The benefits of corticosteroids in preventing fetal CHB remain controversial [9,14]. Two separate retrospective studies reported possible corticosteroid protective effects during pregnancy against fetal CHB [8,10], while another study found that the use of corticosteroids did not affect recurrence of fetal CHB in mothers with past history of fetal CHB [4]. Moreover, the PR Interval and Dexamethasone Evaluation (PRIDE) study, a multicenter, open-label, nonrandomized study, found irreversibility of third-degree heart block and progression of second- to third-degree block despite treatment with dexamethasone [15]. These conflicting results could reflect differences between patients, the use of different types of corticosteroids and doses, and timing or duration of administration. In our study, we focused on the relationship of dose and timing of corticosteroid therapy with the incidence of fetal CHB. Based on our findings, we propose that anti-SS-A antibodies positive women with stable condition of CTD under the corticosteroids therapy before conception who continue the same therapy after conception are not at higher risk of fetal CHB, and that tapering or discontinuation of corticosteroids after conception in these women could increase the risk of fetal CHB. On the other hand, for women who were not treated with corticosteroids before conception, commencement of corticosteroids therapy (equivalent doses of PSL, at $\geq$10 mg/day) before 16-week gestation could reduce the risk of fetal CHB.

Although these three observations seem important clinically, our study has some limitations. First, the assays used to determine the titer of anti-SS-A antibodies were not standardized. Second, anti-SS-A antibodies were not investigated routinely in prenatal check-up, therefore many asymptomatic women with anti-SS-A antibodies could have been missed in this study. Third, this survey could have oversampled fetal CHB cases than non-fetal CHB cases, since anti-SS-A antibodies were more likely to be measured in women with a CHB child. For this reason, we excluded women confirmed to be positive for anti-SS-A antibodies after conception to reduce the possibility of selection bias.

In this study, we analyzed the maternal factors associated with the development of fetal CHB. The same data could be useful for analysis of the features of fetuses with CHB. Further studies using these data are planned in the near future.

In conclusion, the present study identified high titer of anti-SS-A antibodies as an independent risk factor for fetal CHB, and administration of corticosteroids (equivalent doses of PSL, at $\geq$10 mg/day) after conception before 16-week gestation to protect against the development of fetal CHB.

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Conflict of interest

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ORIGINAL ARTICLE

Nationwide epidemiological survey of 169 patients with adult Still's disease in Japan

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Abstract

Objectives. A nationwide survey was conducted to assess the number of patients, clinical aspects, treatment, and prognosis of adult Still's disease (ASD) in Japan.
Methods. A primary questionnaire was sent to randomly selected medical institutions in order to estimate the number of patients. We sent a secondary questionnaire to the same institutions to characterize the clinical manifestations and treatment of ASD.
Results. The estimated prevalence of ASD was 3.9 per 100,000. Analysis of 169 patients showed a mean age at onset of 46 years. The main clinical symptoms were fever, arthritis, and typical rash in agreement with previous surveys. Oral glucocorticoids were used to treat 96% of the patients, while methotrexate was used in 41% and biological agents were used in 16%. Lymphadenopathy and macrophage activation syndrome were significantly associated with increased risk of relapse ($P < 0.05$, each). Patients who achieved remission after tocilizumab therapy had significantly longer disease duration (6.2 years) than patients who did not (1.9 years) ($p < 0.05$).
Conclusions. The 2010–2011 nationwide survey of ASD identified important changes in treatment and improvement of prognosis compared with previous surveys.

Keywords

Adult Still's disease, Ferritin, Methotrexate, Multicenter study, Tocilizumab

History

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Introduction

Adult Still's disease (ASD) was first reported by Bywaters in 1971 [1] as an idiopathic systemic inflammatory disease with three main symptoms: quotidian fevers, arthritis, and evanescent rash. ASD is difficult to diagnose due to the lack of specific clinical manifestations and serum biomarkers. The ASD classification proposed by Yamaguchi et al. [2] in 1992 is used worldwide based on its high sensitivity and specificity. Two major epidemiological surveys were conducted in Japan by the research group of the Ministry of Health and Welfare of Japan in 1988 and 1994 [3,4]. Since the last survey, hyperferritinemia has been added to the Yamaguchi's criteria for reference and this has allowed easier and earlier diagnosis of ASD, and probably changed its clinical manifestations and prognosis. In addition, while ASD is generally treated with glucocorticoids, glucocorticoid-resistant ASD has recently been treated with methotrexate (MTX) or biological agents that are effective against rheumatoid arthritis (RA). Such drugs seem to have improved the course and prognosis of ASD over the past two decades. Indeed, some studies have shown the efficacy of immunosuppressive agents like cyclosporine A (CyA) and biologic agents like tumor necrosis factor (TNF) inhibitors or anti-interleukin (IL)-6 receptor antibody in the treatment of small cohort of patients with ASD [5–7]. Therefore, the research group for autoimmune diseases of the Ministry of Health, Labour and Welfare of Japan conducted another nationwide survey of ASD between 2010 and 2011 to estimate the number of ASD patients in Japan and to assess the clinical manifestations, treatment, course, and prognosis of this disease.

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Patients and methods

The survey was performed in two parts: the primary survey was designed to estimate the number of ASD patients treated at medical institutions, while the secondary survey assessed the clinical manifestations of ASD. In the primary survey, we randomly selected medical institutions that were stratified according to the number of beds and posted a questionnaire to the Department of Internal Medicine or Rheumatology about the number of ASD patients treated between January 1 and December 31, 2010.

The diagnosis of ASD depended on physician's judgment. We subsequently sent another questionnaire to the same hospitals in 2011 to obtain detailed information about the patients. To comply with the Personal Information Protection Law in Japan, all information that could identify an individual were made anonymous.

Subjects of the survey included ASD patients aged 16 years or older, who met Yamaguchi's criteria, and attended and/or were admitted to the hospital between January 1 and December 31, 2010. In the secondary survey, clinical information was obtained through a structured interview with the patient, physical

examination, laboratory tests, and review of medical records. Medications were determined by combining the information provided by patients and medical records. Laboratory data on all parameters were obtained at the time when the maximum serum ferritin was detected. Articular X-rays were taken and reviewed in all patients.

Statistical analysis

Demographic characteristics are presented as mean ± SD (median) for continuous variables and as frequencies and percentages for categorical variables. The associations between serum ferritin level and other variables such as leukocyte count, lactate dehydrogenase (LDH), aspartate aminotransferase (AST), and albumin were examined in ASD patients using Spearman's rank correlation analysis. Comparison of patients who achieved remission with patients who did not achieve remission after treatment with tocilizumab (TCZ), anti-IL-6 receptor antibody, was performed by Mann–Whitney U test, Fisher's exact test, and Cochran–Armitage test. We defined remission as the absence of articular, systemic, and laboratory evidence of disease activity under the current therapy [8]. Logistic regression analysis was employed to assess the association between clinical features and induction therapy with the risk of relapse in patients with ASD. For this analysis, variables of clinical features were age at onset (per 10 years old), gender, typical rash, lymphadenopathy, splenomegaly, disseminated intravascular coagulation (DIC), macrophage activation syndrome, abnormal liver function, and ferritin level (≧3,000 or not). Variables in relation to medications for induction therapy were administration of oral glucocorticoid only, pulse glucocorticoid therapy, MTX, CyA, non-steroidal anti-inflammatory drugs (NSAIDs), and TCZ. For treating missing data, we used the multiple imputation method. Two hundred imputed datasets were generated using the multiple imputation by chained equations method and their results were synthesized using the ordinary Rubin's rule [9]. We also evaluated potential predictive factors which associate with complication of MAS by logistic regressions. All analyses were performed using SPSS for Windows, version 18.0 (IBM Japan Inc., Tokyo, Japan) and R ver. 3.0.3 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Primary survey

A total of 7,999 Departments of Internal Medicine and 936 Departments of Rheumatology were subsequently stratified into the following seven categories according to the hospital size and number of beds: university hospitals, hospitals with ≧500 beds (excluding university hospitals), 400–499 beds, 300–399 beds, 200–299 beds, 100–199 beds, and ≦99 beds (Table 1). We randomly selected hospitals from among 100% of the university hospitals, the hospitals with 500 beds or over, and the departments of rheumatology, as well as from among 80% of the hospitals with 400–499 beds, 40% of the hospitals with 300–399 beds, 20% of the hospitals with 200–299 beds, 10% of the hospitals with 100–199 beds, and 5% of the hospitals with 99 beds or less. Among the 8,935 departments, we sent the primary questionnaire to 2,586 departments and we received 500 replies (19%). The reported number of patients was 956 (Table 1). The estimated number of patients with ASD across Japan was calculated by the following formula: Sum of [number of reported patients (C)/Returns (B) X Total number of departments (A) in each category]. The total number of ASD patients in 2011 was estimated to be 4,760 in Japan. According to the census conducted in 2010, the population of Japan on December 1, 2011 was 127,799,000; hence, the estimated prevalence rate of ASD was 3.9 patients per 100,000 people.

Secondary survey

Clinical characteristics

We received 40 replies to the secondary questionnaire survey and obtained clinical information on 169 patients, including 121 females, with a male:female ratio of 1:2.57 (Table 2). The information about 94 (55.6%) out of 169 patients with ASD was obtained from university hospitals in secondary survey. Estimated number of ASD patients in university hospitals was 1,516 (31.8%) out of 4,760 (estimated number of ASD patients in Japan). Thus, prevalence of the patients in university hospitals was higher in the secondary survey than that in the primary survey.

All patients satisfied Yamaguchi's criteria. Eight of the one sixty-nine patients developed ASD when they were at the age of younger than sixteen, while the other one fifty-eight patients had adult-onset ASD. The mean age at onset was 46 ± 19 years (Table 2). Disease duration at presentation of the patients was 0.4 ± 1.6 years (mean ± SD; median: 0.1 year). Observation period of this study was 4.9 ± 4.6 years (mean ± SD; median: 3.3 years). Two (1.3%) out of one fifty-eight patients had a family history of juvenile Still's disease, while none had family history of ASD. Seven (4.5%) out of one fifty-seven patients had family

Table 1. Reported patients in primary survey.

Category by specialty and number of beds	Total number of departments (A)	Surveys for target departments	Returns (B)	Response rate (%)	Number of reported patients with ASD (C)	Estimated number of patients with ASD (D)
Internal medicine						
University hospital	147	147	34	23	122	527
500 beds and over	384	384	60	16	118	755
400–499 beds	321	256	39	15	13	107
300–399 beds	662	265	39	15	7	119
200–299 beds	1023	204	39	19	10	262
100–199 beds	2367	236	55	23	7	301
Under 100 beds	3095	158	35	22	0	0
Subtotal	7999	1650	301	18	277	2072
Rheumatology						
University hospital	48	48	15	31	309	989
500 beds and over	67	67	16	24	79	331
400–499 beds	48	48	10	21	6	29
300–399 beds	67	67	12	18	12	67
200–299 beds	130	130	28	22	257	1193
100–199 beds	270	270	52	19	10	52
Under 100 beds	306	306	66	22	6	28
Subtotal	936	936	199	21	679	2688
Total	8935	2586	500	19	956	4760

ASD adult Still's disease

Table 2. Clinical characteristics of patients with ASD .

	Present study ($n = 169$) Values (Frequency)	Previous survey in 1988 ($n = 90$) Values (Frequency)
General characteristics		
Infant onset:Adult onset	8 (4.8%):158 (95.2%)	NA
Age at onset, years	46 ± 19 (median 46)	NA
Female (%)	121/168 (72.0%)	60/90 (66.7%)
Family history		
Juvenile idiopathic arthritis	2/158 (1.3%)	NA
ASD	0/159 (0.0%)	NA
RA	7/157 (4.5%)	NA
Other autoimmune disease	3/157 (1.9%)	NA
Clinical characteristics		
Fever > 39.0°C, ≧ 1 week	152/166 (91.6%)	71/90 (78.9%)
Arthralgia > 2 weeks	138/166 (83.1%)	90/90 (100.0%)
Arthritis	77/152 (50.7%)	62/86 (72.1%)
Typical rash	102/164 (62.2%)	72/83 (86.7%)
Sore throat	96/162 (59.3%)	58/83 (69.9%)
Lymphadenopathy	72/161 (44.7%)	59/86 (68.6%)
Splenomegaly	52/161 (32.3%)	56/86 (65.1%)
Pericarditis	5/161 (3.1%)	9/87 (10.3%)
Pleuritis	6/161 (3.7%)	11/89 (12.4%)
Interstitial pneumonia	4/161 (2.5%)	NA
Myalgia	42/162 (25.9%)	50/89 (56.2%)
Drug allergy	29/165 (17.6%)	44/82 (53.7%)
Complications		
Amyloidosis	0/127 (0.0%)	NA
DIC	8/127 (6.3%)	NA
Macrophage activation syndrome	19/127 (15.0%)	NA

There were some missing data in the database of this study. Total number of enrolled patients in this study was 169. Information about disease onset and gender was not obtained from 3 patients and 1 patient, each.

DIC disseminated intravascular coagulation, *NA* not applicable

Infant onset was defined as ASD developed at the age of less than sixteen. Adult onset was defined as ASD developed at sixteen years old or older.

history of RA and 3 patients had family history of autoimmune diseases (Graves' disease, $n = 1$; Sjögren's syndrome, $n = 2$) (Table 2). Clinical manifestations detected in the 169 ASD patients were mainly fever (> 39°C for at least 1 week, 91.6%), arthralgia (persisting for at least 2 weeks, 83.1%), and typical rash (62.2%). The features corresponded to the results of previous survey in Japan [3].

Arthritis was found in 44.4% of patients with arthralgia ($n = 138$) (Table 3). The number of ASD patients with monoarthritis, oligoarthritis, and polyarthritis was 3, 33, and 41, respectively. Polyarthritis was the most common in patients with ASD. The involved joints were the wrists (27.0%), knees (27.0%), and shoulders (15.8%) in order of descending prevalence. Articular X-rays of suffered joints were carried out in each patient by the attending physician's decision. Because we assumed that joint destruction did not exist when no articular X-ray was taken in the patients, we count such patients as having no joint destruction. Fifteen patients (11.7%) showed joint destruction, such as bone erosion (11 patients, 8.6%), joint space narrowing (10 patients, 7.8%), and/or ankylosis (2 patients, 1.6%). Three patients showed other radiographic abnormalities (osteoporosis, spur formation at the distal interphalangeal joints, and unspecified changes in 1 case each), while 110 other patients (85.9%) showed no radiographic abnormalities. Of 128 ASD patients with available X-ray findings,

Table 3. Articular manifestations on each joints during 6 months after disease onset in patients with ASD.

	Patients who were assessed by X-ray ($N = 128$)							
Joint	No of Pts with erosion in each joint	Erosion (%)	No of Pts with JSN in each joint	JSN (%)	No of Pts with ankylosis in each joint	Ankylosis (%)	No of Pts who had each joint destruction (erosion or JSN or ankylosis)	each joint destruction (%)
TMJ	0	0.0	0	0.0	0	0.0	0	0.0
Shoulder	0	0.0	1	0.8	0	0.0	1	0.8
SCJ	0	0.0	0	0.0	0	0.0	0	0.0
Elbow	2	1.6	1	0.8	0	0.0	2	1.6
Wrist	7	5.5	7	5.5	1	0.8	10	7.8
Hip	3	2.3	1	0.8	0	0.0	3	2.3
Knee	3	2.3	3	2.3	1	0.8	6	4.7
Ankle	2	1.6	1	0.8	0	0.0	3	2.3
MCP	1	0.8	4	3.1	0	0.0	4	3.1
PIP	3	2.3	6	4.7	0	0.0	8	6.3
DIP	1	0.8	3	2.3	0	0.0	3	2.3
ITJ	0	0.0	1	0.8	0	0.0	1	0.8
MTP	0	0.0	1	0.8	0	0.0	1	0.8

Pts patients, *JSN* joint space narrowing, *No* number, *TMJ* Temporomandibular joint, *SCJ* Sternoclavicular joint, *MCP* Metacarpophalangeal, *PIP* Proximal interphalangeal, *DIP* Distal interphalangeal, *ITJ* Intertarsal joints, *MTP* Metatarsophalangeal

Several joints were affected at the same time in some of the patients.

the radiographic abnormalities of erosion, joint space narrowing, and ankylosis were commonly seen in the wrists, knees, and proximal interphalangeal joints (Table 3). Several joints were affected at the same time in some of the patients. Erosions and joint space narrowing were detected in several joints in 8 patients and in 6 patients, each. Other findings included sore throat (57.3%), lymphadenopathy (44.7%), splenomegaly (32.3%), and drug allergy (17.6%) (Table 1). With respect to complications, 15.8% of the patients had macrophage activation syndrome (MAS) and 6.3% developed DIC, while none of the patients had secondary amyloidosis (Table 2).

Laboratory findings

The results of various laboratory tests are summarized in Table 4. In general, the laboratory tests demonstrated an inflammatory response, with high leukocyte count ($\geq$10,000/mm^3, 79.4%), polymorphonuclear cells ($\geq$80%, 71.5%), erythrocyte sedimentation rate (ESR; $\geq$40 mm/hr, 68.9%), and C-reactive protein (CRP; positive CRP, 91.5%), together with liver dysfunction (abnormal liver function tests, 73.9%) and hyperferritinemia (presence of hyperferritinemia, 88.5%). Severe hyperferritinemia (ferritin, $\geq$3,000 ng/mL) was noted in 60% of the patients. Serum rheumatoid factor (RF) and antinuclear antibody were negative in 79.9% and 74.2% of the patients, respectively. Serum IL-6 levels were high in all of the 15 patients tested. Plasma IL-18 levels were also elevated in 11 out of the 12 patients tested (91.7%).

Various factors, such as leukocyte count ($r=0.289$, $P<0.001$), AST ($r=0.561$, $P<0.001$), LDH ($r=0.677$, $P<0.001$), and hypoalbuminemia ($r=-0.445$, $P<0.001$), correlated significantly with serum ferritin level at time of peak hyperferritinemia.

Treatment

The drugs used to treat 166 ASD patients are listed in Table 5. The most common was oral glucocorticoid, which was used in 160 patients (96.4%), followed by NSAIDs in 73 patients (44.0%). With respect to immunosuppressants, MTX was used in 68 patients (41.0%), followed by CyA in 45 patients (27.1%). Fifty-two patients (31.3%) were treated with glucocorticoid pulse therapy (Table 5, left column). Biological drugs were used on 33 occasions in 27 patients (16.3%) (Table 5, left column, and Table 6). Among them, four patients received two biologic agents (Patients 1, 5, 15, and 24) and one patient received three biologics (Patient 14, Table 6). As induction therapy for ASD ($n=161$), oral glucocorticoid alone was the most common choice and used in 82 patients (50.9%), among whom 47 (29.2%) were treated with glucocorticoid pulse therapy. MTX was combined with glucocorticoid in 37 patients (23.0%) and CyA was administered with steroids in 30 patients (18.6%) (Table 5, middle column).

With regard to treatment of relapses ($n=67$), oral glucocorticoid monotherapy was the most common, being used in 28 patients (41.8%). Twelve patients (17.9%) were treated with steroid pulse therapy. MTX was combined with glucocorticoid in 17 patients (25.4%), and CyA was administered with glucocorticoid in 8 patients (11.9%) (Table 5, right column).

Table 6 lists the demographic profiles of 27 patients treated with biologic agents, comprising TNF inhibitors in 12 patients (infliximab in 7 patients, etanercept in 4 patient, and adalimumab in 1 patient) and TCZ in 21 patients. We obtained clinical information on 19 patients out of 21 patients who received TCZ. Nine patients were treated with TCZ for induction therapy; however, only 2 achieved remission and 5 patients stopped TCZ because of adverse events (allergic reaction, hypotension, MAS, rash, and infection). On the other hand, 10 patients were treated with TCZ for maintenance therapy (2 patients) or for relapse (8 patients), among whom 7 patients achieved remission and 1 developed fungal infection. Comparison of clinical features of patients who achieved remission ($n=9$) with patients who did not achieve remission ($n=10$) by TCZ indicated significantly longer disease duration (6.2 ± 5.6 years) in the former compared with the latter (1.9 ± 2.8 years, $P=0.03$). Age, gender, treatment period, and prevalence of patients with oral glucocorticoid or with other immunosuppressant were not related to TCZ-treated patients who did or did not achieve remission.

Clinical outcome

Of the 146 patients with available data on the clinical course, 58 (39.7%) and 50 patients (34.2%) showed monocyclic and polycyclic systemic patterns, respectively, while 15 (10.3%) and 23 patients (15.8%) showed monocyclic and polycyclic systemic

Table 4. Laboratory findings in patients with ASD.

	Present survey in 2011 ($n=169$) Values (Frequency)	Previous survey in 1988 ($n=90$) Values (Frequency)
Leukocytosis (Leucocytes $\geqq$ 10,000/μL)	131/165 (79.4%)	80/90 (88.9%)
Glanulocytosis (Neutrophils $\geqq$ 80%)	118/165 (71.5%)	74/89 (83.1%)
Anemia (Hemoglobin $\leqq$ 10 g/dL)	68/169 (40.2%)	53/90 (58.9%)
Thrombocytopenia (Platelets $< 15\times10^4$)	23/169 (13.6%)	NA
Elevated ESR (ESR $\geqq$ 40mm/hr)	113/164 (68.9%)	85/89 (95.5%)
Hypoproteinemia[#]	32/169 (18.9%)	NA
Hypoalbuminemia[#]	107/139 (77.0%)	44%
Abnormal liver function[†]	122/165 (73.9%)	74/87 (85.1%)
Positive CRP	151/165 (91.5%)	NA
Hyperferritinemia[*]	146/165 (88.5%)	28/34 (82.4%)
Serum ferritin levels above 3,000 ng/mL	99/165 (60.0%)	NA
Positive RF	33/164 (20.1%)	5/89 (5.6%)
Positive ANA	42/163 (25.8%)	6/88 (6.8%)
Elevation of serum IL-6 (pg/mL)	15/15 (100.0%)	NA
Elevation of plasma IL-18 (pg/mL)	11/12 (91.7%)	NA

ESR erythrocyte sedimentation rate, *CRP* C-reactive protein, *RF* rheumatoid factor, *ANA* anti-nuclear antibodies, *IL-6* interleukin-6, *IL-18* interleukin-18, *NA* not applicable

[†]Abnormal liver function was defined as any elevated liver enzymes (aspartate aminotransferase, alanine aminotransferase, and lactate dehydrogenase) above the normal range in each medical facility.

[#]Hypoproteinuria and hypoalbuminemia were defined as total protein and serum albumin levels lower than a normal reference value in each medical facility.

[*]Hyperferritinemia was defined as serum ferritin levels higher than a normal reference value in each medical facility.

Table 5. Treatment of patients with ASD.

Medication for ASD ($N = 166$)		Induction therapy for ASD ($N = 161$)		Therapy for relapse of ASD ($N = 67$)	
Drugs	Numbers of patients (%)	Therapy	Numbers of patients (%)	Therapy	Numbers of patients (%)
Medication	163 (98.2%)	Pulse GC therapy	47 (29.2%)	Pulse GC therapy	12 (17.9%)
Pulse GC therapy	52 (31.3%)	GC only	82 (50.9%)	GC only	28 (41.8%)
GC	160 (96.4%)	GC + MTX	21 (13.0%)	GC + MTX	10 (14.9%)
NSAIDs	73 (44.0%)	GC + CyA	20 (12.4%)	GC + CyA	8 (11.9%)
MTX	68 (41.0%)	GC + NSAIDs	9 (5.6%)	GC + TCZ	3 (4.5%)
CyA	45 (27.1%)	GC + MTX + CyA	6 (3.7%)	MTX + TCZ	2 (3.0%)
TAC	12 (7.2%)	GC + MTX + TAC	3 (1.9%)	GC + MTX + NSAID	2 (3.0%)
Salazosulfapyridine	4 (2.4%)	TCZ	3 (1.9%)	GC + MTX + TAC	2 (3.0%)
Mizoribine	3 (1.8%)	GC + MTX + NSAID	2 (1.2%)	Other therapy	10 (14.9%)
Azathioprine	3 (1.8%)	Other therapy	15 (9.3%)		
Cyclophosphamide	2 (1.2%)				
Leflunomide	1 (0.6%)				
Auranofin	2 (1.2%)				
Gold	1 (0.6%)				
Etoposide	1 (0.6%)				
Infliximab	7 (4.2%)				
Etanercept	4 (2.4%)				
Adalimumab	1 (0.6%)				
Tocilizumab	21 (12.7%)				

ASD adult Still's disease, *GC* glucocorticoid, *MTX* methotrexate, *CyA* cyclosporine, *NSAIDs* non-steroidal anti-inflammatory drugs, *TAC* tacrolimus, *TCZ* tocilizumab.

Other therapy means the therapies carried out in only one patient, respectively.

As induction therapy, the group of other therapy includes 5 patients treated with MTX + GC+ some other drugs and 2 patients treated with CyA + GC+ some other drugs. As therapy for relapse, the group of other therapy includes 3 patients treated with MTX + GC+ some other drugs.

patterns with chronic articular involvement, respectively. Furthermore, a self-limited pattern was seen in 11 patients. During the observation period, 66 out of 169 patients (39.1%) experienced relapse. However, there were no deaths during the observation period. At the last medical examination, 145 out of 164 patients (88.4%) had achieved remission.

Relationship between clinical features/induction therapy and risk of relapse

Association of clinical features at baseline and induction therapy with risk of relapse was investigated by logistic regression analysis. Relapse was observed in 66 out of 169 patients with ASD. According to the previous report [10], serum ferritin levels of above 3000 ng/mL in a patient with compatible symptoms should lead to suspicion of ASD in the absence of a bacterial or viral infection. Therefore, we defined cutoff levels of ferritin as more than 3000 ng/ml. Univariate analysis showed that lymphadenopathy (odds ratio [OR] = 1.99, 95% confidence interval [CI]: 1.04–3.78; $p = 0.037$), and complication of MAS (OR = 2.88, 95%CI: 1.04–8.00; $p = 0.043$) were associated with risk of relapse in patients with ASD; however, marked hyperferritinemia (≥3,000 ng/mL), complication of DIC, and medications of induction therapy were not. Multivariate analysis identified lymphadenopathy as the only significant factor for risk of relapse after adjustment for age, sex, other clinical features, and medications for induction therapy (OR = 2.40, 95%CI: 1.08–5.33; $p = 0.032$).

Potential predictive factors for complication of MAS in patients with ASD

We conducted logistic regression analyses to explore potential predictive factors for complication of MAS in ASD. Here, the number of MAS events was small (only 19), so we evaluated univariate associations. As a result, AST (OR = 1.84, 95%CI: 1.24–2.74; $p = 0.003$), LDH (OR = 5.07, 95%CI: 1.98–12.97; $p = 0.001$), and hyperferritinemia (OR = 4.36, 95%CI: 1.30–14.68; $p = 0.017$) were significantly associated with complication of MAS. These factors have been known to be included in laboratory findings of MAS and the strong associations were also observed in the clinical data.

Discussion

According to the 1994 nationwide survey conducted by the research group of the Ministry of Health and Welfare of Japan [4], the prevalence rate of ASD was approximately 2 patients per 100,000 and the male:female ratio was 1:2. The current survey conducted in 2010 showed a prevalence rate of 3.7 per 100,000 people in Japan. The tendency for ASD to show female predominance was still noted. A French retrospective study published in 1995 showed an estimated prevalence rate of ASD of 0.16 cases per 100,000 people and no difference in prevalence between males and females [11]. These results suggest a higher prevalence rate in Japan than that in France. Yamaguchi's classification criteria were used in the 1994 survey, but serum ferritin was not included at that time [4]. The research group of the Ministry of Health and Welfare of Japan assessed the diagnostic value of serum ferritin and concluded that the inclusion of ferritin did not improve the diagnostic accuracy. Hence, serum ferritin is only used for reference in Yamaguchi's criteria. In the present survey, 89% of ASD patients showed hyperferritinemia and serum ferritin level was more than 3,000 ng/mL in 60% of patients. In the 1988 epidemiological survey of ASD, the major clinical manifestations consisted of fever (100%), high fever (81%), arthralgia (100%), typical rash (87%), sore throat (70%), lymphadenopathy (69%), splenomegaly (65%), pleuritis (12%), and pericarditis (10%) (Table 2) [3]. Laboratory findings included leukocytosis (89%), liver dysfunction (85%), negative RF (94%), and negative antinuclear antibody (93%) (Table 4). In the present survey, fewer patients were positive for each of these items (Table 4). Since earlier diagnosis would have been possible due to the wide-spreading knowledge of this disease, the prevalence of clinical and laboratory findings decreased in ASD patients of this study in 2011 compared with the 1988 epidemiological survey of ASD. Also, drug allergy was only found in 18% of the patients in this survey compared with 54% in the 1988 survey (Table 2). Initiation of steroids at an early stage of the disease might have resulted in a decrease in the number of ASD patients with drug allergy.

Recent advances have demonstrated the major role of proinflammatory cytokines, such as IL-6 and IL-18, in the pathogenesis of ASD [12,13]. IL-6 levels are associated with disease activity, and IL-18 levels are thought to be a marker of disease severity and

Table 6. Demographic profile of 27 patients treated with biologic agents.

Patient	Age (years)	Gender	Disease duration (years)	Biologic agent	Therapy	Treatment period (months)	Adverse events	Treatment progress	With GC	With other immunosuppressant
1–1	21	M	0.3	TCZ	Induction therapy	5	Allergy	Withdrawal (adverse event)	Yes	None
1–2	21	M	0.3	ADA	Induction therapy	NA		Ongoing (active)	NA	NA
2	20	M	0.1	TCZ	Induction therapy	10	Hypotension	Withdrawal (adverse event)	No	None
3	47	M	0.1	TCZ	Induction therapy	6		Withdrawal (remission)	Yes	CyA
4	26	F	0.1	TCZ	Induction therapy	1	MAS	Withdrawal (adverse event)	Yes	None
5–1	48	M	0.1	TCZ	Induction therapy	7		Withdrawal (unavailable)	No	None
5–2	48	M	1.2	IFX	Therapy at relapse	9		Ongoing (remission)	Yes	MTX
6	36	F	1.7	TCZ	Induction therapy	38		Ongoing (remission)	No	None
7	48	F	0.2	TCZ	Induction therapy	2	Generalized rash	Withdrawal (adverse event)	No	CyA
8	71	F	5.7	TCZ	Therapy at relapse	12		Ongoing (remission)	Yes	MTX
9	57	F	9.4	TCZ	Therapy at relapse	5		Ongoing (remission)	Yes	None
10	23	M	8.5	TCZ	Therapy at relapse	12		Ongoing (active)	Yes	None
11	78	F	1.8	TCZ	Therapy at relapse	18	Fungal infection	Ongoing (remission)	Yes	None
12	65	F	NA	IFX	Maintenance therapy	NA		NA	Yes	MTX
13	46	M	4.6	TCZ	Maintenance therapy	10		Ongoing (remission)	Yes	MTX
14–1	35	F	1.4	IFX	Induction therapy	17		Withdrawal (unavailable)	Yes	MTX
14–2	35	F	2.8	TCZ	Induction therapy	20	Infection	Withdrawal (adverse event)	Yes	MTX
14–3	35	F	4.8	ETN	Maintenance therapy	3		Withdrawal (unavailable)	Yes	MTX
15–1	34	F	NA	IFX	NA	NA		NA	NA	NA
15-2	34	F	15.1	TCZ	Therapy at relapse	22		Ongoing (remission)	Yes	MTX
16	39	F	NA	TCZ	Therapy at relapse	10		Ongoing (remission)	Yes	TAC
17	72	F	4.3	TCZ	Therapy at relapse	96		Ongoing (active)	None	MTX
18	42	M	1.7	IFX	Maintenance therapy	41		Withdrawal (remission)	Yes	MTX
19	50	F	0.2	TCZ	Induction therapy	8		Withdrawal (economic reason)	Yes	MTX
20	76	M	NA	TCZ	NA	NA		NA	NA	NA
21	32	F	NA	ETN	NA	NA		NA	NA	NA
22	52	M	NA	ETN	Maintenance therapy	NA		Withdrawal (remission)	Yes	CyA
23	60	F	NA	TCZ	NA	NA		NA	NA	NA
24–1	49	F	3.9	TCZ	Therapy at relapse	11		Withdrawal (unavailable)	Yes	MTX
24–2	49	F	4.8	IFX	Therapy at relapse	10		Ongoing (remission)	Yes	MTX
25	50	F	NA	IFX	Maintenance therapy	NA		Ongoing (remission)	Yes	MTX
26	37	F	2.5	ETN	Therapy at relapse	NA		Ongoing (active)	Yes	TAC
27	24	F	7.3	TCZ	Maintenance therapy	40		Ongoing (remission)	Yes	None

M male, *F* female, *GC* glucocorticoid, *MTX* methotrexate, *CyA* cyclosporine A, *TAC* tacrolimus, *ETN* etanercept, *IFX* infliximab, *ADA* adalimumab, *TCZ* tocilizumab, *MAS* macrophage activation syndrome, *NA* not applicable.

Patient 1 was treated with TCZ and ADA. Patients 5, 15, and 24 were treated with TCZ and IFX. Patient 14 was treated with IFX, TCZ, and ETN.

MAS in patients with ASD [12,13]. Serum IL-6 and plasma IL-18 were elevated in patients with ASD in this study, despite the small number of patients tested for these parameters.

Pouchot et al. [8] reported that 41% (16 of 39 patients) of ASD patients had abnormal X-ray findings of joint space narrowing at the carpometacarpal or intercarpal joints of the wrist, which progressed to ankylosis in 25% of the patients. In the present study, 12% of patients exhibited radiographic abnormalities (erosion, 9%; joint space narrowing, 8%), and only 2% showed ankylosis. With respect to the radiographic joint damage, the prognosis of joints in ASD seems to be relatively better than that before 1990s. A study of 90 ASD patients in 1990 reported that the polycyclic systemic pattern was the most common, being seen in 41% of patients, while more than half of the patients experienced relapse (55%) and 4 patients died (4%) [3]. At that time, glucocorticoid and NSAIDs were used for treating 92% and 79% of the patients, respectively, while only 10% were treated with immunosuppressants (cyclophosphamide and azathioprine) and neither MTX nor CyA was used. In the present survey, the monocyclic systemic pattern was the most common (in 40% of the patients), while 39% of patients experienced relapse and none of the patients died. The majority of the large observational studies of ASD were performed before MTX became widely used and before the marketing of biologic agents. The long-term benefits of MTX in limited joint destruction have been demonstrated in RA but not in ASD. One possible reason for the improved prognosis is that ASD was better controlled by steroid therapy combined with immunosuppressants (MTX or CyA) or biological agents, all of which have become available after the previous survey. ASD patients occasionally develop severe complications such as MAS or reactive hemophagocytotic syndrome (RHS). According to a retrospective study of 50 patients with ASD, 6 patients (12%) experienced RHS [14]. Another retrospective observational study showed that RHS was complicated in 8 out of 57 patients (14.0%) in ASD patients [15]. In our survey, 16% of patients developed MAS or RHS, which was similar to that in the previous reports. We evaluated potential predictive factors which were associated with complication of MAS in ASD. In our study patients with ASD, AST, LDH, and hyperferritinemia were associated with complication of MAS. Although the above factors have been already known to be included in laboratory findings of MAS and might not be novel findings, the information obtained in this epidemiological study would be meaningful. Secondary amyloidosis was also identified as a complication of ASD in previous surveys, but was not recognized in any of the patients in the present survey [2,15,16]. This could be due to early treatment, which resulted in inhibition of production and deposition of amyloid proteins associated with chronic inflammation.

Glucocorticoids were the most commonly used medications on the treatment of ASD. Furthermore, glucocorticoid pulse therapy, DMARDs, or biologic agents were added to control the disease based on the degree of disease activity and severity. The prognosis of ASD is known to be relatively good; however, 39% of the patients with ASD experienced relapse while on therapy or after discontinuation of treatment during the observation period (the mean observation period between the first and last examinations was 4.9 years in this survey). We investigated whether clinical features and induction therapy were associated with risk of relapse. Previous studies reported significant correlation between hyperferritinemia and disease activity, and recommended the use of hyperferritinemia as a marker to monitor the response to treatment in ASD [17,18]. Analysis of our survey data showed no relation between serum ferritin levels above 3,000 ng/mL and risk of relapse. Furthermore, pulse glucocorticoid therapy, and MTX, CyA, NSAIDs, and TCZ for the induction therapy did not reduce the risk of relapse. Univariate analysis showed that lymphadenopathy and MAS were associated with increased risk of relapse in patients with ASD, suggesting that these two complications could be considered as risk factors for relapse in patients with ASD.

Twenty-one ASD patients in the present survey were treated with TCZ, and clinical information was available for 19 of these 21 patients. Patients who achieved remission after treatment with TCZ had longer duration of disease compared with those who did not. A few studies have reported the efficacy of TCZ in refractory ASD [6,7]. Furthermore, 94% of 35 patients with ASD reported in the literature were resistant to other immunosuppressive agents, such as MTX, TNF blockers, and anakinra. TCZ induced remission and allowed reduction of the dose or discontinuation of corticosteroids [19]. Based on the above results and those of the present survey, we recommend the use of TCZ for treatment in patients with refractory and long-standing ASD.

There have been some papers about successful treatment experience with TCZ in patients with ASD [6,7,20,21]. MAS is a life-threatening syndrome with excess immune activation. MAS occurs either in ASD or in systemic juvenile idiopathic arthritis (sJIA) [14,22]. Major findings are fever, hepatosplenomegaly, rash, lymphadenopathy, neurologic symptoms, cytopenias, hyperferritinemia, and abnormal liver function. The propriety of TCZ therapy in MAS complicated with ASD patients has been controversial. In some cases of ASD and sJIA, MAS aggravated during TCZ therapy [6,19,21,23]. Although the contribution of TCZ to occurrence of MAS has not been determined, careful observation should be required during TCZ therapy in patients with active ASD.

Conclusion

We conducted a nationwide survey of ASD, which showed changes in treatment and improvement of prognosis compared with previous surveys. Our findings suggest that lymphadenopathy and MAS are potential risk factors for relapse in patients with ASD. The use of immunosuppressants like MTX or CyA and biologics for ASD has increased in recent years. We also recommend the use of TCZ for treatment of relapse in patients with long-standing ASD.

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Conflict of interest

None.

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Effects of Bosentan on the Skin Temperature of Hands and Feet in Patients with Connective Tissue Diseases Complicated with Raynaud's Phenomenon: A Prospective, Open-Label, Uncontrolled, Single-Center Study

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Abstract- Objective: To assess the effects of bosentan on Raynaud's phemonenon and the skin temperature of hands and feet in patients with connective tissue diseases (CTDs) complicated with digital ulcers or pulmonary arterial hypertension (PAH).

Methods: An open-label, non-controlled, single-center, prospective study, which was designed to exclude the seasonal bias. Bosentan was commenced from 62.5mg twice daily for four to six weeks, followed by 125mg twice daily for 10 to 12 weeks (total period was 16 weeks). Bosentan was reduced or discontinued if adverse events were appearing. Patients without adverse events for 16 weeks continued the trial for 52 weeks.

Results: In 13 enrolled patients, six were patients with suspected PAH and eight had digital ulcers. Ten patients were diagnosed with systemic sclerosis (eight with limited cutaneous and two with diffuse cutaneous form), two with mixed connective tissue disease and one with systemic sclerosis (diffuse cutaneous form)-polymyositis overlap syndrome.

After 16-week bosentan therapy, the frequency and the duration of Raynaud's phenomenon was significantly decreased (P=0.009, P = 0.008, respectively). Not the numbness, but the cold sensation of hands and feet was also improved (P = 0.021). Skin temperature measured by thermography was not increased after 16-week treatment, but the significant increases were seen after 52 weeks, respectively (P = 0.038 & P = 0.025). Nasal bleeding in one patient and liver dysfunction in four patients was investigated.

Coclusions: It was suggested that the long-term treatment of bosentan could improve the decreased skin temperature in CTD patients with secondary Raynaud's phenomenon.

Keywords: digital ulcers, endothelin receptor antagonist, pulmonary arterial hypertension, secondary Raynaud's phenomenon, systemic sclerosis, thermography.

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I. Introduction

Endothelins are consisted with 21-amino acid and induce potent vasoconstriction (1). There are three isoforms in endothelins (ET1-3) and their receptors are divided into ETA, ETB1, ETB2 and ETC. Bosentan is an antagonist of ETA and ETB and is clinically indicated for pulmonary arterial hypertension (PAH) and ongoing digital ulcers (2). PAH is one of serious complications in some connective tissue diseases (CTDs), such as mixed connective tissue disease (MCTD), systemic sclerosis (SSc) and systemic lupus erythematosus, and influences to their prognosis (3).

On the other hand, Raynaud's phemonenon is another symptom in CTDs that is not commonly critical, but often impairs quality of life and may lead occasionally digital ulcers. Raynaud's phemonenon is induced by cold temperature or emotional stress. It gets worse in winter, and is diminished since the end of winter and usually disappears during the summer. To judge the effectiveness of medicines for Raynaud's phemonenon, the timing to evaluate is very important. For example, it is not fair to estimate the efficacy in spring or summer for the therapy starting from midwinter. However the point to evaluate Raynaud's phemonenon has not been clear in most of the reports (4-11). Herein, to exclude the seasonal bias, we set observation time strictly and investigated the efficacy of bosentan on Raynaud's phemonenon and the skin temperaturein patients with CTDs.

II. Materials and Methods

a) Study design

The probe was planned as an open-label, non-controlled, single-center, prospective study. Patients were recruited from the outpatient clinic of the Department of Rheumatology and Applied Immunology, the Saitama Medical University Hospital. Bosentan was

commenced since the end of November, from 62.5mg twice daily for four to six weeks, followed by 125mg twice daily for 10 to 12 weeks (total period was 16 weeks). Bosentan was reduced or discontinued if adverse events were appearing. Patients without adverse events for 16 weeks continued the trial for 52 weeks. Prior medications administered for more than 12 weeks were permitted to continue (Table 1).

Table 1 : Patient background

No.	Disease	Gender	Age (years)	Disease Duration (years)	PAH (WHO FC)	No. of DU or DUS	Prior Medication
1	lcSSc	F	77	21	0	2U, 1S	Beraprost Sarpogrelate
2	lcSSc	F	76	17	III	0	Sarpogrelate
3	lcSSc	F	71	25	II	0	Beraprost
4	lcSSc	F	71	15	0	1U	Beraprost
5	lcSSc	F	65	16	II	0	Beraprost Sarpogrelate
6	lcSSc	F	62	1	0	6S	Beraprost
7	lcSSc	F	53	18	0	2U, 1S	Beraprost Sarpogrelate
8	lcSSc	M	53	1	II	0	NA
9	dcSSc	M	71	16	III	1S	Beraprost Sarpogrelate
10	dcSSc	F	45	11	0	2U	Sarpogrelate
11	lcSSc PM	F	59	24	0	1U, 1S	Beraprost Sarpogrelate
12	MCTD	F	58	11	II	0	NA
13	MCTD	F	45	7	0	1U	Beraprost Sarpogrelate

The study protocol conformed to the principles of the Declaration of Helsinki and was approved by the institutional review board of the Saitama Medical University Hospital (09-028-1).

b) Patients

Patients with SSc (12) and systemic lupus erythematosus (SLE) (13) were diagnosed according to the American College of Rheumatology criteria, MCTD according to the criteria proposed by the Special Research Committee for MCTD of the Japanese Ministry of Health and Welfare (Kasukawa criteria) (14) and polymyositis (PM) according to the Bohan and Peter's criteria(15). PAH was suspected from more than four out of six clinical and laboratory findings, including exertional dyspnea, systolic pulsation on the left sternum, increase of the pulmonary segment of the second cardiac sound, enlargement of the base of the pulmonary artery or protrusion of the left second aortic arch in the chest X-ray, right ventricular hypertrophy or load as diagnosed by the electrocardiogram, right ventricular enlargement, right ventricular load or right ventricular pressure greater than 35 mmHg by the Doppler echocardiogram. Patients with ischemic heart diseases, valvular diseases unrelated with PAH and congenital heart diseases were excluded.

c) Clinical evaluation

Raynaud's phemonenon was evaluated by the diaries as follows; the number of the attacks daily, the duration of the attacks daily and an assessment of severity of cold sensation and numbness of hands and feet by a visual analogue scale (VAS) of 100 mm. The number of digital ulcers and scars were recorded at the baseline, at Week 16 and at Week 52, or at the time of dropped-out. Thermography was carried out just before starting bosentan, after 16 weeks and after 52 weeks receiving bosentan. After sitting on the chair for 20 min in the room at 26°C, 50 ± 10% humidified, the skin temperature of hands and feet was measured by the thermography (Nihon Kohden, Tokyo, Japan). We compared the mean temperature of twelve points on the regions between the back side of interphalangeal joints and the base of thumbnails, between the back side of distal interphalangeal joints and the base of the other fingernails, between the back side of interphalangeal joints and the base of first toenails, between the back side of distal interphalangeal joints and the base of the other toenails and on the center of the back of hands and feet before and after the administration of bosentan (Fig 3a, b).

d) Statistical anlysis

Wilcoxon's signed rank test was used for comparisons between paired data. P values of less than 0.05 were considered significant. Statistical analyses were performed using IBM SPSS statistics software version 18.0 (IBM SPSS Japan, Tokyo, Japan).

III. Results

a) Patients

Three patients were recruited in 2009, five in 2010, two in 2011 and three in 2012. Enrolled in this study were 13 patients, of which half a dozen patients had suspected PAH (four; NYHA functional class II and two; class III) and eight had digital ulcers. No one underwent right heart catheterization. Ten patients were diagnosed with SSc (eight with limited cutaneous and two with diffuse cutaneous form), two with MCTD and one with SSc (diffuse cutaneous form)-PM overlap syndrome. The patient background was summarized in Table 1. Raynaud's phenomenon was present in all patients and ten patients were accompanied with nail fold bleeding. Bosentan was discontinued in one patient due to nasal bleeding at Week 6. Since liver dysfunction appeared at the dosage of 250mg bosentan in four patients, the dosage was decreased to 125mg. Of those patients, two discontinued at Week 16 and two continued for 52 weeks. Eight patients could be increased to 250mg of bosentan, of which, one patient transferred to the nearby clinic after week 16 and seven continued for 52 weeks.

b) Raynaud's phenomenon

After 16-week treatment with bosentan, the frequency and the duration of Raynaud's phenomenon were significantly decreased ($P=0.009$ and $P = 0.008$, respectively, Fig. 1a, b); both frequency and duration of Raynaud's phenomenon improved in nine patients and only the duration improved in one patient. Two patients did not experience any changes. Not the numbness, but the cold sensation with VAS was also significantly improved (Fig. 1c,d). After the treatment of 52-week administration of bosentan, the frequency and the duration of Raynaud's phenomenon were significantly decreased as well (data not shown).

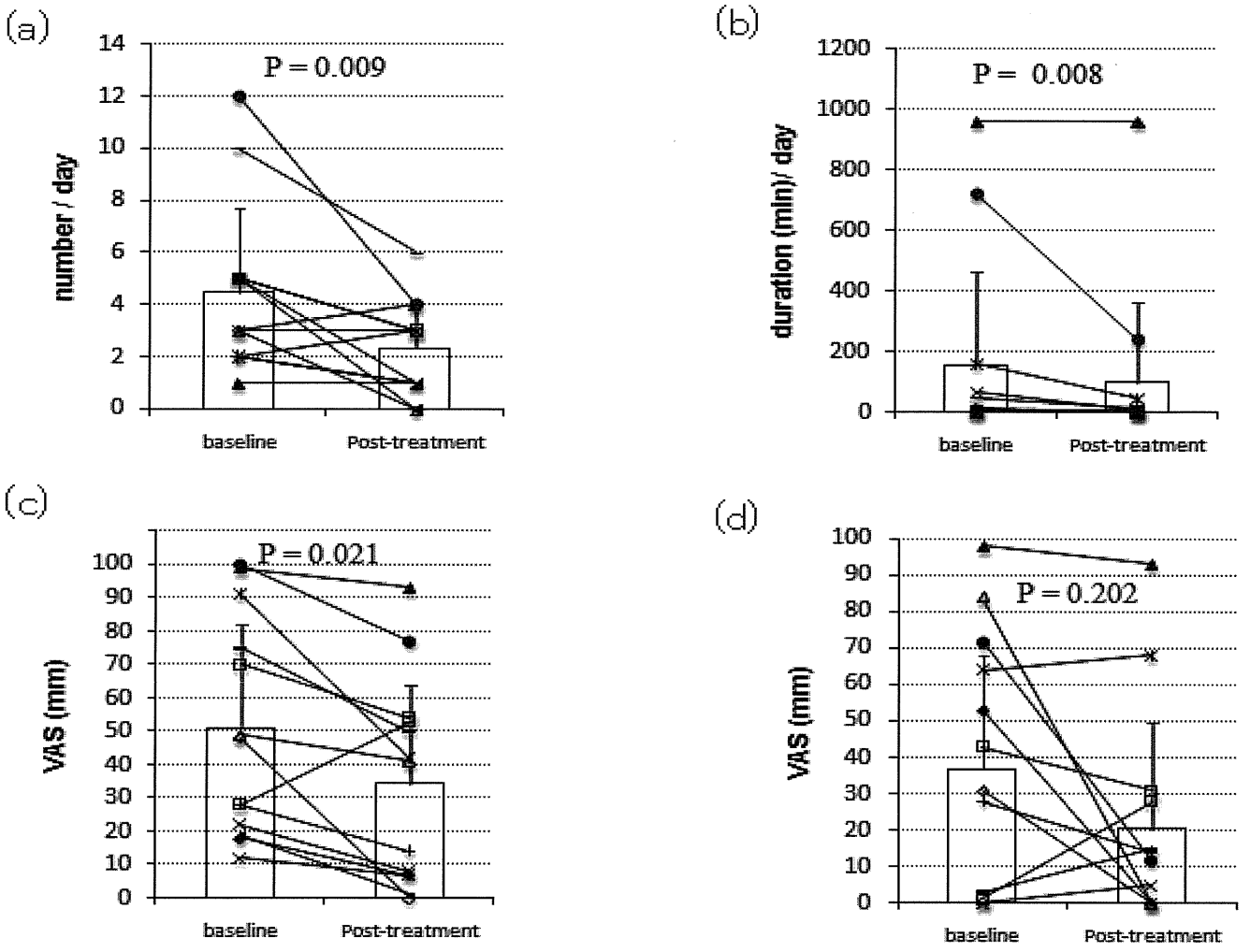

Figure1 : Effect of bosentan treatment on Raynaud' s phenomenon after 16-week treatment
One patient was dropped out at week six. Open columns and error bars mean average and standard deviation (n=12)
(a) the daily frequency of Raynaud's phenomenon, (b) the daily duration of Raynaud's phenomenon, (c) severity of the cold sensation of hands and feet by visual analogue scale (VAS), (d) severity of numbness of hands and feet by VAS.

c) Digital ulcers

Digital ulcers of all patients became scarred or disappeared after bosentan administration. Namely, nine digital ulcers improved to six scars in seven patients and ten digital ulcer scars decreased to six in five patients after the treatment for 16 weeks. New digital ulcers were not recognised throughout the treatment.

d) Thermography

The skin temperature of ten patients were monitored by thermography at Week 16 and nine patients at Week 52. No significant increase of the skin temperature was detected at Week 16, but the significant increase was seen at Week 52, respectively (Fig. 2, P = 0.038 & P = 0.025). Representative results were shown in Fig. 3c and d.

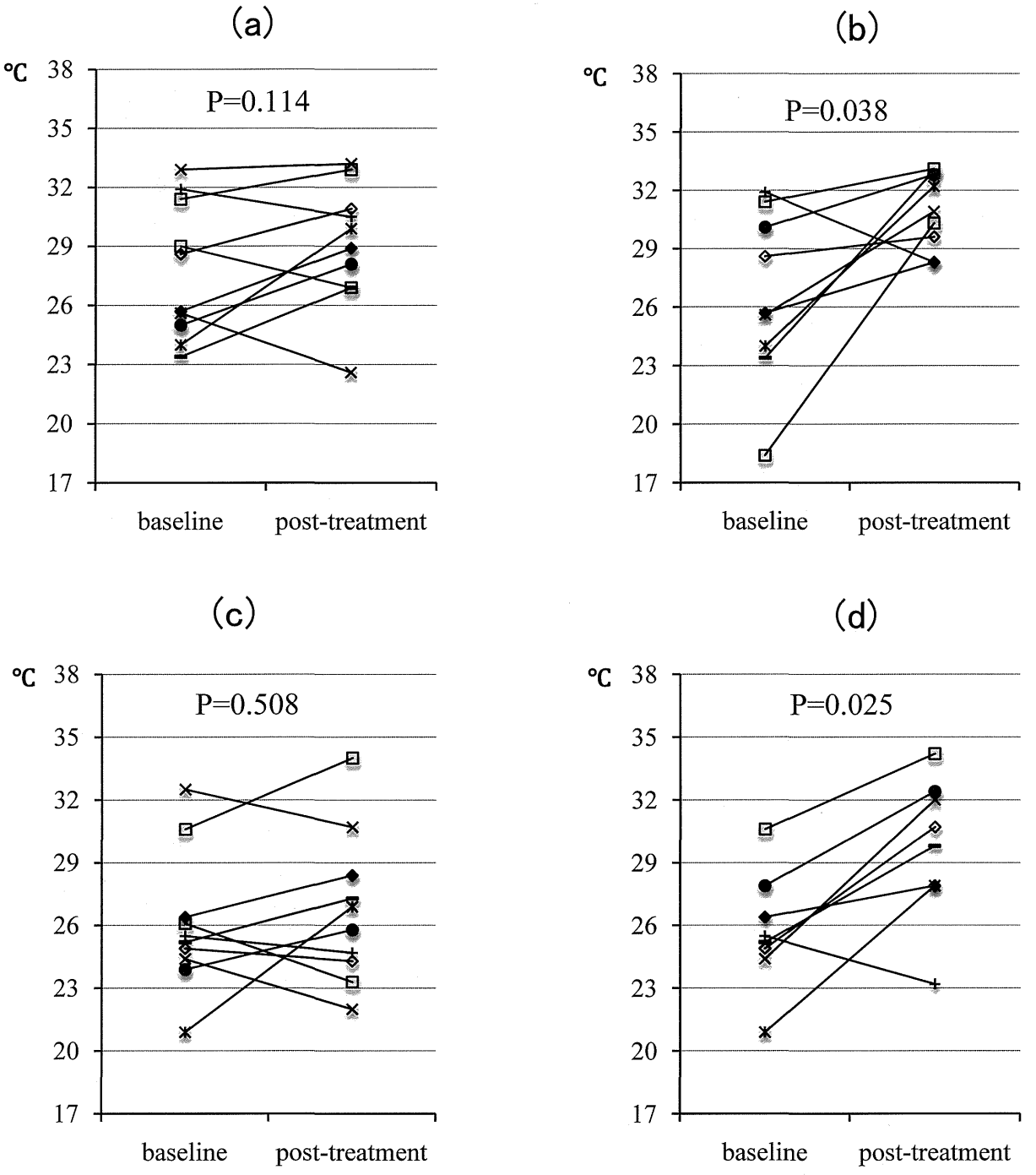

Figure 2 : The skin temperature of hands and feet measured by thermography before and after bosentan treatment. Hands (a) and feet (c) before and after the 16 week treatment (n=10), hands (b) and feet (d) before and after the 52 week treatment (n=9).

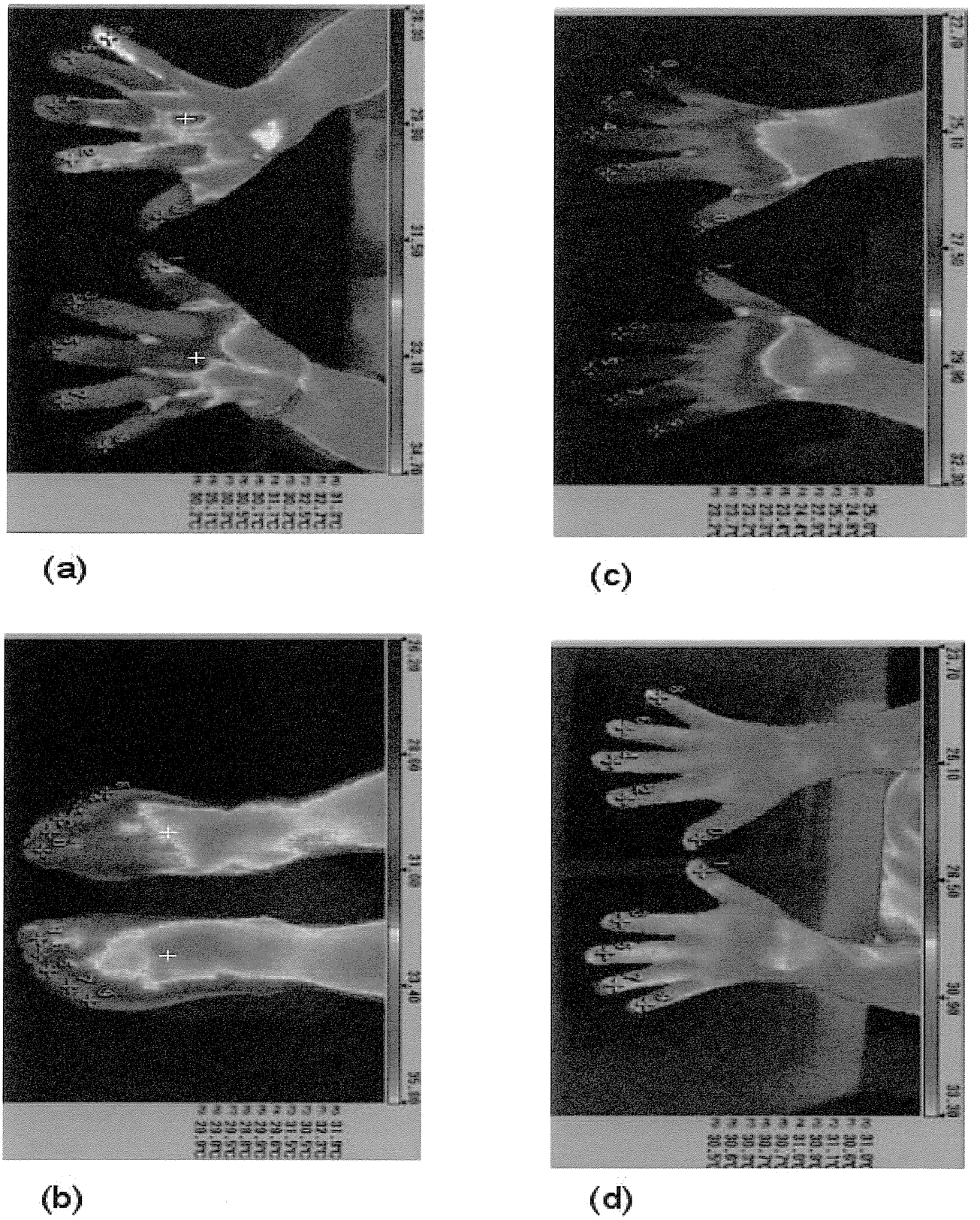

Figure 3: Thermography findings The skin temperature of twelve points in hands (a) and feet (b) was checked out by thermography. Representative thermographic pictures of the good responder (case 8) at week 0 (c) and week 52 (d).

IV. Discussion

Concerning the remedy for Raynaud's phenomenon, calcium channel blockers (CCBs) (16), oral prostacyclin analogues (4), or serotonin receptor antagonists (17, 18) have been prescribed. As CCBs lower blood pressure strongly, patients with hypotension cannot be administered them sufficiently. The effect of latter two agents is almost insufficient as well. ET participates in not only pulmonary circulation, but also peripheral circulation. Accordingly, the ET-receptor antagonist, bosentan has an anti-PAH effect, but also is expected to have an improving effect of peripheral circulatory disturbance. In fact, many researchers reported that bosentan was subjectively effective for Raynaud's phenomenon (5-7, 19, 20), oppositely, others did that the medicine was ineffetive (8-10). These reports were shown in table 2.

Table 2 : Comparison between literatures and the present study

Study	Design	Disease: patient number	Entry time	Evaluation time	Subjective effect	Objective effect	Remarks
Ramos-Casals et al.[19]	Case report	dcSSc: 1, lcSSc: 3	Unknown; 1, January; 2, December;1	Unknown: 1, May: 3	Effective	NT	None
Funauchi et al.[5]	Single center, retrospective	dcSSc: 7, lcSSc: 6, MCTD: 2	Unknown, various	Various	Effective	NT	None
Hettema et al.[6]	Single center, prospective	lcSSc: 15	Unknown	Week 8, 16	Effective	Not improved by photoelectric plethysmography	The outdoor temperature was higher at Week16 than baseline.
Giordano et al.[7]	Single center, retrospective	dcSSc: 4, lcSSc: 10	Unknown	Week 4, 12, 24, 48	Effective at Week 12, 24, 48, not at Week4	Effective by videocapillaroscopy at Week 48	None
Selenko-Gebauer et al.[20]	Single center, retrospective	lcSSc: 1, MCTD: 1, pre-SSc: 1	November	Week 16 (March)	Effective	Effective by thermography	None
The present study	Single center, prospective	dcSSc: 2, lcSSc: 8, dcSSc-PM: 1, MCTD: 2	End of November	Week 16, 52	Effective	Effective by thermography at Week52, not at Week16	None
Rosato et al.[9]	Single center, open-label, prospective	Bosentan (PAH+): dcSSc: 14, lcSSc: 16, nifedipine (PAH-): dcSSc: 15, lcSSc: 15	Winter?	Week 4, 8, 16	Ineffective	Effective by laser doppler perfusion imaging at Week 8 and 16, not at Week4, ineffective by videocapillaroscopy	None
Moore et al.[10]	Single center, prospective	dcSSc: 6, lcSSc : 12	Unknown,	Week 24	Ineffective	Ineffective by videocapillaroscopy at Week24	None
Nguyen et al.[8]	Single center, randomized, prospective, double-blind	Bos: dcSSc: 3, lcSSc: 6, placebo: dcSSc: 1, lcSSc: 7	Winter	Week 16	Ineffective	NT	DcSSc was more in the bosentan group than placebo. Effectivity of the placebo group (57%) was fairly high.

Bos: bosentan; dc SSc: diffuse cutaneous systemic sclerosis; lc SSc: limited cutaneous systemic sclerosis; MCTD: mixed connective disease; NT: not tested; PAH: pulmonary arterial hypertension; PM: polymyositis

As mentioned above, the timing of the evaluation is very important to judge restrictly the effectiveness of treatments for Raynaud's phemonenon. For example, one of patients was estimated the efficacy in May in Ramos-Casals and co-workers' report (19), and Hettema et al. (5) reported the improvement of Raynaud's phemonenon at Week 8 and Week 16, but the outdoor temperature was significantly higher at Week 16, it was thought that seasonal improvement might be appended to their final results. Funauchi et al. (6) reported that Raynaud's phenomenon improved somewhat in 13 out of 15 patients with a median of eight weeks of treatment and that Raynaud's phenomenon disappeared in eight of them after a median of 14 weeks. They did not indicate when bosentan had initiated. Giordano et al. (7) reported 14 patients decreased in daily numbers and daily duration of Raynaud's phemonenon at 12 weeks, 24 weeks and 48 weeks, but not at four weeks after the administration of bosentan. They did not indicate when bosentan had initiated either. Therefore the improvement at 24 weeks must be influenced with seasonal recovery and the result at 12 weeks was not clear either. In contrast to these, Nguyen et al. reported that bosentan did not improve the frequency, duration, pain or severity of Raynaud's phemonenon after 16-week treatment as compared with placebo (8). The trial was the only one double-blinded test of bosentan for Raynaud's phenomenon. It is superior to other reports in the point which was able to exclude the placebo-effect. But the protocol permitted participants to start from anytime in winter. Starting examination from the latter of winter, considerable participants could bring spontaneous improvement after 16 weeks. Actually, because even the placebo group showed 57% reduction of the daily frequency of Raynaud's phemonenon attacks after 16 weeks, not a few patients might be affected by not only placebo effect but also a seasonal improvement. In other double-blinded studies (16,21), the examination period was six or seven weeks and the improvement rates of placebo groups were much lower. On the other hand, Selenko-Gebauer et al. (20) were initiated bosentan in November and evaluated the outcome after 16 weeks, it seemed that the evaluation points were fairly strict. Their cases were improved, however the participants were only three. We also started bosentan from the end of November and estimated the effectiveness at the end of March, in which the temperature is same as that in November at Saitama where our hospital located, and investigated the significant improvement. Although placebo effects could not be excluded, the present study suggested that bosentan was effective to Raynaud's phenomenon.

Bosentan has been evaluated the objective effectiveness for peripheral circulation. Selenko-Gebauer et al. (20) reported that the temperature of hands by the thermography increased after 16-week treatment, but the result was only three analyses including one patient of pre-scleroderma. Rosato et al. (9) reported that bosentan improved the blood flow of fingers by a Lisca laser Doppler perfusion imager after eight- and 16-week treatment. Hettema et al. reported that the blood flow determined by photoelectric plethysmography during cooling and rewarming did not improve after 16-week treatment (5). Giordano et al. reported that visibility and sludging of nailfold by the videocapillaroscopy improved after 48-week treatment (7). Moore et al. reported that 24-week administration showed no improvement of nail fold capillary density and dimensions by the videocapillaroscopy either (10). Our data showed no significant improvement of skin temperature by the thermography after 16-week treatment, but 52-week treatment demonstrated the significant increase. Generalizing the present findings and the other reports, it was thought that bosentan needs the long-term use to improve peripheral circulatory disturbance significantly.

Although bosentan has been indicated for the prevention of new digital ulcers, a long-term use of bosentan might not be recommended for Raynaud's phenomenon alone from a viewpoint of medical economy because the prognosis of Raynaud's phenomenon is generally much better than that of digital ulcers or PAH. When we focus on the medicines except conventional drugs or bosentan, it was reported that the efficacy of phosphodiesterase-5 (PDE-5) inhibitors is equal to or more than bosentan as for the treatment of Raynaud's phenomenon (21, 22). PDE-5 inhibitors might be more practicable because they are more inexpensive than bosentan. As for ERAs except bosentan, ambricentan blocks selectively the binding of endothelin-1 to ETA which induces vasoconstriction.It was reported that ambricentan decreased the number of Raynaud's phenomenon and healed digital ulcers in patients with SSc who had failed bosentan (11). Macitentan blocks both ETA and ETB as well as bosentan. The former is a non-competitive antagonist and inhibits ETA strongly compared to ETB, while the latter is a competitive antagonist. Additionally, it was reported that macitentan suppresses the proliferation of sclerodermic fibroblasts (23). These reports indicate that it is worth evaluating the efficacy of new ERAs released after bosentan on the peripheral circulatory disturbance including Raynaud's phenomenon.

In conclusion, the present study suggested that long-term use is required to pull out the full potential of bosentan on peripheral circulatory disturbance in CTDs.

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