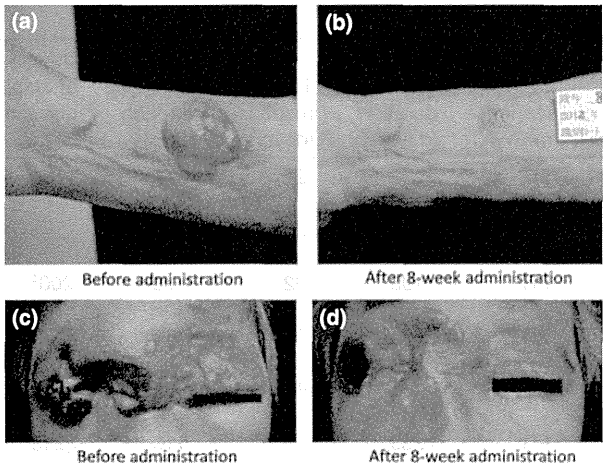


**Figure 1.** Right forearm and palpebral part of patient 11 before (a,c) and after 8-week administration of interferon (IFN)-γ (b,d). The patient was an 81-year-old woman. The patient was diagnosed with stage IIB mycosis fungoides and received concomitant 308-nm excimer light therapy. The overall skin response was stable disease. The tumors were markedly improved after 8-week administration of IFN-γ (right forearm, 34 mm × 27 mm × 10 mm before administration, 0 mm × 0 mm × 0 mm after 8-week administration; forehead and superior palpebral part, 130 mm × 78 mm × 13 mm before administration, 54 mm × 80 mm × 3 mm after 8-week administration, in 3-way measurements).

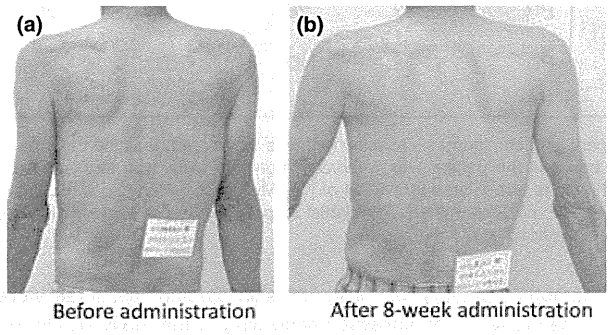


**Figure 2.** Photographs of patient 13 before (a) and 8 weeks after interferon-γ administration (b). The patient was a 62-year-old man. The patient was diagnosed with stage IIA mycosis fungoides and received concomitant topical corticosteroids. The overall skin response was partial response. Obvious improvement was observed for the patches after 8-week administration.

Drug-related AE leading to treatment interruption or dose reduction were three events of decreased white blood cell count (two with grade 2 and one with grade 3), two of decreased neutrophil count (grade 3 and grade 4), one of influenza-like illness (grade 3) and one of dyspnea (grade 3). The patient with grade 4 decreased neutrophil count (patient 13) was treated with granulocyte-colony stimulating factor, and thereafter recovered from the event. Two serious AE were

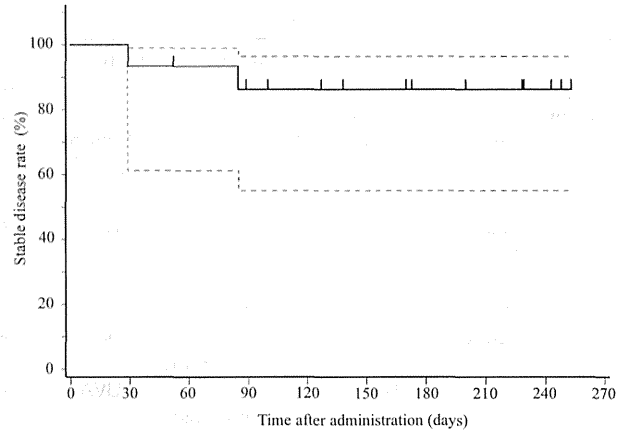


**Figure 3.** Kaplan–Meier estimate for duration of stable disease ($n$ = 15). Duration of stable disease was defined as the time between the date treatment was initiated and the date criteria were first met for progressive disease (PD). The solid line shows the ratio of patients who did not experience PD. The dotted line shows the 95% confidence interval estimated with the Greenwood formula.

**Table 3.** Drug-related adverse events occurring in two or more patients ($n$ = 15)

| Events | Overall *n* | Grade 3 or 4 *n* |
|---|---|---|
| Influenza-like illness | 15 | 2 |
| White blood cell count decreased | 3 | 1 |
| Decreased appetite | 3 | 1 |
| Neutrophil count decreased | 2 | 2 |
| Constipation | 2 | 0 |
| Hepatic function abnormal | 2 | 0 |
| Erythemas | 2 | 0 |

reported in two patients: aggravation of MF and aggravation of cataract, neither of which was considered directly related to the study drug. The patient experiencing aggravation of MF (patient 15) was withdrawn from the study after 22 days and died 50 days after the study initiation. The aggravation of MF was due to disease progression resulting from insufficient efficacy of the study drug. The patient experiencing aggravation of cataract (patient 14) had been diagnosed with cataract 2 years prior to the study. No abnormal ECG changes were found except for one patient who had an abnormal baseline.

## DISCUSSION

The study aimed to evaluate the efficacy and safety profiles of IFN-γ in Japanese patients diagnosed with stage IA–IIIA MF. IFN-γ was administrated i.v. into 15 patients at a dose of 2 million JRU once daily over 5 days a week for the first 4 weeks, followed by subsequent intermittent injections.

On the basis of the evaluation criteria for chemotherapeutics for malignant skin carcinomas, the objective response rate was 73.3% (11/15, CR rate of 0%); 90% (9/10) for stage IA–IIA, 25% (1/4) for stage IIB and 100% (1/1) for stage IIIA. Biogamma and Ogamma were previously marketed IFN-γ products for treatment of MF. The objective response rate in the clinical studies in Japanese patients with MF was 57.7% (15/26, CR rate of 7.7%) for Biogamma; 63% (12/19) for stage IA–IIA, 50% (2/4) for stage IIB and 33% (1/3) for stage IVA.[15] The objective response rate was 57.9% (22/38, CR rate of 7.9%) for Ogamma; 63% (17/27) for stage IA–IIA, 38% (3/8) for stage IIB, 100% (2/2) for stage III and 0% (0/1) for stage IVB.[16] Although there was a difference in the efficacy for each stage probably resulting from the small number of patients enrolled, the overall objective response rates of these IFN-γ products are similar to each other. IFN-α is not approved for treatment of MF/SS in Japan, although it is a widely accepted treatment for CTCL. The objective response rate of Imunomax-γ is also similar to that of IFN-α (54%, CR rate of 17%).[17,18] Considering these findings, although no patient achieved CR in this study, Imunomax-γ is an effective medication for treatment of MF.

In the treatment of MF/SS, it is important to prevent or slow disease progression. This study showed that the time to response for the 11 patients achieving the objective response was short (26–32 days). Furthermore, 13 of 15 patients did not experience PD throughout the study, and the maximum duration of SD was over 253 days. These findings demonstrate that Imunomax-γ has the ability to prevent or slow progression of MF/SS.

For patients with stage IA–IIA MF, the guidelines for the management of cutaneous lymphomas (2011) recommend topical therapies including topical corticosteroids and UV therapies as first-line therapy, and IFN-α products, IFN-γ products, and etretinate alone or in combination with topical therapies as second-line therapy.[7] For patients with stage IIB–IIIB MF, concomitant use of IFN-α products, IFN-γ products, or etretinate and topical therapies such as UV therapies or radiation therapy is recommended as a first-line treatment. Although the objective response rate for the patients with stage IIB MF was only 25% in this study, some tumors completely disappeared in response to IFN-γ (Fig. 1). Localized radiation therapy should be considered when tumorous lesions are resistant to treatment with IFN-γ in combination with UV therapies. Imunomax-γ was effective in patients with stage IA–IIIA regardless of concurrent treatment including corticosteroids and UV therapies. A good objective response, 60.0%, was also obtained as assessed according to the mSWAT. Although a small discrepancy was identified in the rating of skin response, overall evaluation results of the response to the study drug were similar between the two scoring systems. This study offers proof that IFN-γ can be used as monotherapy or combination therapy with topical treatment of patients with early to late stage MF.

The most common drug-related AE was influenza-like illness occurring in all patients enrolled. One event of influenza-like illness required dose reduction but did not lead to discontinuation of the study. The other events of influenza-like illness did not require dose reduction or treatment interruption with prophylactic use of antipyretic analgesics. None of the hematological events occurring during the study led to discontinuation of the study. Therefore, influenza-like illness and hematological events are considered to be manageable. One patient was withdrawn from the study due to drug-related grade 1 cough, which was resolved without any treatment after discontinuation and considered not due to interstitial pneumonia. Taken together, IFN-γ is safe and well-tolerated, providing a promising therapeutic option for MF.

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Case report

# Dots/globules on dermoscopy in nail-apparatus melanoma

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## Introduction

Nail-apparatus melanoma is a rare malignant tumor. Approximately 1–3% of melanomas in Caucasians,[1–3] 15–20% in African-Americans,[4] 10–30% in Japanese,[5,6] 17% in Chinese,[7] and 33% in American Indians[8] are nail-apparatus melanomas. Dermoscopy is extremely useful in the diagnosis of malignant melanoma. However, dermoscopic features on nails are different from those on other sites. In addition, toes and fingers are common sites of amelanotic melanoma,[9] whose dermoscopic features are not typical for melanomas. For these reasons, the diagnosis of nail-apparatus melanoma may be more difficult than other types of melanoma.

In 2002, Ronger *et al.*[10] prescribed seven dermoscopic schemas of melanonychia. These schemas include blood spots, brownish background discoloration, regular and irregular lines, grayish background and thin gray lines, (micro)-Huchinson sign, and microscopic grooves. The presence of some of these schemas leads to a high suspicion of melanoma.

## Case report

A 35-year-old man presented with left fourth digital longitudinal melanonychia, which had developed 5 years before. At the first admission, we recognized 9 mm-wide black lines. On dermoscopy, we could see a brown background discoloration, irregular lines, and dots/globules. However, we could not see the Huchinson's sign. Furthermore, there was no deformity of the nail plate. The lesion enlarged from 9 to 12 mm in two months (Fig. 1), therefore total excision of the nail was performed. Histopathologically, the epithelium of the nail matrix showed marked acanthosis and elongation of rete ridges. Melanoma cells with atypical nuclei were scattered in the epidermis and dermis with little inflammatory cell infiltration. Some atypical melanocytes rich in melanin were found clumped



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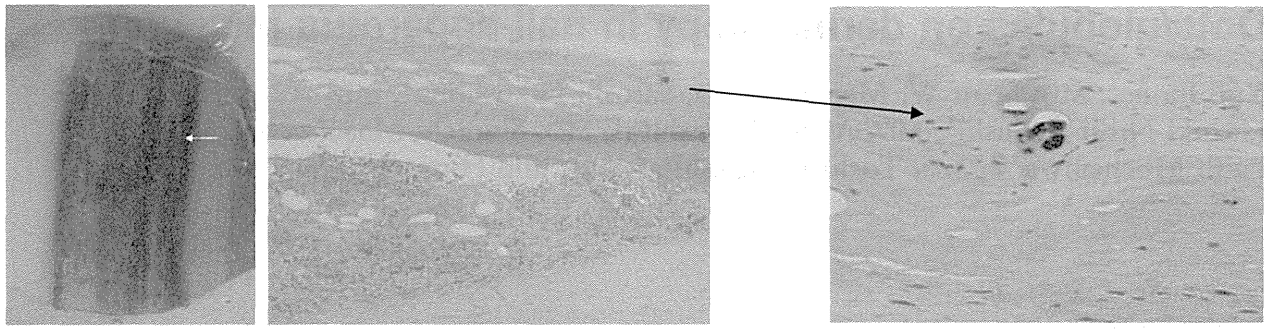

**Figure 1** Dermoscopic examination of left fourth fingernail. There was brown coloration of the background, irregular lines and dots/globules; however, Huchinson's sign or nail deformity were not seen (Heine Dermaphoto Aufsatz® ×10), Histopathological examination of dots/globules with a dermoscopic view. Melanoma cells with atypical nuclei were scattered in the epidermis and dermis with inflammatory cell infiltration (H&E, ×20). Atypical melanocytes rich in melanin were found clumped within the nail plate (H&E, ×100)

within the nail plate (Figs. 2 and 3). This corresponds to the dot/globules seen on dermoscopy. There was no destruction of the nail; however, atypical melanoma cells glowed within the subungual space with many melanin granules on the basal cell layer. This histopathology showed invasive malignant melanoma whose tumor thickness was 1.1 mm. We finally made a diagnosis of malignant melanoma: pT2aNoMo, Stage IB.

## Materials and methods

All recorded clinical and dermoscopic photographs were collected retrospectively from Kumamoto University Hospital between January 2003 and December 2009. All photographs were taken by Heine Delta 20® (Heine, Herrsching, Germany) with Nikon Coolpix® 4500 (Nikon, Tokyo, Japan) or Heine Dermaphot Aufsatz® with Canon D-50® (Canon, Tokyo, Japan). In all cases, the diagnosis of melanoma was proven by histopathological examination including immunohistochemical studies. The analysis of dermoscopic features was done by at least three dermatological specialists. Seven dermoscopic features of melanonychia, brownish background discoloration, regular and irregular lines, grayish background and thin gray lines, and micro-Huchinson's sign were found. In addition to these features, there were dots/globules on the nail.

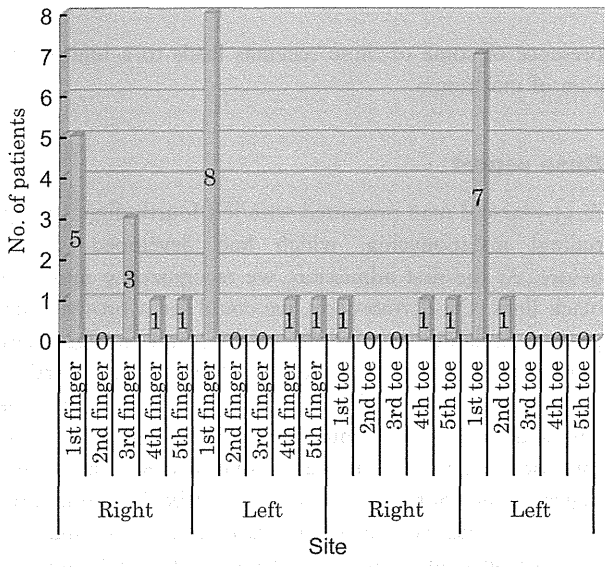


**Figure 2** Primary sites of 31 nail-apparatus melanomas and melanoma *in situ*

## Results

We had 242 patients with melanoma in seven years; 50 cases (20.7%) occurred in toe- or fingernails. Of these 50 cases, we reviewed dermoscopic features of about 31 cases without nail destruction. All 31 patients were diagnosed histopathologically. Twelve women and eight men had lesions on fingernails, while five women and six men had lesions on toenails. The most common digit involved was the first digit; 20 of 31 cases (67.6%) occurred on the first finger- or toenail (Fig. 4). The age at diagnosis ranged from 36 to 83 years, average 65.3 ± 13.8 years. The pattern of involvement was similar in both sexes ($P > 0.05$) and in both hands and feet ($P > 0.05$). Stage classification of the 31 cases is as follows (Table 1); *in situ*, 16 cases; stage IA, five cases; stage IB, three cases; stage IIA, two cases; stage IIB, two cases; stage IIC, one case; stage IIIB, two cases. The positive rates of the eight dermoscopic features are outlined in Table 2. Brown background discoloration, regular lines, irregular lines, grayish background and thin gray lines, micro-Huchinson's sign, and microscopic grooves were 45.2, 90.3, 3.2,

**Table 1** Clinical and dermoscopic features of 31 cases of melanoma *in situ* and melanoma

| No | year | M/F | site | tumor thickness | Stage | blood spots | bronw color | regular lines | irregular lines | grayish background | Hutchinson sign | grooves | dots / globules | nail deformity | Others |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 36 | M | left 4th finger | 1.1 | pT1bN0M0 | − | + | − | + | − | − | + | + | − | |
| 2 | 71 | F | left 1st toe | 0.9 | pT1aN0M0 | − | + | − | + | − | + | + | + | − | |
| 3 | 75 | M | left 1st toe | 2.3 | pT3bN2bM0 | + | + | − | + | − | + | + | − | + | |
| 4 | 55 | M | right 1st toe | 1.6 | pT2bN0M0 | + | + | − | + | − | + | + | + | + | |
| 5 | 75 | M | left 1st toe | 2.2 | pT3bN0M0 | + | + | − | + | − | + | + | + | + | |
| 6 | 54 | M | right 1st finger | is | is | − | + | − | + | − | + | + | − | − | |
| 7 | 83 | F | right 3d finger | 10.5 | pT4bN0M0 | − | − | − | − | − | + | + | − | + | |
| 8 | 82 | F | left 1st toe | is | is | + | + | − | + | + | + | + | − | + | tinea unguium |
| 9 | 81 | F | left 1st finger | 0.8 | pT1aN0M0 | − | + | − | + | − | + | + | − | + | |
| 10 | 68 | F | left 1st finger | 2.4 | pT3bN0M0 | + | − | − | − | − | + | + | − | + | |
| 11 | 55 | M | right 1st finger | is | is | − | + | + | − | − | + | + | − | − | |
| 12 | 79 | M | left 1st finger | 0.8 | pT1bN0M0 | − | + | − | + | − | + | + | + | + | |
| 13 | 81 | F | left 1st finger | 0.8 | pT1aN0M0 | − | + | − | + | − | + | + | − | + | |
| 14 | 55 | M | right 1st finger | 4.5 | pT4bN1M0 | + | + | − | + | − | + | − | − | − | |
| 15 | 80 | M | left 1st finger | 0.4 | pT1b.0.0 | + | + | − | + | − | − | + | − | + | |
| 16 | 40 | F | right 3rd finger | is | is | − | + | − | + | − | + | + | − | − | |
| 17 | 56 | F | right 5th finger | is | is | + | + | − | + | − | + | + | + | + | |
| 18 | 65 | M | left 1st finger | is | is | − | + | − | + | − | + | − | − | − | |
| 19 | 65 | F | left 2nd toe | is | is | − | + | − | + | − | + | + | − | − | |
| 20 | 73 | F | right 3rd finger | is | is | − | + | − | + | − | + | + | − | − | |
| 21 | 36 | F | left 1st finger | is | is | − | + | − | + | − | − | − | + | − | |
| 22 | 52 | F | right 4th toe | is | is | + | + | − | + | − | + | + | + | − | |
| 23 | 81 | M | left 1st finger | 0.8 | pT1bN0M0 | + | + | − | + | − | + | + | − | + | |
| 24 | 62 | F | right 1st finger | 2 | pT2bN0M0 | + | − | − | + | + | + | + | − | − | |
| 25 | 62 | M | left 1st toe | 0.7 | pT1bN0M0 | + | + | − | + | − | + | + | − | − | |
| 26 | 69 | M | left 1st toe | is | is | − | + | − | + | − | − | − | − | − | |
| 27 | 55 | F | right 4th finger | is | is | − | + | − | + | − | + | − | − | − | |
| 28 | 63 | M | left 1st toe | is | is | − | + | − | + | − | + | + | − | − | |
| 29 | 57 | F | left 5th finger | is | is | − | + | − | + | − | − | + | − | − | |
| 30 | 74 | F | right 1st finger | is | is | + | + | − | + | − | + | + | − | + | tinea unguium |
| 31 | 83 | F | right 5th toe | is | is | + | + | − | + | − | + | + | − | + | |
| | 65.3±13.8 | | | | | 14 (45.2%) | 28 (90.3%) | 1 (3.2%) | 28 (90.3%) | 2 (6.4%) | 26 (83.9%) | 26 (83.9%) | 8 (25.8%) | 14 (45.2%) | |

is, *in situ* melanoma.

90.3, 6.4, 83.9, 83.9%, respectively. Brown discoloration of the background and irregular lines were the most common features, followed by the (micro-) Huchinson's sign. Both cases without irregular brown discoloration of the background had the Huchinson's sign. The diagnosis rate of malignant melanoma was 100% when we confirmed either brown discoloration of the background or (micro)-Huchinson's sign. We confirmed dots/globules in eight cases (25.8%; Table 2).

## Discussion

There were many early-stage melanomas in this study because we reviewed nail-apparatus melanoma without nail destruction. If we added 19 melanomas with nail destruction, this would come to 20.6% of all melanomas presenting to our hospital over seven years. This rate of nail-apparatus melanoma is similar to other Japanese reports.[5,6]

Twenty cases (64.5%) occurred on fingernails, and 11 (35.5%) occurred on toenails. Thirteen (41.9%) cases occurred on the first finger, and eight (25.8%) cases occurred on the first toe. In total, 67.7% of all cases occurred on first finger- or toenails. Leung *et al.*[11] reported that first digits had the greatest number of melanonychia in the Chinese, and he mentioned that the reason may be that the first finger- and toenails are larger and more active than others so they may be easily injured.[12]

The brown background discoloration and irregular lines were the most frequent of Ronger's seven features (90.3%), followed by (micro-) Huchinson's sign (83.9%). These rates were relatively higher than other reports.[9,10] Both cases without discoloration of the background had Huchinson's signs. These three signs were very useful dermoscopic features of nail-apparatus melanoma. Blood spots were seen in 5 of 16 cases (31.3%) with *in situ* melanomas and nine of 15 cases (60.0%) with invasive melanomas. There was no significant difference between *in situ* and invasive melanoma.

In eight of 31 cases (25.8%), we confirmed dots/granules besides Ronger's seven features; five cases were invasive melanoma and three were *in situ*. This is the phenomenon where blackish brown pigment dots/globules lined up over the nail plate lengthwise (Fig. 1), and clumping was found in tissue with melanin granules in the nail plate (Figs. 2 and 3). We suggest that this finding is similar to the Pagetoid phenomenon and casting off the melanin in melanoma histopathology. Ohara[13] reported that dots/globules are often seen in melanonychia of a child; however, in adult melanonychia, it is rare to see it. We think that this is one of the important dermoscopic features in nail-apparatus melanoma, especially invasive melanoma without nail deformity. Amin *et al.*[14] reported that the rate of Pagetoid spread was certainly low in subungual lentigo compared with melanoma *in situ* or invasive melanoma. They also reported that the rate of focal/florid Pagetoid phenomena is high in *in situ* melanoma and low in invasive melanoma. In this study, there was no significant difference between *in situ* melanoma and invasive melanoma in dots/globules ($P = 0.35466$); there were significant differences only in the nail deformity ($P = 0.01967$), using the chi-square test (Table 2). We think that the Pagetoid spread on histopathology was reflected as dots/globules in dermoscopy, and this sign may be one of the important features of subungual melanoma.

In a diagnosis of melanonychia and nail-apparatus melanoma, dermoscopy was very useful. We should have a high clinical suspicion for melanoma when there is irregular brown discoloration of the background and (micro-) Huchinson's sign. In addition, invasive melanoma is likely when there are blood spots and some nail deformity. Dermoscopic findings of dots/globules, however, should increase our clinical suspicion of melanoma.

**Table 2** Clinical characteristics and dermoscopy features of subjects

| | **Invasive melanoma ($n = 15$)** | ***In situ* melanoma ($n = 16$)** | ***P*-value** |
|---|---|---|---|
| Types of events | | | |
| Sex (female/male) | 6/9 | 11/5 | |
| Age (years) | 69.6 ± 13.4 | 61.2 ± 13.3 | |
| Stages (UICC) (no. of patients) | | | |
| *In situ* | | 16 | |
| IA | 3 | | |
| IB | 5 | | |
| IIA | 2 | | |
| IIB | 2 | | |
| IIC | 1 | | |
| IIIA | 0 | | |
| IIIB | 2 | | |
| IIIC | 0 | | |
| IV | 0 | | |
| Features of dermoscopy | | | |
| Blood spots | 9 (60.0%) | 5 (31.2%) | NS |
| Brown coloration | 12 (80.0%) | 16 (100.0%) | NS |
| Regular lines | 0 (0%) | 1 (6.2%) | NS |
| Irregular lines | 13 (86.7%) | 15 (93.7%) | NS |
| Grayish background | 1 (6.7%) | 1 (6.2%) | NS |
| (micro)-Huchinson's sign | 13 (86.7%) | 13 (81.2%) | NS |
| Grooves | 14 (93.3%) | 12 (75.0%) | NS |
| Dots/globules | 5 (33.3%) | 3 (18.7%) | NS |
| Nail deformity | 10 (66.7%) | 4 (25.0%) | 0.01967 |

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# Serum Adhesion Molecule Levels as Prognostic Markers in Patients with Early Systemic Sclerosis: A Multicentre, Prospective, Observational Study

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## Abstract

***Objective:*** To assess the utility of circulating adhesion molecule levels as a prognostic indicator of disease progression in systemic sclerosis (SSc) patients with early onset disease.

***Methods:*** Ninety-two Japanese patients with early onset SSc presenting with diffuse skin sclerosis and/or interstitial lung disease were registered in a multicentre, observational study. Concentrations of intercellular adhesion molecule (ICAM) −1, E-selectin, L-selectin, and P-selectin in serum samples from all patients were measured by enzyme-linked immunosorbent asssay (ELISA). In 39 patients, adhesion molecule levels were measured each year for four years. The ability of baseline adhesion molecule levels to predict subsequent progression and severity in clinical and laboratory features were evaluated statistically.

***Results:*** At their first visit, serum levels of ICAM-1, E-selection, P-selectin were significantly elevated and serum L-selectin levels were significantly reduced in patients with SSc compared with healthy controls. Overall, serum ICAM-1 levels at each time point were significantly inversely associated with the %vital capacity (VC) of the same time and subsequent years by univariate analysis. The initial serum ICAM-1 levels were significantly inversely associated with the %VC at the fourth year by multiple regression analysis. The initial serum P-selectin levels were significantly associated with the health assessment questionnaire disability index (HAQ-DI) at the fourth year by multiple regression analysis. Initial adhesion molecule levels were not significantly associated with other clinical features including skin thickness score. Baseline adhesion molecule levels were not significantly associated with subsequent rate of change of clinical parameters.

***Conclusion:*** In patients with SSc, serum levels of ICAM-1 and P-selectin may serve as prognostic indicators of respiratory dysfunction and physical disability, respectively. Further longitudinal studies of larger populations are needed to confirm these findings.

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## Introduction

Systemic sclerosis (SSc) is a connective tissue disease characterized by tissue fibrosis in the skin and internal organs, and vascular involvement [1,2]. Interstitial lung disease (ILD) develops in more than half of SSc patients and is one of the major SSc-related causes of death. Joint contracture due to extensive skin sclerosis and/or severe internal organ involvement results in impaired physical function.

SSc patients exhibit increased numbers and activation of monocytes/macrophages and T cells in the circulation and tissues

[3,4]. Infiltration of these cells into the skin or internal organs may promote endothelial damage and fibrosis, most likely through the production of soluble mediators including cytokines and chemokines. Leukocyte recruitment into inflammatory sites is generally achieved using multiple cell adhesion molecules [5].

E-selectin, (CD62E), L-selectin (CD62L), and P-selectin (CD62P) primarily mediate leukocyte capture and rolling on the endothelium [6]. L-selectin is constitutively expressed on most leukocytes [6]. Whereas P-selectin is rapidly mobilized to the surface of activated endothelium or platelets, E-selectin expression is induced within several hours after activation with inflammatory cytokines [6]. These selectins share a highly conserved N-terminal lectin domain that can interact with sialylated and fucosylated oligosaccharides such as sialyl Lewis X [7]. Although various candidates have been identified as potential ligands for selectins, P-selectin glycoprotein ligand 1 (PSGL-1) is the best characterized ligand, which is recognized by all three selectins [8]. PSGL-1 is a mucin-like, disulfide-linked homodimer expressed by all subsets of leukocytes and is a high-affinity ligand for E- and P-selectins [9]. PSGL-1 has also been shown to bind to L-selectin, but its affinity is lower than E- and P-selectins [10].

Intercellular adhesion molecule (ICAM)-1 (CD54) is a member of the Ig superfamily that is constitutively expressed not only on endothelial cells, but also on fibroblasts and epithelial cells [11]. It can be upregulated transcriptionally by several proinflammatory cytokines, such as interleukin (IL) -1, interferon (IFN) -γ, and tumor necrosis factor (TNF) -α [11]. ICAM-1 binds to leukocyte function associated antigen-1 (LFA-1) and macrophage adhesion ligand-1 (Mac-1). LFA-1 and Mac-1 expressed on leukocytes bind to ICAM-1 to mediate firm adhesion and transmigration of leukocytes across vascular endothelia in processes such as extravasation and the inflammatory response [5].

In most patients, severe organ involvement occurs within the first three years of disease and skin sclerosis seldom progresses after five or six years [12,13]. Therefore, predicting disease progression is particularly important for SSc patients at their first visit. However, except for SSc-related autoantibodies [14] there are no definitive serum biomarkers available to estimate disease progression. We hypothesized that some adhesion molecules may be related to underlying biologic process which is ongoing and which will change clinical features over time.

In the present study, we focused on major 4 adhesion molecules (ICAM-1, E-selectin, L-selectin, and P-selectin). We sought to determine if baseline serum adhesion molecule levels could predict the progress of symptoms in early SSc patients.

## Methods

### Patients

Patients were grouped according to the degree of skin involvement based upon the classification system proposed by LeRoy *et al.* [diffuse cutaneous SSc (dcSSc) versus limited cutaneous SSc (lcSSc)] [15]. In this study, 92 Japanese patients with early SSc (disease duration defined by the period from the first symptom including Raynaud's phenomenon attributable to SSc to our first assessment ≤ three years) who had dcSSc and/or ILD were registered at nine major scleroderma centers in Japan (Gunma University Hospital, Kanazawa University Hospital, Keio University Hospital, Kumamoto University Hospital, Nagasaki University Hospital, Tokyo University Hospital, Tokyo Women's Medical University Hospital, Toho University Omori Medical Center, Tsukuba University Hospital). Patients with other inflammatory, infectious, or malignant diseases were not included in this study.

Among the patients, 49 patients had dcSSc with ILD, 30 patients had dcSSc without ILD, and 13 patients had lcSSc with ILD. Sixty-four patients were female and twenty-eight patients were male. The median age was 53 (range, 14–76). The median disease duration was 19 months (range, 1–60 months). All patients fulfilled the criteria for SSc proposed by the American College of Rheumatology [16]. With respect to the specificity of anti-nuclear antibodies (Abs) in the serum, 56 patients were positive for anti-topoisomerase I Ab and 11 patients were positive for anti-centromere Ab. Age and gender-matched 24 healthy persons (17 females and 7 males, median age 49 (range, 20–65) ) were also included as normal controls in this study.

Among 92 patients, 39 patients could be followed every year for four years. Twenty-three patients had dcSSc with ILD, seven patients had dcSSc without ILD, and nine patients had lcSSc with ILD. Twenty-seven patients were female and twelve patients were male. The median age was 54 (range, 14–75). The median disease duration was 20 months (range, 1–60). With respect to the specificity of anti-nuclear Abs, 25 patients were positive for anti-topoisomerase I Ab and three patients was positive for anti-centromere Ab. The ethical committee at each centre (Institutional Review Board, Gunma University Hospital; Kanazawa University Ethical Committee; Keio University Ethical Committee; Ethics Committee for Clinical Research and Advanced Medical Technology at the Faculty of Life Sciences, Kumamoto University; Ethics Committee of Nagasaki University Hospital; the Ethical Committee of the Faculty of Medicine, University of Tokyo; the Ethics Committee of Tokyo Women's Medical University, the Ethics Committee of Toho University Omori Medical Center; Ethics Committee University of Tsukuba Hospital) approved all protocols and informed written consent was obtained from all patients.

### Clinical Assessments

Patients had a physical examination and laboratory tests were performed at their first visit and at each subsequent year for four years. The degree of skin involvement was determined according to the modified Rodnan total skin thickness score (MRSS), as described elsewhere [17]. Organ system involvement was defined as described previously [18] with some modifications: ILD = bibasilar interstitial fibrosis or ground-glass shadow on computed tomogram (CT); pulmonary arterial hypertension (PAH) = clinical evidence of pulmonary hypertension and elevated right ventricular systolic pressure (>45 mmHg) documented by echocardiography in the absence of severe pulmonary interstitial fibrosis; esophagus = apparent dysphagia, reflux symptoms, or hypomotility shown by barium radiography; heart = pericarditis, congestive heart failure, or arrhythmias requiring treatment; kidney = malignant hypertension and rapidly progressive renal failure unexplained by certain diseases other than SSc; joint = inflammatory polyarthralgias or arthritis; and muscle = proximal muscle weakness and elevated serum creatine kinase. A health assessment questionnaire-disability index (HAQ-DI) modified for Japanese patients [19] including digital ulcer, pitting scar, maximal oral aperture (the maximum vertical length of opened mouth), and skin pigmentation/depigmentation was also evaluated. Erythrocyte sedimentation rate (ESR) and pulmonary function including vital capacity (VC) were also tested.

### ELISA

Fresh venous blood samples were taken from 92 patients and 24 healthy controls at their first visit (baseline). In 39 patients, blood samples were also taken at each subsequent year for four years. Samples were centrifuged shortly after clot formation. All serum

samples were stored at −70°C prior to use in assays. Serum levels of ICAM-1, E-selectin, L-selectin, and P-selectin were measured by ELISA (R&D systems, Inc. Minneapolis, MN). Limit of detection was as follows; ICAM-1 31.2 pg/ml, E-selectin 93.8 pg/ml, L-selectin 78.1 pg/ml, and P-selectin 125 pg/ml.

### Statistical Analysis

JMP® Statistical Discovery Software (SAS Institute, Cary, NC) was used for analysis. Since Shapiro-Wilk test did not indicate that serum adhesion molecule concentration showed normal distribution, the data were converted to logarithm so that the data exhibited normal distribution. Then, statistical analyses were performed using the t-test for the comparison of sample levels between two groups. The Pearson product-moment correlation coefficient was used to examine the relationship between two continuous variables. Potential prognostic factors for estimating subsequent MRSS, %VC, and HAQ-DI were statistically examined by multiple regression analysis. A p-value $<0.05$ was considered statistically significant. All values are expressed as the median (range) otherwise indicated.

## Results

### Serum Levels of Adhesion Molecules were Elevated in SSc Patients

Serum samples were taken from normal controls (n = 24) and all patients (n = 92) at their first visit. Serum levels of ICAM-1 were significantly increased in SSc patients compared with healthy controls ($p<0.0001$, Figure 1). Serum levels of E-selectin and P-selectin were also significantly elevated in the SSc patients ($p<0.01$ vs. $p<0.0001$, respectively, Figure 1). By contrast, serum L-selectin levels were significantly reduced in patients with SSc ($p<0.01$, Figure 1). Serum levels of ICAM-1 were significantly associated with levels of E-selectin in patients ($r = 0.51$, $p<0.0001$). However, other combinations of adhesion molecules were not significantly associated with each other.

At the initial visit, serum ICAM-1 levels were significantly elevated in patients with ILD compared with patients without it (median (range) ng/ml; 179.3 (91.6–556.7) vs. 165.9 (89.7–263.5), $p<0.05$). There were significant inverse associations between serum ICAM-1 levels and %VC in patients with SSc ($r = -0.41$ $p<0.001$). In addition, serum ICAM-1 levels were significantly elevated in patients with anti-topoisomerase I Ab than in patients without it (median (range) ng/ml; 183.5 (104.9–556.7) vs. 156.5 (89.7–331.1), $p<0.01$). There was also a significant association between serum P-selectin levels and HAQ-DI ($r = 0.30$, $p<0.01$). However, no significant correlations were found between the levels of any of the adhesion molecules measured and any other clinical or laboratory findings. Steroid treatment did not significantly affect the levels of these adhesion molecules (steroid (+) vs. steroid (−) (median (range) ng/ml); ICAM-1 160.8 (91.1–556.7) vs. 170.0 (89.7–474.8), $p = 0.14$; E-selectin 41.2 (19.3–161.0) vs. 36.1 (16.4–117), $p = 0.22$; L-selectin 800.5 (213.2–3989.7) vs. 745.7 (343.2–1423.9), $p = 0.62$; P-selectin 132.8 (36.2–699.5) vs. 122.6 (56.2-492.8), $p = 0.53$.

### Longitudinal change of Clinical Features

To assess progression of SSc over time, clinical features of thirty-nine patients who were able to be followed-up every year for four years were analyzed (Table 1). To assess the degree of skin involvement in patients, MRSS values were calculated, and %VC was used to assess lung involvement. HAQ-DI was also obtained in order to evaluate the functional abilities of the patients. For the patient population as a whole, the median MRSS value decreased from 16 to 10 during the first year. The median MRSS was 12 at the end of year two, 9 at the end year three, and 8 at the end year four. Median values for %VC did not significantly change during the four-year evaluation period. In this regard, the %VC was 96 at first visit, 91 at the end of the first year, 95 at the end of the second year, 91 at the end of the third year, and 90 at the end of the fourth year. The median HAQ-DI was 0.125 at the first visit and at the end of year one and three, whereas it was 0.25 at the end of year two and four. ILD and renal crisis were newly detected during the evaluation period in 2 and 4 patients, respectively. No patients had PAH during the period. Most patients were treated with oral prednisolone during the follow-up period. Additionally, a part of patients were treated with immunosuppresive agents including cyclophosphamide, cyclosporin A, azathioprine, and methotrexate.

### Longitudinal Change of Adhesion Molecule Levels

The yearly changes in serum adhesion molecule levels for each case are shown in Figure 2. The dotted horizontal lines indicate median values of healthy controls. Overall, the levels of each adhesion molecule in the serum showed considerable variations in each patient. However, the median values of ICAM-1, E-selectin, L-selectin, and P-selectin measured did not change significantly over time (Figure 2). Nonetheless, the median levels of ICAM-1, E-selectin, and L-selectin tended to slightly increase during the course. The variation in adhesion molecule levels over time was not significantly associated with the variation of the dose of steroid, MRSS, %VC, and HAQ-DI during the four years' course of the study (data not shown).

### Association between Each Adhesion Molecule Level and Subsequent Severity of Clinical Features

We evaluated if baseline serum adhesion molecule levels are associated with baseline and subsequent clinical features of SSc by univariate analysis. Baseline serum ICAM-1 levels were significantly inversely associated with %VC values at baseline ($r = -041$, $p<0.05$) and subsequent every year until 4 years (Table 2). Additionally, serum ICAM-1 levels at the first year were significantly negatively associated with %VC values at the third and fourth year (Table 3). Similarly, ICAM-1 levels at the second year were significantly inversely associated with %VC at the second, third, and fourth year. ICAM-1 levels of the third year were significantly negatively correlated with %VC of the third and fourth year. Baseline serum P-selectin levels were significantly associated with HAQ-DI values at baseline ($r = 0.51$, $p = 0.001$) and the first, second, and fourth year (Table 2). However, serum P-selectin levels at the subsequent years did not significantly associate with HAQ-DI at the same time or subsequent years (Table 4). Otherwise, no significant associations between serum levels of adhesion molecules and subsequent clinical features were found. These data indicate that serum level of ICAM-1 is a useful biomarker for estimating the current and subsequent respiratory dysfunction. Additionally, baseline serum P-selectin level may reflect the current and subsequent physical disability.

### Association between the Level of Each Adhesion Molecule and the Severity of Clinical Features Analyzed by Multiple Regression Analysis

Next, we utilized multiple regression analysis to evaluate the ability of serum adhesion molecule levels to predict clinical or laboratory factors such as MRSS, %VC, and HAQ-DI of patients four years after the first visit. Selected variables were as follows: each adhesion molecule level, anti-topoisomerase I Ab,

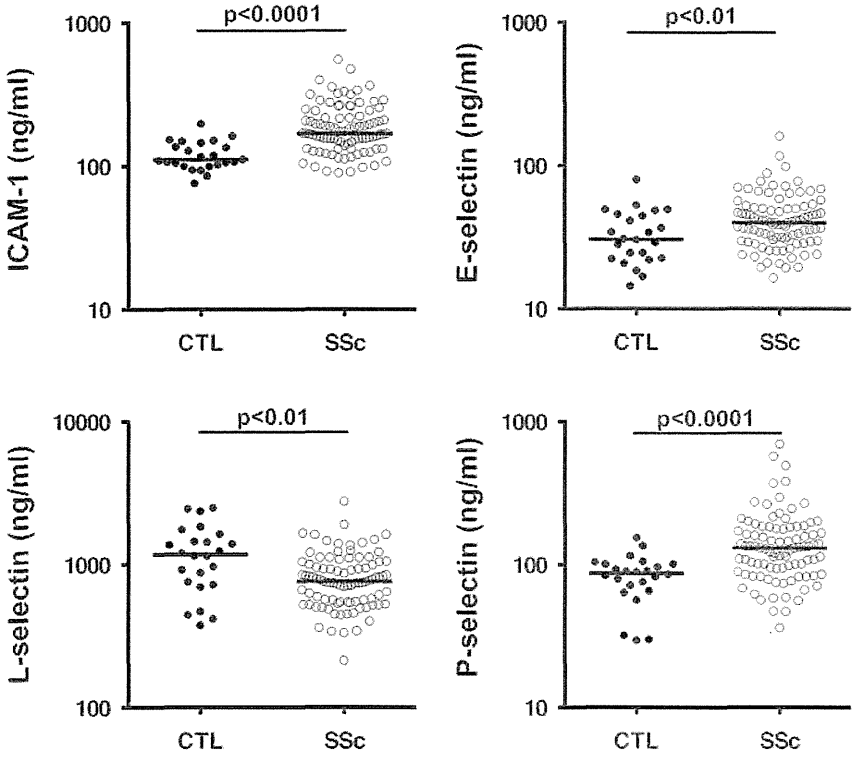


**Figure 1. Serum adhesion molecule levels in healthy controls (CTL) and early systemic sclerosis (SSc) patients with diffuse skin sclerosis and/or interstitial lung diseases.** The horizontal bar in each group indicates the median value.
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anticentromere Ab, MRSS, %VC, presence of ILD, HAQ-DI, ESR, corticosteroid treatment, and cyclophosphamide treatment at the first visit. We performed stepwise regression analysis that specified the $\alpha$ level for either adding or removing a regression as 0.15. As a result, the multiple regression equation predicting the %VC of 4 year follow-up = 230.2+0.62×%VC of baseline+−60.1×$\log_{10}$ (serum ICAM−1 levels (ng/ml)) of baseline ($R^2 = 0.73$, root mean square error (RMSE) = 12.1, $p<0.0001$, Table 5). Using our equation, we found that the %VC value at the fourth year was significantly associated with the %VC of baseline ($p = 0.0001$) and was significantly inversely associated with the initial ICAM levels ($p = 0.015$). Multi-colineality was not detected between independent factors (variance inflation factor (VIF) = 1.20). The multiple regression equation predicting the HAQ-DI of 4 year follow-up = −2.75+2.22×$\log_{10}$ (serum P-selectin levels (ng/ml)) of baseline+−0.0060×%VC of baseline +0.29× HAQ-DI ($R^2 = 0.41$, RMSE = 0.345, $p = 0.001$, Table 6). Using our equation, we found that the HAQ-DI value of 4-year follow-up was significantly associated with P-selectin levels of baseline ($p = 0.028$). The HAQ-DI value at the fourth year tended to be negatively associated with the %VC of baseline ($p = 0.057$) and tended to be positively associated with the initial HAQ-DI

**Table 1.** The course of clinical and laboratory features in patients with SSc.

| | Baseline | 1 year follow-up | 2 year follow-up | 3 year follow-up | 4 year follow-up |
|---|---|---|---|---|---|
| MRSS | 16 (2–39) | 10 (0–38) | 12 (0–35) | 9 (1–25) | 8 (0–29) |
| And meto | 96 (53–143) | 91 (62–143) | 95 (61–143) | 91 (56–137) | 90 (58–136) |
| HAQ-DI | 0.125 (0–1.5) | 0.125 (0–1.75) | 0.25 (0–2.5) | 0.125 (0–1.875) | 0.25 (0–1.75) |
| ILD | 30 (77%) | 30 (77%) | 31 (79%) | 32 (82%) | 32 (82%) |
| Renal crisis | 0 (0%) | 2 (5.1%) | 0 (0%) | 1 (2.6%) | 1 (2.6%) |
| Corticosteroid therapy | 26 (67%) | 32 (82%) | 33 (85%) | 34 (87%) | 32 (82%) |
| Cyclophosphamide therapy | 4 (10%) | 8 (21%) | 4 (10%) | 6 (15%) | 8 (21%) |
| Cyclosporin A therapy | 0 (0%) | 1 (3%) | 2 (5%) | 4 (10%) | 4 (10%) |
| Azathioprine therapy | 0 (0%) | 0 (0%) | 1 (3%) | 1 (3%) | 1 (3%) |
| Methotrexate therapy | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 1 (3%) |

Values are represented as median (range) or as number of positive cases with percentage within parentheses.
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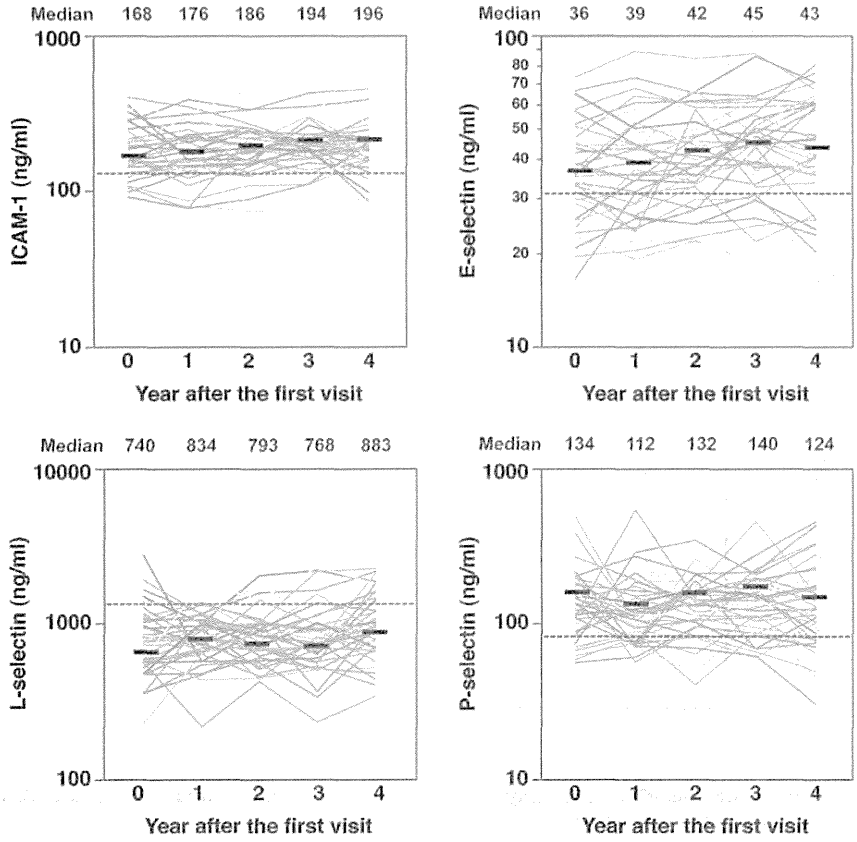


**Figure 2. Longitudinal change of serum adhesion molecule levels in each patient during the four years of the study.** The horizontal dotted line indicates the median value in healthy controls. The horizontal bar at each time point indicates the median value.
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($p = 0.10$). Multi-colineality did not exist among independent factors (VIF = 1.05−1.41). MRSS at the fourth year was not significantly associated with any adhesion molecule levels or clinical factors of baseline.

### Association between Each Adhesion Molecule Level and Subsequent Change of Clinical Parameters

Finally, we evaluated if baseline serum adhesion molecule levels are associated with subsequent percent change of clinical parameters by univariate analysis. However, baseline serum adhesion molecule levels were not significantly associated with the percent change of MRSS, %VC, and HAQ-DI values every year until 4 years (Table S1). Additionally, the percent changes of clinical parameters including MRSS, %VC, and HAQ-DI during 4-years were not significantly associated with any baseline adhesion molecule levels by multiple regression analysis (data not shown).

## Discussion

In this study, serum levels of ICAM-1, E-selectin, and P-selectin were significantly elevated in early SSc patients with diffuse skin sclerosis and/or ILD. By contrast, serum L-selectin levels were significantly reduced in these SSc patients. In this multicentre, longitudinal, prospective study, a multiple regression equation was defined to predict symptoms four years after initial diagnosis using baseline serum levels of four adhesion molecules and multiple clinical or laboratory factors presenting at the time of the first visit. Our findings suggest that elevated serum ICAM-1 levels are useful to predict the subsequent respiratory dysfunction. Furthermore, serum P-selectin levels at baseline may reflect the subsequent physical disability.

Our findings indicate that serum ICAM-1 levels were inversely associated with the current and subsequent respiratory functions in patients with early SSc. Elevated serum levels of ICAM-1 measured in early SSc patients in our study are consistent with previous several reports investigated in SSc patients [20-22] or dcSSc patients [23]. In one of those studies, circulating ICAM-1 levels were especially elevated in patients with diffuse rapidly progressive disease or digital ulcers [20]. In one report, serum levels of ICAM-1, P-selectin, and to a lesser degree, E-selectin correlate well with their in situ expression and with clinical disease activity [21]. Another study demonstrated that ICAM-1 levels were significantly higher in dcSSc patients and were correlated with the presence of contracture of phalanges, pulmonary fibrosis, joint involvement, and increased erythrocyte sedimentation rate [22]. In this study, we investigated the association between ICAM-1 levels and clinical features focused on early SSc patients with diffuse skin sclerosis and/or ILD in larger and multicenter population. As a result, serum ICAM-1 levels were specifically inversely associated with respiratory dysfunction.

Among various adhesion molecules, ICAM-1 has been most thoroughly investigated in the pathogenesis of SSc [24]. ICAM-1 is induced through IL-1β, IFN-γ, and TNF-α and initiates the binding of leukocytes to endothelium. Several previous studies have shown that SSc fibroblasts exhibit increased surface ICAM-1

**Table 2.** The associations between baseline adhesion molecule levels and subsequent clinical parameters in patients with SSc.

| | Baseline | 1 year follow-up | 2 year follow-up | 3 year follow-up | 4 year follow-up |
|---|---|---|---|---|---|
| $Log_{10}$ (ICAM-1 (ng/ml)) (baseline) vs. MRSS (baseline~4 year) | r = -0.12 | r = −0.02 | r = −0.02 | r = −0.15 | r = −0.072 |
| | p = 0.48 | p = 0.90 | p = 0.90 | p = 0.37 | p = 0.66 |
| $Log_{10}$ (ICAM−1 (ng/ml)) (baseline) vs. %VC (baseline~4 year) | r = −0.41* | r = −0.40* | r = −0.41* | r = −0.57** | r = −0.59** |
| | p = 0.019 | p = 0.033 | p = 0.036 | p = 0.0027 | p = 0.0009 |
| $Log_{10}$ (ICAM−1 (ng/ml)) (baseline) vs. HAQ-DI (baseline~4 year) | r = 0.065 | r = 0.027 | r = −0.14 | r = 0.11 | r = 0.060 |
| | p = 0.69 | p = 0.87 | p = 0.39 | p = 0.49 | p = 0.72 |
| $Log_{10}$ (E-selectin (ng/ml)) (baseline) vs. MRSS (baseline~4 year) | r = 0.16 | r = 0.12 | r = 0.012 | r = 0.012 | r = 0.16 |
| | p = 0.33 | p = 0.46 | p = 0.94 | p = 0.94 | p = 0.33 |
| $Log_{10}$ (E-selectin (ng/ml)) (baseline) vs. MRSS (baseline~4 year) | r = −0.30 | r = −0.30 | r = −0.29 | r = −0.06 | r = −0.30 |
| | p = 0.13 | p = 0.25 | p = 0.14 | p = 0.77 | p = 0.13 |
| $Log_{10}$ (E-selectin (ng/ml)) (baseline) vs. HAQ-DI (baseline~4 year) | r = 0.12 | r = 0.056 | r = 0.12 | r = 0.19 | r = 0.012 |
| | p = 0.94 | p = 0.73 | p = 0.48 | p = 0.25 | p = 0.94 |
| $Log_{10}$ (L-selectin (ng/ml)) (baseline) vs. MRSS (baseline~4 year) | r = −0.06 | r = −0.10 | r = −0.12 | r = −0.08 | r = −0.18 |
| | p = 0.57 | p = 0.37 | p = 0.27 | p = 0.51 | p = 0.17 |
| $Log_{10}$ (L-selectin (ng/ml)) (baseline) vs. %VC (baseline~4 year) | r = −0.051 | r = 0.049 | r = −0.052 | r = −0.16 | r = −0.26 |
| | p = 0.68 | p = 0.76 | p = 0.71 | p = 0.32 | p = 0.13 |
| $Log_{10}$ (L-selectin (ng/ml)) (baseline) vs. HAQ-DI (baseline~4 year) | r = −0.13 | r = −0.062 | r = −0.12 | r = −0.058 | r = −0.07 |
| | p = 0.21 | p = 0.57 | p = 0.26 | p = 0.63 | p = 0.63 |
| $Log_{10}$ (P-selectin (ng/ml)) (baseline) vs. MRSS (baseline~4 year) | r = 0.14 | r = 0.30 | r = 0.13 | r = 0.079 | r = 0.23 |
| | p = 0.39 | p = 0.060 | p = 0.43 | p = 0.63 | p = 0.15 |
| $Log_{10}$ (P-selectin (ng/ml)) (baseline) vs. %VC (baseline~4 year) | r = −0.16 | r = −0.20 | r = 0.022 | r = −0.13 | r = −0.077 |
| | p = 0.37 | p = 0.47 | p = 0.91 | p = 0.53 | p = 0.70 |
| $Log_{10}$ (P-selectin (ng/ml)) (baseline) vs. HAQ-DI (baseline~4 year) | r = 0.51** | r = 0.52** | r = 0.54** | r = 0.31 | r = 0.36* |
| | p = 0.0010 | p = 0.0006 | p = 0.0004 | p = 0.058 | p = 0.026 |

*p<0.05, **p<0.01.
doi:10.1371/journal.pone.0088150.t002

**Table 3.** The associations between ICAM−1 levels and subsequent %VC in patients with SSc.

| | %VC (baseline) | %VC (1 year follow-up) | %VC (2 year follow-up) | %VC (3 year follow-up) | %VC (4 year follow-up) |
|---|---|---|---|---|---|
| $Log_{10}$ (ICAM−1 (ng/ml)) (baseline) | r = −0.41* | r = −0.40* | r = −0.41* | r = −0.57** | r = −0.59** |
| | p = 0.019 | p = 0.033 | p = 0.036 | p = 0.0027 | p = 0.0009 |
| $Log_{10}$ (ICAM−1 (ng/ml)) (1 year follow-up) | | r = −035 | r = −0.36 | r = −0.56** | r = −0.46** |
| | | p = 0.080 | p = 0.079 | p = 0.0042 | p = 0.014 |
| $Log_{10}$ (ICAM−1 (ng/ml)) (2 year follow-up) | | | r = −0.43* | r = −0.58** | r = −0.50** |
| | | | p = 0.028 | p = 0.0022 | p = 0.0074 |
| $Log_{10}$ (ICAM−1 (ng/ml)) (3 year follow-up) | | | | r = −0.55** | r = −0.39* |
| | | | | p = 0.0048 | p = 0.040 |
| $Log_{10}$ (ICAM−1 (ng/ml)) (4 year follow-up) | | | | | r = −0.30 |
| | | | | | p = 0.12 |

*p<0.05, **p<0.01.
doi:10.1371/journal.pone.0088150.t003

**Table 4.** The associations between P-selectin levels and subsequent HAQ-DI in patients with SSc.

| | HAQ-DI (baseline) | HAQ-DI (1 year follow-up) | HAQ-DI (2 year follow-up) | HAQ-DI (3 year follow-up) | HAQ-DI (4 year follow-up) |
|---|---|---|---|---|---|
| $Log_{10}$ (P-selectin (ng/ml)) (baseline) | r = 0.51** | r = 0.52** | r = 0.54** | r = 0.31 | r = 0.36* |
| | p = 0.0010 | p = 0.0006 | p = 0.0004 | p = 0.058 | p = 0.026 |
| $Log_{10}$ (P-selectin (ng/ml)) (1 year follow-up) | | r = −0.18 | r = −0.064 | r = 0.018 | r = −0.12 |
| | | p = 0.29 | p = 0.70 | p = 0.91 | p = 0.49 |
| $Log_{10}$ (P-selectin (ng/ml)) (2 year follow-up) | | | r = 0.015 | r = −0.074 | r = −0.25 |
| | | | p = 0.92 | p = 0.66 | p = 0.12 |
| $Log_{10}$ (P-selectin (ng/ml)) (3 year follow-up) | | | | r = 0.25 | r = 0.017 |
| | | | | p = 0.12 | p = 0.92 |
| $Log_{10}$ (P-selectin (ng/ml)) (4 year follow-up) | | | | | r = 0.018 |
| | | | | | p = 0.92 |

*p<0.05, **p<0.01.
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expression, suggesting an augmented potential for binding to T cells [25]. Another study demonstrated that ICAM-1 and vascular cell adhesion molecule (VCAM)-1 have important roles in the retention of myeloid cells in the skin of SSc patients [26,27]. In tight-skin 1 mouse, a genetic model of skin fibrosis, it has been demonstrated that ICAM-1 expression contributes to the development of skin fibrosis, especially via ICAM-1 expressed on skin fibroblasts [28]. ICAM-1 deficiency ameliorates lung fibrosis induced by intratracheal bleomycin administration [29]. A recent study has shown that L-selectin and ICAM-1 regulate Th2 and Th17 cell accumulation in the skin and lungs, leading to the development of fibrosis in a bleomycin-induced fibrosis model [30]. These previous reports indicate that ICAM-1 is contributing to the development of inflammation and fibrosis in SSc via inducing the infiltration and activation of leukocytes. Furthermore, increased circulating ICAM-1 may be reflecting the vascular activation and inflammation in SSc. This may be the reason why serum ICAM-1 levels are highly associated with current and subsequent respiratory dysfunction.

Circulating ICAM-1 has been considered as the result of proteolytic cleavage of cell-bound ICAM-1 close to the cell membrane [31,32]. ICAM-1 cleavage is regulated by tumor necrosis factor-α-converting enzyme and multiple kinases, including mitogen-activated protein kinase, S locus receptor kinase, and phosphoinositide 3-kinase pathways [33,34]. There are some reports demonstrating the critical roles of these enzymes in SSc patients or animal model of SSc [35-38]. Soluble ICAM-1 is functionally active and retains the ability to inhibit leukocyte-endothelial cell interaction [39,40]. On the other hand, soluble ICAM-1 has also been reported to promote angiogenesis [41] and induce the production of TNF-α, IFN-γ, IL-6, and macrophage inflammatory protein-2 [42,43]. Thus soluble ICAM-1 may also have proinflammatory potential.

In the current study, serum P-selectin levels were found to be increased, consistent with previous reports [21,44,45]. However, another previous study showed normal levels of P-selectin in SSc [46]. Since our population was selected for early active SSc, serum P-selectin levels are likely elevated at least at early active stage. The baseline P-selectin levels were significantly associated with HAQ-DI at the fourth year as determined by multiple regression analysis. Recently, we reported that baseline serum CXCL8 (IL-8) levels were significantly associated with subsequent HAQ-DI in early SSc patients [47]. Therefore, we compared the utility between P-selectin and CXCL8 for predicting the subsequent HAQ-DI by multiple regression analysis in the current population. As a result, P-selectin was more useful serum indicator of subsequent HAQ-DI (data not shown). The roles of P-selectin in the pathogenesis of SSc remain unclear. In lung fibrosis mouse model induced by intratracheal bleomycin administration, P-selectin deficiency did not significantly affect the fibrosis of lungs [48]. On the other hand, another study showed that the loss of P-selectin augmented the fibrosis of both skin and lungs induced by intracutaneous bleomycin injection [30].

**Table 5.** Factors predicting %VC of 4 year follow-up determined by multiple regression analysis.

| | Estimate | Standard error | P value |
|---|---|---|---|
| Intercept | 230.2 | 83.4 | 0.012 |
| %VC of baseline | 0.62 | 0.13 | 0.0001 |
| $Log_{10}$ (serum ICAM-1 levels of baseline) ng/ml | −60.1 | 22.7 | 0.015 |

The multiple regression equations predicting %VC of 4 year follow-up are as follows; %VC of 4 year follow-up = 230.2+0.62×%VC of baseline+−60.1×$log_{10}$ (serum ICAM-1 levels (ng/ml) of baseline). $R^2$ (determination coefficient) = 0.73, root mean square error = 12.1, p<0.0001.
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**Table 6.** Factors predicting HAQ-DI of 4 year follow-up determined by multiple regression analysis.

| | Estimate | Standard error | P value |
|---|---|---|---|
| Intercept | −2.75 | 1.62 | 0.099 |
| $Log_{10}$ (serum P-selectin levels of baseline) ng/ml | 2.22 | 0.96 | 0.028 |
| %VC of baseline | −0.0060 | 0.0030 | 0.057 |
| HAQ-DI of baseline | 0.29 | 0.17 | 0.100 |

The multiple regression equations predicting HAQ-DI of 4 year follow-up are as follows; HAQ-DI of 4 year follow-up = $-2.75+2.22\times\log_{10}$(serum P-selectin levels (ng/ml) of baseline)+−0.0060×%VC of baseline +0.29×HAQ-DI of baseline. $R^2$ (determination coefficient) = 0.41, root mean square error = 0.345, p = 0.001.
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We detected that serum E-selectin levels are also significantly elevated in SSc patients in consistent with previous studies [46,49-51]. Although serum E-selectin levels were significantly associated with the presence of pulmonary fibrosis in a previous study [50], we could not find any significant association with clinical features in our population selected as those with early SSc patients with diffuse skin sclerosis and/or ILD.

Previous findings regarding serum L-selectin levels were not consistent. Significantly reduced levels of serum L-selectin have been reported in patients with SSc [52]. In a recent report, serum L-selectin levels were reduced and were negatively associated with skin damage in patients with dcSSc [53]. By contrast, serum levels of L-selectin were significantly elevated in patients with SSc in another study [54]. Another group reported that the levels were not significantly different between SSc patients and normal controls [45]. However, our multicentre, larger studies indicate that serum L-selectin levels are decreased in early SSc patients with diffuse skin sclerosis and/or ILD. Serum L-selectin levels have been known to increase during acute inflammatory conditions as a result of shedding from activated leukocytes and/or leukocytes transmigrating endothelial cells. Although we can not explain why our patients with SSc showed reduced serum L-selectin levels, chronic inflammation such as chronic heart or renal diseases likely results in downregulation of leukocyte expression of cell-surface L-selectin and thus lower circulating L-selectin levels [55,56].

Some limitations exist in this study. The population of longitudinal study is relatively small. Additionally, this is an observational study and, therefore, the treatment protocol is heterogeneous. Nonetheless, our data indicate that serum levels of ICAM-1 and P-selectin may be useful for predicting the subsequent severity of ILD and physical dysfunction, respectively. The predictive biomarkers are generally important if they predict the rates of change in the investigated outcomes rather than their absolute levels. However, the association between serum levels of adhesion molecules and the rates of change of investigated outcomes were not significant. Therefore, this study indicates that serum levels of ICAM-1 and P-selection are useful to predict the subsequent severity of respiratory dysfunction and physical disability, respectively, but not the subsequent rate of their change. Further longitudinal studies in a larger population will be needed to confirm the utility of these adhesion molecules as prognostic indicators in SSc patients.

## Supporting Information

**Table S1** The associations between baseline adhesion molecule levels and subsequent percent change of clinical parameters in patients with SSc.
(DOCX)

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## Author Contributions

Conceived and designed the experiments: MH MK SS KT. Performed the experiments: MH. Analyzed the data: MH KI. Contributed reagents/materials/analysis tools: MH YA HE MF DG HI OI YK MK FO HT ST SS KT. Wrote the paper: MH.

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LETTER

# Successful treatment of skin fistulas in systemic sclerosis patients with the combination of topical negative pressure therapy and split-thickness skin grafting

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**Keywords**

Scleroderma, Skin ulcer, Treatment

**History**

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**To the Editor,**

Vascular involvements including skin ulcers, gangrenes and fissures are frequently seen in patients with systemic sclerosis (SSc). Because they tend to be unresponsive to medical management, treatments sometimes need to be continued for more than 1 year and amputation of digits is sometimes performed, which severely decreases patients' quality of life. Thus, developing new therapeutic approaches against refractory vascular involvements in SSc is urgently needed. Recently, we experienced skin ulcers/gangrenes with severe fistulation in two SSc patients successfully treated with the combination of topical negative pressure (TNP) therapy and split-thickness skin grafting (STSG). Using this approach, we could shorten the duration of treatments and could avoid amputation.

Case 1 was a 64-year-old woman with a 20-year history of limited cutaneous SSc (lcSSc), positive for anti-centromere antibodies. Her fingers had been amputated several times because of repeated ulcers and gangrenes. On the first visit, she suffered from a digital ulcer of the left third finger (Fig. 1a). The ulcer was accompanied by a fistula reaching to the tendon, and pus discharged from the fistula. Conservative therapies, including fistulotomy, intravenous prostacyclin, oral antibiotics and povidone–iodine sugar ointment were performed for a month, but the wound was unresponsive (Fig. 1b, c). Before considering amputation, we tried TNP therapy to facilitate granulation of the wound, using a VAC therapy system (KCI, San Antonio): the system contains polyvinyl-alcohol and polyurethane ethylene foam dressing, an integrated vacuum tube, and pressure setting. Foam dressing was placed in the wounds and sealed with clear film. A small cut was made in the film to allow an integrated vacuum tube to project into the foam dressing (Fig. 2a, b). The tube was sealed in place and connected to the pressure setting. The dressing was renewed every 72 h and a regimen of continuous pressure at −100 mmHg was maintained. This resulted in good granulation tissue after 1 month (Fig. 2c). Subsequently, we performed STSG, harvested from the thigh, to improve epithelialization. The skin defect was almost completely healed 5 months after the first visit (Fig. 2d).

The second case was a 73-year-old woman with a 3-year history of lcSSc, positive for anti-centromere antibodies. She developed skin gangrene and a fistula of the left big toe (Fig. 3a). MRI revealed a subcutaneous abscess around the tendon without osteomyelitis. Because of unresponsiveness to conservative therapies including intravenous prostacyclin, intravenous antibiotics, oral bosentan and povidone–iodine sugar ointment, we performed a fistulotomy (Fig. 3b). However, 1 month later, there was still large skin defect (Fig. 3c). Before considering amputation, we tried the TNP therapy as described above. After 1 month of TNP therapy, the patient's ulcer developed a good wound bed (Fig. 1d). Therefore, the wound was treated with STSG, harvested from the thigh. Four months after the first visit, the raw surface was almost completely healed (Fig. 1e).

In these cases, blood examination, CT and MRI denied the presence of vasculitis and thrombosis. It is known that patients with diffuse cutaneous SSc (dcSSc) tend to have anti-topoisomerase I antibodies and rapid skin/visceral fibrosis, while lcSSc patients tend to have anti-centromere antibodies. Vascular abnormalities including Raynaud's phenomenon, telangiectasia, skin ulcers or gangrenes and pulmonary hypertension are more pronounced and fibrosis is limited in these patients [1]. Thus, the ulcers/gangrenes in our cases are likely to occur as the vascular manifestation of anti-centromere antibody-positive lcSSc.

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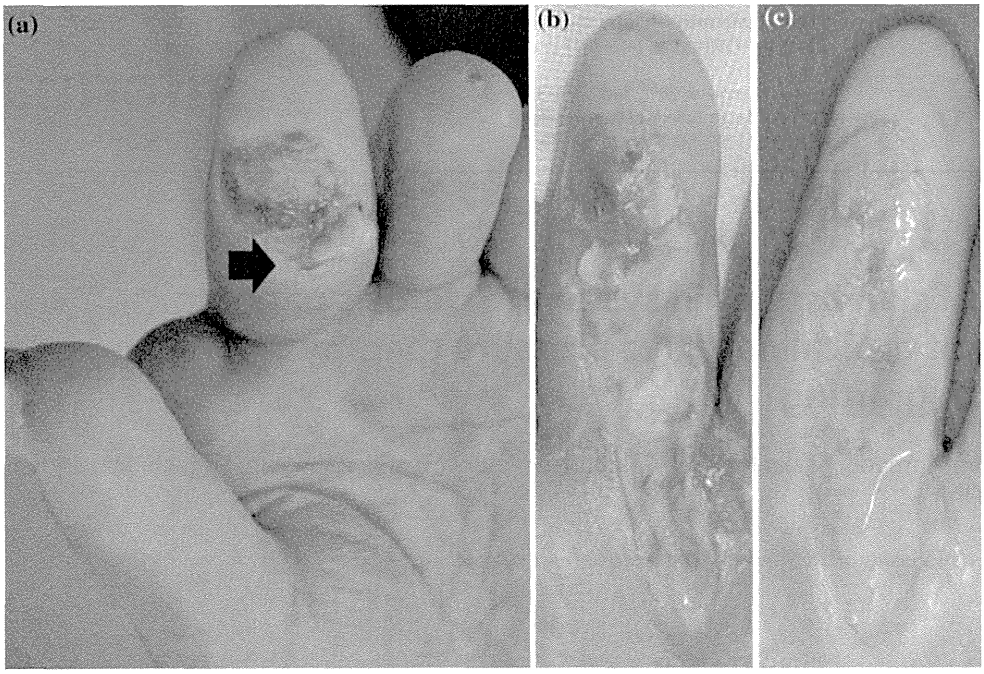


Fig. 1 The ulcer and fistula of left third finger in case 1. (a) Before treatments; the *arrow* indicates pus from the fistula. The second and fourth fingers were amputated previously, (b) just after the fistulotomy and (c) 1 month after the

It is important to develop alternative therapies for skin ulcers/gangrenes in SSc, because they tend to be resistant to conventional management. Although TNP therapy is not included in the EULAR recommendations for the treatment of SSc yet [2], it induces blood flow, cell proliferation and angiogenesis, reduces the wound surface area, and modulates inhibitory contents in wound fluid [3]. In previous reports, TNP therapy was effective for digital ulcers [4] and toe gangrenes [5] in SSc patients. In this manuscript,

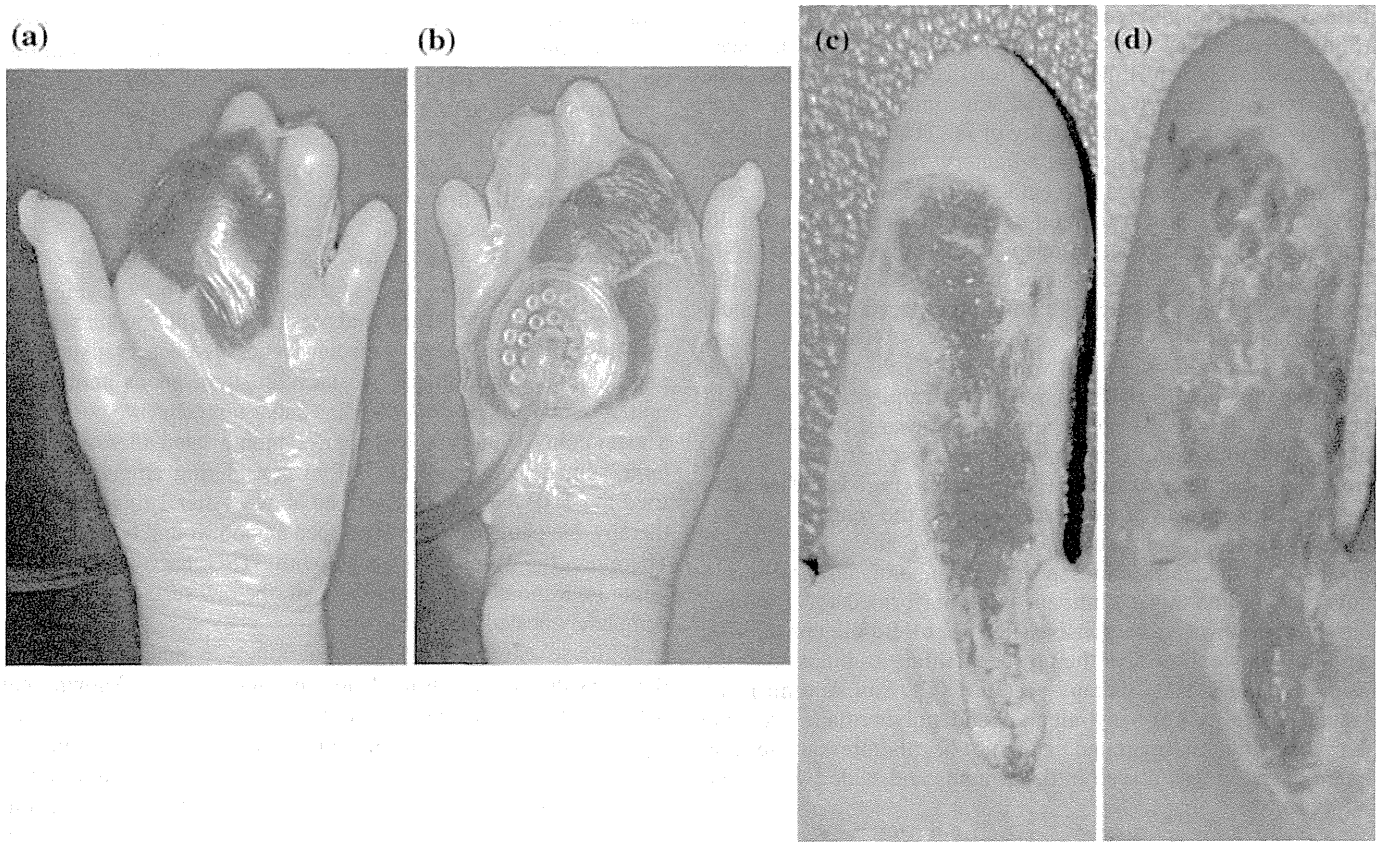


Fig. 2 The effect of topical negative pressure therapy and split-thickness skin grafting in case 1. Topical negative pressure therapy (a) palmar side, (b) dorsal side of hand, (c) 1 month after the topical negative pressure therapy and (d) 2 weeks after the split-thickness skin grafting

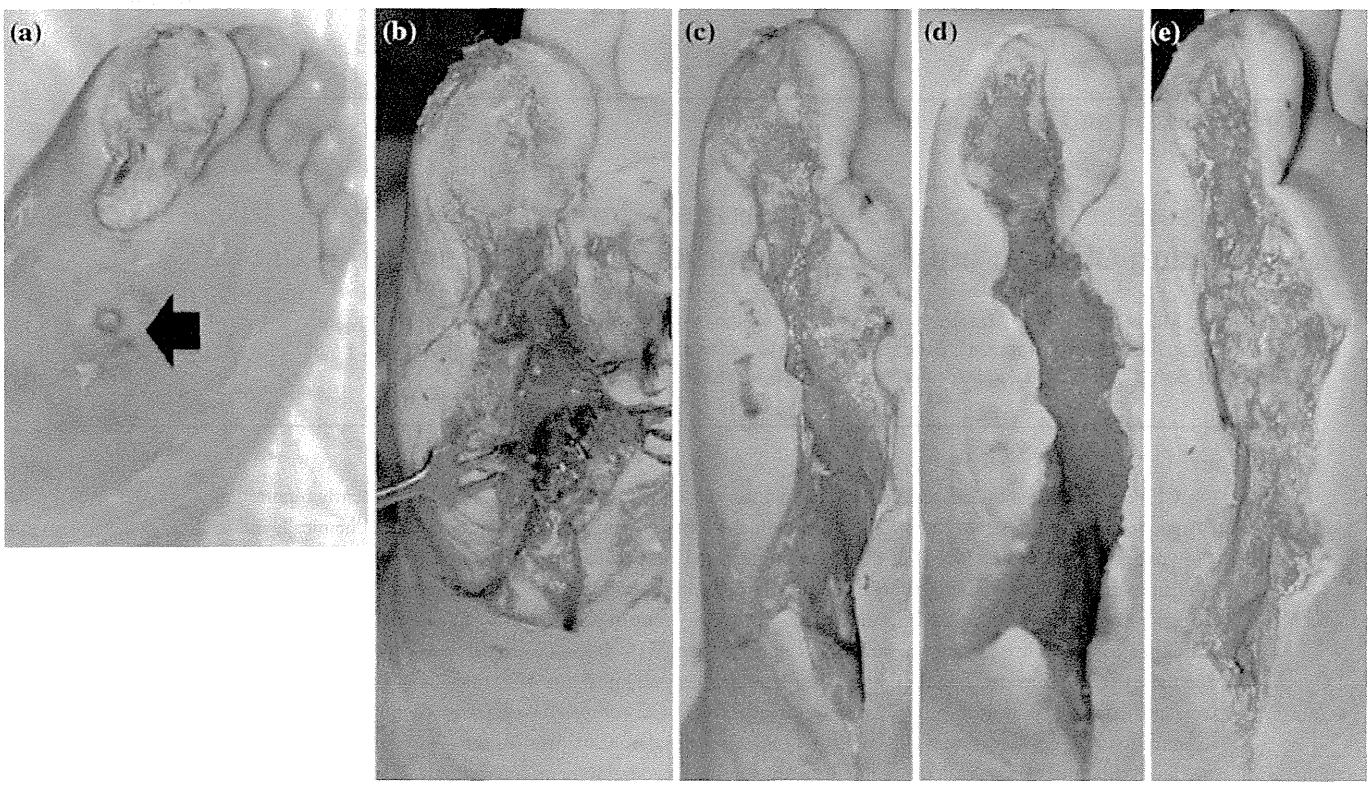


Fig. 3 The ulcer and fistula of left big toe in case 2. (a) Before treatments; the *arrow* indicates the external opening of fistula, (b) just after the fistulotomy, (c) 1 month after the fistulotomy, (d) 1 month after the topical negative pressure therapy and (e) 1 month after the split-thickness skin grafting

we suggest that TNP therapy and STSG therapy is useful for treating not only ulcers and gangrenes but also fistulas, and helps avoid amputation. To add the combination therapy to the therapeutic options against refractory ulcers, gangrenes and fistulas of SSc, more patient trials will be needed in the future.

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## Conflict of interest

None.

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