuppressive therapy with high dose PSL, 10–30 mg/kg of intravenous pulse cyclophosphamide (IVCYC) every 3–4 weeks, and 2–4 mg/kg/day of cyclosporin A (CSA) for patients with A/SIP. A/SIP was diagnosed based on a history of rapidly worsening respiratory symptoms, progressive radiological findings or hypoxemia, and compatible findings in high resolution computed tomography images.

Results. Before December 2000, 12 patients with DM among 83 PM/DM patients developed A/SIP, and 9 patients died despite treatment using high dose PSL with or without a choice of CSA, cyclophosphamide, or azathioprine. Thereafter, 10 patients with DM among 27 PM/DM patients developed A/SIP, and they were given combination therapy with PSL, CSA, and IVCYC. Five patients survived and are doing well for more than 2 years, although the remaining 5 patients died of respiratory failure within 3 months. DM patients with A/SIP showed the following characteristic features: mild myositis, palmar papule, fever, and negative or low titer of antinuclear antibody.

Conclusion. Immediate institution of intensified immunosuppressive therapy should be considered for patients with A/SIP complicating DM. However, even early recognition of A/SIP and immediate commencement of a regimen including CSA and IVCYC in addition to high dose PSL may not be sufficient for some of those patients. (J Rheumatol 2005;32:1719–26)

Key Indexing Terms:
PALMAR PAPULE POLYMYOSITIS DERMATOMYOSITIS PNEUMOMEDIASTINUM

Polymyositis/dermatomyositis (PM/DM) is a disease of autoimmune origin, predominantly affecting the proximal girdle muscles[1,2]. The presence of the pathognomonic rashes, namely the heliotrope rash and Gottron's papules, distinguishes DM from PM[1-3]. PM and DM share overlapping systemic features, including Raynaud's phenomenon, polyarthritis, dysphagia, cardiac dysfunction, and interstitial pneumonia (IP).

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IP is an important factor adversely influencing the prognosis of patients with PM/DM, and is related to the presence of some autoantibodies against aminoacyl transfer ribonucleic acid (tRNA) synthetases such as the anti-Jo-1 antibody[4]. Some patients with PM/DM, especially with amyopathic DM (ADM), develop acute or subacute interstitial pneumonia (A/SIP), with rapid worsening within a month (acute) or within 2–3 months (subacute)[5]. The prognosis of patients with ADM who develop A/SIP is extremely poor, and they usually do not respond to the corticosteroid treatment, including high dose prednisolone (PSL) and pulse corticosteroid therapy, cyclosporin A (CSA), and cyclophosphamide (CYC), and often die of respiratory failure within a few months[5-7]. Indeed, the condition has been estimated to be fatal in about 70% of the patients despite therapy of high dose corticosteroid plus CSA[8].

We reviewed patients with PM/DM who had visited our department until November 2000. Then we prospectively investigated the efficacy of an intensified immunosuppressive therapy regimen consisting of a combination of high

dose PSL, CSA, and intravenous pulse CYC (IVCYC). Finally, we examined the clinical features of PM/DM patients who developed A/SIP, in order to clarify the characteristic features of those patients who are at high risk of developing A/SIP, for early recognition of A/SIP and immediate institution of intensive therapy.

MATERIALS AND METHODS

Patients. A total of 110 consecutive patients with a diagnosis of PM (n = 35 including 5 men) or DM (n = 75, 24 men) were enrolled for this study. All were seen at our department, either as inpatients or outpatients, between 1985 and 2002. Patients were evaluated according to the criteria of Bohan and Peter[1], and cases with both definite and probable PM/DM were included in the study. To include patients with "hypomyopathic" DM or ADM, patients exhibiting biopsy-confirmed hallmark cutaneous manifestations of classic DM, including the heliotrope rash and Gottron's papules/signs, were enrolled according to the criteria proposed by Sontheimer[3].

Diagnosis of A/SIP. A/SIP was diagnosed based on (1) a history of worsening respiratory symptoms within 3 months; and (2) worsening chest radiograph or computerized tomography (CT) findings, or decrease in the level of PaO_2 within 3 months; and (3) findings in high resolution CT (HRCT) images of the lungs compatible with nonspecific interstitial pneumonia (NSIP), diffuse alveolar damage (DAD), or organizing pneumonia (OP)[9].

Treatment. In December 2000, we started a prospective pilot study of a combination immunosuppressive therapy regimen consisting of high dose corticosteroids (> 0.5 mg/kg/day PSL), 10–30 mg/kg IVCYC every 3–4 weeks, and 2–4 mg/kg/day CSA (to achieve a trough level of 150–250 ng/ml) for patients who had A/SIP associated with DM, only if HRCT of the lungs suggested NSIP or DAD. On the other hand, when the chest HRCT suggested OP associated with PM/DM, 0.5 mg/kg/day of PSL alone was typically started. The dose of PSL was tapered by 10% weekly after 2–3 weeks of treatment at the initial dose. The dose of IVCYC was adjusted to achieve a peripheral blood total leukocyte count nadir not less than 2000/μl.

Statistical analysis. For group comparisons using binary data, Fisher's exact test was used. Comparisons based on continuous data were by Mann-Whitney U test. The results were regarded as significant when the p value was < 0.05.

RESULTS

A/SIP complicating DM was associated with death within months of the diagnosis despite conventional immunosuppressive therapies. First, we investigated the prevalence of A/SIP in patients with PM/DM. Until November 2000, we had seen 83 patients with PM/DM, and surprisingly, all the patients with A/SIP in our series had a diagnosis of DM. Twelve patients with DM developed A/SIP, and 9 (75%) patients died of respiratory failure within months. Then we reviewed the response of those patients with A/SIP to immunosuppressive therapy. Most of them failed to respond to the treatment using high dose PSL with or without one of the following immunosuppressant drugs: CSA, CYC (oral or intravenous), azathioprine, and mizoribine (an inhibitor of inosine monophosphate dehydrogenase; Figure 1). PSL alone, PSL plus CSA, or PSL plus CYC was effective in only one patient each. Thus, DM patients with complicating A/SIP showed an extremely unfavorable course, and the disease was often fatal despite the treatment with immunosuppressive agents, either singly or sequentially, immediately after establishment of the diagnosis. Therefore, in December 2000, we decided to treat patients with PM/DM who developed A/SIP with a combination of PSL, CSA, and IVCYC.

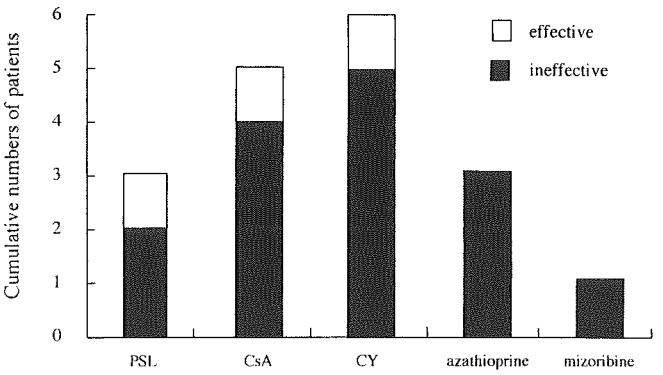

Figure 1. Lack of efficacy of PSL with or without another immunosuppressive drug administered singly in DM patients with A/SIP. Until November 2000, 12 DM patients who developed A/SIP had been treated with high dose PSL alone, or PSL in combination with another immunosuppressive agent administered singly, including CSA, CYC, azathioprine, or mizoribine. Some patients who showed no response to the first drug were given a second drug, thus the total cumulative number of patients reached 18.

Only half of the DM patients with A/SIP who were administered combination therapy with PSL, CSA, and IVCYC survived. From December 2000 to December 2002, 27 patients with PM/DM were recruited to our hospital. Among them, A/SIP was diagnosed in 10 patients, and again exclusively in patients with DM. All their initial HRCT findings for IP suggested DAD (Patients 3 and 7 in Table 1) or NSIP, hence all of them were treated with the combination therapy regimen of PSL, CSA, and IVCYC. Corticosteroid pulse therapy was performed when the development or worsening of A/SIP was observed under treatment with high dose corticosteroids. The responses to the combination therapy regimen are summarized in Table 1. Since the introduction of this combination regimen, the survival rate of DM patients with A/SIP seemed to have improved to 50%. Only one patient (Patient 7) showed both apparent muscle weakness and an elevated serum CK concentration to above twice the value of the upper limit. There were no significant differences between the patients who survived and those who eventually died in terms of the levels of serum CK, lactate dehydrogenase, or KL-6, or in terms of the dose of IVCYC, the serum trough level of CSA, or the time interval between the onset of A/SIP and the start of treatment. However, the patients who eventually died showed a decreased PaO_2 (necessitating the use of oxygen in Patients 7 and 9) at the start of the therapy, tended to develop pneumomediastinum (60% in dead vs 20% in surviving patients), and died within a few months of the diagnosis despite repeated pulses of steroid therapy. All sera from those 10 patients were examined for the presence of antinuclear antibodies (ANA) by

Table 1. Comparison of clinical features between surviving and dead patients with DM and A/SIP.

Patient	Age/Sex	Muscle Weakness	Pneumo-mediastinum	CK, IU/l	PaO_2, Torr	LDH, IU/l	KL-6, U/ml	PSL, mg/day	Steroid Pulse	Maximum IVCYC, mg	CSA Trough, ng/ml	A/SIP Therapy*, days	Outcome**, Days
Surviving													
1	55 M	+	+	235	90.6	230	2710	60	–	750	98–244	8	> 1206
2	33 F	–	–	987	94.0	436	308	60	+	1500	101–178	5	> 1058
3	45 F	+	–	219	86.1	267	885	50	+	1000	119–449	11	> 797
4	34 M	–	–	81	88.3	208	465	60	–	1500	113–227	43	> 788
5	68 F	–	–	133	79.6	238	580	30	–	1000	86–201	45	> 763
Dead													
6	50 F	+	–	103	63.0	419	1160	60	+	1000	40–59	8	55
7	29 F	+	+	1052	10†	1450	4900	100	+	750	86–121	13	48
8	66 F	–	–	411	72.2	296	773	60	+	1000	18–391	34	53
9	58 F	–	+	298	3†	491	803	50	+	1000	75–293	19	52
10	47 F	+	+	338	84.4	306	431	45	+	900	151–348	4	67

* Duration from onset of A/SIP to start of combination therapy. ** Duration from start of combination therapy to outcome. † Oxygen supplementation with 10 l/min and 3 l/min, respectively.

both immunofluorescence and RNA-immunoprecipitation tests, which resulted in negative tests for all the patients.

Representative radiographic changes of A/SIP in the chest CT during treatment with the combination regimen of PSL, CSA, and IVCYC. Patient 7 did not show apparent lung disease at the time of diagnosis of DM (Figure 2A). However, soon after the start of treatment with PSL 60 mg/day, she developed a subpleural consolidation, which developed into diffuse ground-glass opacities within 9 days. With a diagnosis of A/SIP, CSA and IVCYC as well as corticosteroid pulse therapy were immediately added to PSL; however, despite this treatment, she died of respiratory failure within a month. Patient 3 also showed no evidence of IP at the time of diagnosis of DM; however, later, during treatment with PSL 50 mg/day, she developed linear opacities in the lung fields bilaterally (Figure 2B). Despite immediate addition of CSA and IVCYC as well as corticosteroid pulse therapy to the therapeutic regimen, ground-glass opacities appeared in the CT. This patient did not develop pneumomediastinum, and the ground-glass opacities gradually changed to reticular or linear opacities, and marked resolution of the chest CT abnormalities was observed within a few months. Patient 9 showed reticular opacities and a subpleural consolidation in June 2002 (Figure 2C). Although combination therapy with PSL, CSA, and IVCYC was immediately started, the changes of interstitial lung disease showed marked aggravation, and she died of respiratory failure in August 2002 despite repeated pulses of steroid therapy. Patient 10 showed reticular opacities and a subpleural consolidation in her chest CT in November 2002 (Figure 2D). Despite immediate institution of treatment with PSL, CSA, and IVCYC, progressive reticular opacities developed as well as pneumomediastinum, and she died of respiratory failure in January 2003 despite repeated pulses of steroid therapy.

Clinical characteristics of DM patients with A/SIP. Finally, in order to clarify the clinical characteristics of DM patients with A/SIP for early recognition of patients at high risk of developing fatal lung disease, the clinical characteristics of 22 patients with DM who developed A/SIP were compared with those of 53 DM patients with chronic IP or no IP (Table 2). More than half the patients with A/SIP showed only a modest increase in the serum CK level (less than twice the value of the upper limit), and these patients tended to show negative electromyography or muscle biopsy findings for myositis. Thus, DM patients who developed A/SIP had a tendency to have modest myositis, compared to other DM patients with chronic IP or no IP. Patients with A/SIP more frequently showed the heliotrope rash and, interestingly, palmar papules were observed almost exclusively in these patients (Figure 3). These DM patients with A/SIP also had fever and a negative test for ANA.

DISCUSSION

We describe a subgroup of DM patients with an extremely poor prognosis because of the development of A/SIP. Further, we conducted, for the first time, a pilot prospective study of an intensified immunosuppressive therapy regimen consisting of PSL, CSA, and IVCYC for these patients.

About 50% of Japanese patients with PM/DM develop IP during the course of their disease[4], whereas only around 30% of Caucasian patients with this disease have been reported to develop IP[9,10]. Further, a disproportionately large number of cases of fatal A/SIP among Japanese patients without muscle weakness has been reported from Japan; 16 out of 27 patients (59%) died due to IP[5], although there is a tendency recently that only successfully treated cases have been reported, thus raising the apparent survival rate.

In DM patients, DAD, NSIP, and OP have been found

(A) Patient 7, 29/F

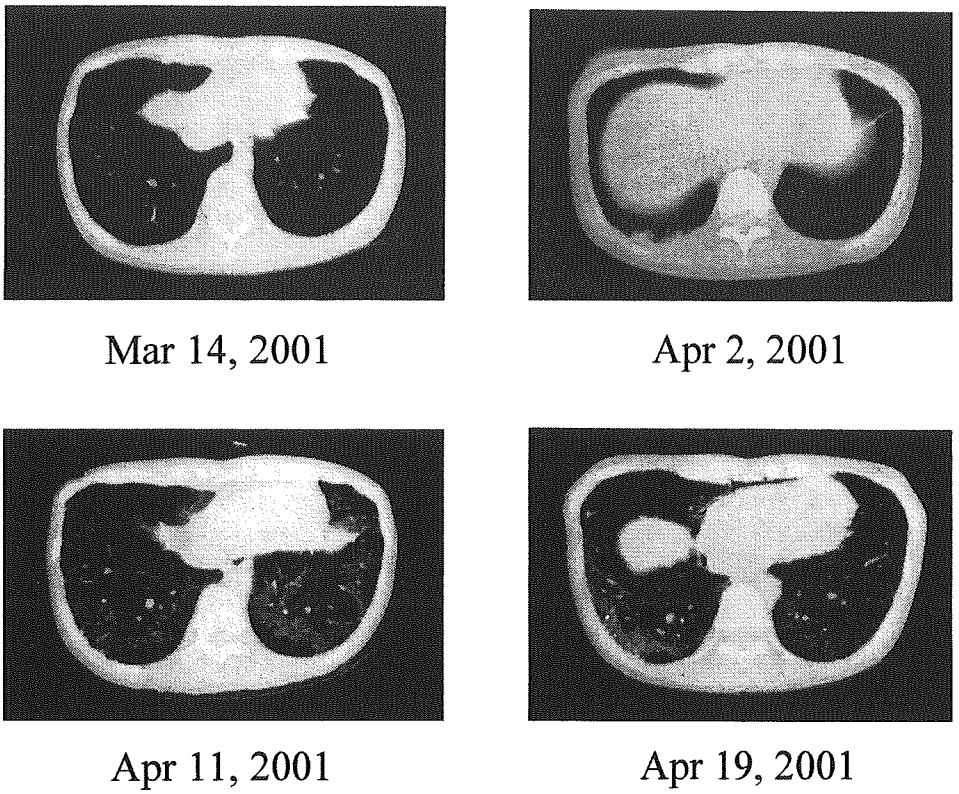

Figure 2. Progression or resolution of chest CT findings in DM patients with A/SIP during PSL + CSA + IVCYC therapy. Paired images from different axial levels are shown sequentially in B. A, B, C, and D correspond to Patients 7, 3, 9, and 10, respectively (see Table 2).

(B) Patient 3, 45/F

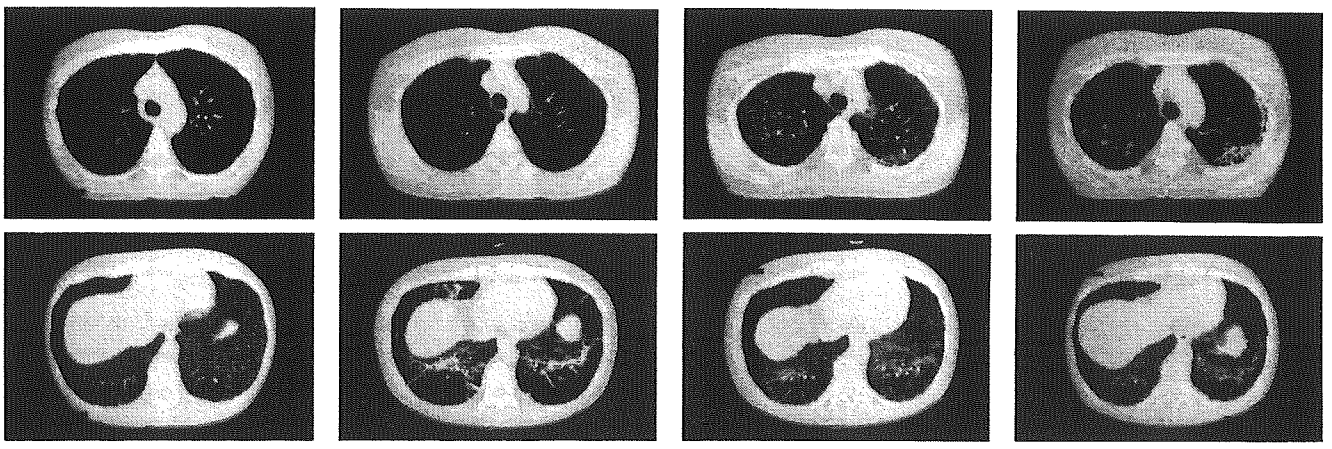

Figure 2B. Patient 3; paired images from different axial levels are shown sequentially.

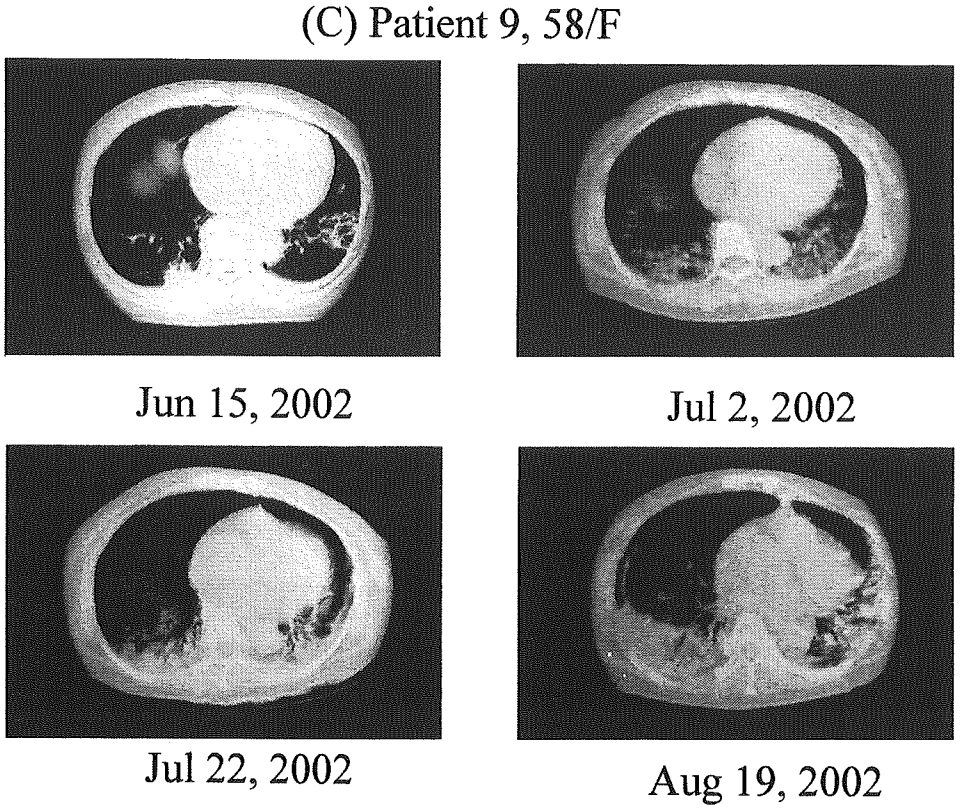

Figure 2C. Chest CT findings, Patient 9.

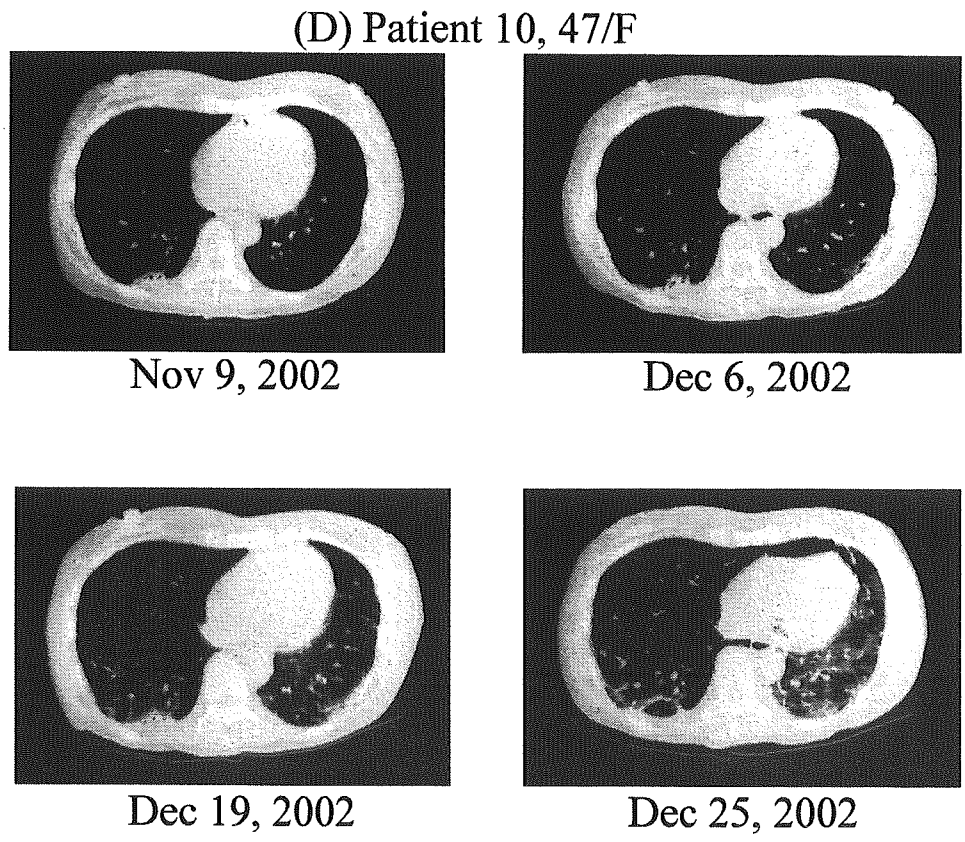

Figure 2D. Chest CT findings, Patient 10.

Table 2. Comparison of clinical features between DM patients with A/SIP and either chronic or no IP. Data are percentages, except serum CK.

	A/SIP, % (n = 22)	Chronic or No IP, % (n = 53)	p
Male	27	30	NS
Muscle weakness	74	76	NS
Serum CK ≥ 2 × upper limit	41	86	< 0.001
Serum CK, IU/l	429	4109	NS
Positive EMG	55	91	< 0.01
Positive muscle biopsy	32	81	< 0.001
Heliotrope rash	68	36	< 0.05
Gottron's papule/sign	91	76	NS
Palmar papule	64	4	< 0.0001
Fever ≥ 38°C	59	34	< 0.05
ANA ≥ × 160	41	73	< 0.05
Anti-Jo-1 positive	0	10	NS

NS: not significant.

during histologic examination for A/SIP[4,7,9-11]. Among these histologic patterns, patients with OP, and also a majority with NSIP, typically show a favorable response to corticosteroids[5,7,12]. In contrast, DAD usually shows exacerbation during immunosuppressive therapy, and is often fatal[6–8,13,14]. Recently, Kuroda, *et al* described 10 Japanese DM patients with A/SIP. Histopathological diagnosis was made in 5 patients: the diagnoses were DAD in 2 patients, who died, and NSIP in 3 patients including 2 survivors[7]. In our series of patients, transbronchial lung biopsy was performed in Patient 3; unfortunately, nondiagnostic samples were obtained. The remaining patients refused consent for lung biopsy procedures and autopsy results were obtained only from Patient 9, which revealed DAD.

Thus, we conducted serial HRCT of the chest once or twice a month in order (1) to confirm the diagnosis; (2) to clarify the mode of IP progression; and (3) to rule out opportunistic infections. We also regularly performed tests for monitoring the serum level of ß-D-glucan, bacterial cultures of the sputum, and polymerase chain reaction for the detection of *Pneumocystis carinii* in the sputum and to determine the copy number of cytomegalovirus in whole blood. Among 10 patients described here, none developed any opportunistic infections. The serial chest HRCT findings in the patients who died (Patients 7, 9, and 10; Figure 2) clearly showed steady progression of the IP despite immunosuppressive therapy, resulting in a fatal outcome; and based on the CT findings, the possible histologic patterns in these cases were consistently deduced to be NSIP or DAD. Importantly, the chest CT findings at the time of starting the combined immunosuppressive therapy were typically not so severe, even in patients who eventually died of respiratory failure within a few months. Therefore, the initial CT findings do not seem to be predictive of the prognosis.

In this study, we identified some characteristic clinical features of DM patients who developed A/SIP: (1) milder myositis, in terms of either the absence of muscle weakness or a serum CK level less than twice the upper limit value; (2) presence of the characteristic rashes of DM, including the heliotrope rash and Gottron's papules/signs; (3) presence of

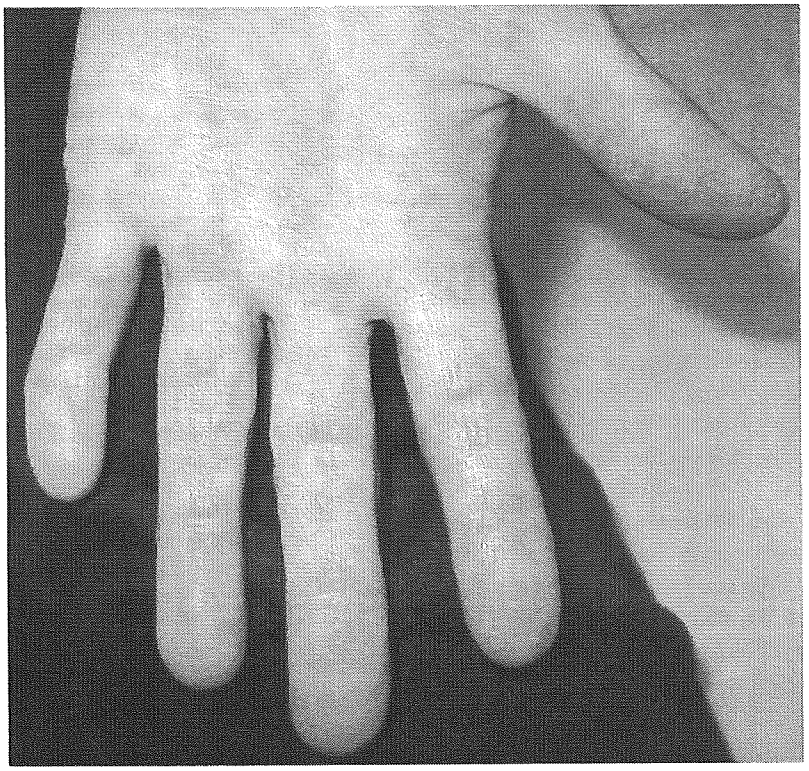

Figure 3. Palmar papules, Patient 10.

palmar papules; (4) presence of fever; and (5) negative tests for serum ANA, anti-Jo-1, and other autoantibodies. Among these 5 features, the association between palmar papules and the risk of development of A/SIP was the most striking. This is a painful papule, raising the possibility of the presence of cutaneous vasculitis. Unfortunately, however, skin biopsy samples from that lesion were not obtained in our patients. Instead, skin samples were always obtained from Gottron's papule on knuckles or elbows in order to confim the diagnosis of DM. Those biopsy samples always revealed perivascular lymphocytic infiltration, but they did not show evidence of vasculitis. However, it is also noteworthy that cutaneous vasculopathy has been reported to be associated with pneumomediastinum[15,16], which was observed in only one patient who survived, but in 3 out of the 5 patients who died in our series. Thus, palmar papule is likely to be an indicator not only of the presence or development of A/SIP, but also of the potential development of pneumomediastinum and a fatal outcome. Muscle histopathology in patients with A/SIP usually reveals mild myositis, and vasculitis was never observed in their muscle specimens.

The efficacy of CSA in patients of PM/DM with IP has been reported, and those reports indicate that CSA should be used early in the course of IP to obtain a favorable response[17-22]. However, the prognosis of a subset of patients with A/SIP complicating DM, especially those with DAD in ADM, is still very poor, despite immunosuppressive therapy with a single agent such as CSA or IVCYC at a standard dose (10–30 mg/kg)[6-8]. IVCYC is also the drug of choice for treatment of various lung diseases including IP associated with PM/DM[23]. The rationale for the combined use of CSA and IVCYC is based on the fact that CSA is a selective T cell inhibitor, whereas IVCYC mainly suppresses B cell functions[24]. Our aim was to improve the survival rate of the patients with DM and A/SIP, rather than to determine which immunosuppressive agent might be more effective. Adverse events were observed exclusively in surviving patients, mostly after a few months of the therapy: nasal septal perforation, subcutaneous abscess, and submucosal dissection of the esophagus in Patient 1; herpes zoster infection in Patient 2; diverticular hemorrhage in the ascending colon in Patient 3; and peripancreatic abscess in Patient 4.

With the use of the combination therapy regimen including PSL, CSA, and IVCYC, the survival rate of patients with A/SIP associated with DM seems to have improved from 25% in the era of conventional therapy to 50%. Nonetheless, it is more important that half of such patients still died of respiratory failure within a few months, despite immediate institution of an aggressive combination therapy. Furthermore, it is of considerable interest that A/SIP tended to develop about the same time as the onset of DM, and has not relapsed in surviving patients followed for more than 2 years (Table 1).

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

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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

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