Figure 1. A, Immunoprecipitates with anti-155/140 autoantibodies from ^{35}S-methionine–labeled K562 cell extracts were subjected to 8% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and analyzed by autoradiography. Lane 1, Anti–transcription intermediary factor 1γ (anti–TIF-1γ) polyclonal antibodies (pAb); lanes 2–7, dermatomyositis (DM) patient sera positive for anti-155/140. **B,** K562 cell extracts were immunoprecipitated with anti-155/140–positive sera, anti-155–positive sera, or normal control sera and subjected to SDS-PAGE and Western blotting with anti–TIF-1γ polyclonal antibodies. **C,** TIF-1γ and TIF-1α were immunodepleted from ^{35}S-methionine–labeled cell extracts using anti–TIF-1γ (lanes 2 and 5) or anti–TIF-1α (lane 3) polyclonal antibodies, and immunoprecipitated with anti-155/140–positive sera (lanes 1–3) or anti–p155–positive sera (lanes 4 and 5). Lanes 1 and 4, Control samples immunodepleted with control IgG. **D,** Recombinant TIF-1γ protein was subjected to Western blotting with anti-155/140–positive sera. Lanes 1 and 2, Anti–TIF-1γ–positive sera; lane 3, control sera; lane 4, anti–TIF-1γ polyclonal antibodies. **E,** Shown is a comparison of the proteins immunoprecipitated with anti-155/140, anti–NXP-2, or anti–melanoma differentiation–associated protein 5 (anti–MDA-5) antibodies in DM patient sera, or with anti–TIF-1α polyclonal antibodies, analyzed by 7% SDS-PAGE. **F,** Immunoprecipitates from cell extracts with anti-155/140–positive sera or control sera were probed with anti–TIF-1α polyclonal antibodies. **G,** Recombinant TIF-1α protein was subjected to Western blotting with anti-155/140–positive sera. Lanes 1 and 2, Anti–TIF-1α–positive sera; lane 3, control sera; lane 4, anti–TIF-1γ polyclonal antibodies. **Asterisks** indicate the same serum samples.

were determined by ELISA. Microtiter plates with 96 wells (Costar) were coated with recombinant TIF-1α or TIF-1γ protein (1 μg/ml) at 4°C overnight. The wells were blocked with 2% bovine serum albumin and 1% gelatin in Tris buffered saline for 1 hour at 37°C. The serum samples that were diluted to 1:100 were first preabsorbed in GST-coated wells and then were added to duplicate wells coated with recombinant TIF-1 proteins for 90 minutes at 20°C. After washing 4 times, the bound antibodies were detected with alkaline phosphatase–conjugated goat anti-human IgG antibodies (Cappel) using p-nitrophenyl phosphate (Sigma-Aldrich) as substrate. Absorbance in each well was read using a microplate reader (Bio-Rad) set to 405 nm.

Statistical analysis. Fisher's exact test was employed for comparison of frequencies. The Kolmogorov-Smirnov test was used to determine normality. P values less than 0.05 were considered significant.

RESULTS

Confirmation that the 155-kd antigen of anti-155/140 antibodies was TIF-1γ. In IP assays, "anti-p155 antibodies" have been reported to precipitate a 155-kd protein (14), while "anti-155/140 antibodies" have been reported to precipitate 2 proteins—a 155-kd protein and a 140-kd protein (15). If these 2 autoantibodies are the same, the difference in the reacting proteins may be due to the types of the substrate cells (for example, K562 cells versus HeLa cells) or the experimental procedures

(for example, degradation of antigen proteins). However, when multiple serum samples were compared simultaneously using the same extract of antigen source, some sera reacted strongly with both proteins, while some reacted strongly with the 155-kd protein but not, or very weakly, with the 140-kd protein (Figure 1A). Therefore, the reactivities for the 155-kd and 140-kd proteins differed among serum samples under the same conditions, suggesting that the antibodies reacted with each protein independently and that the 2 proteins were indeed different. We screened a total of 456 serum samples from DM patients and identified 77 samples that were positive for either the 155-kd or 140-kd protein. Among the 77 samples, 52 were reactive with both the 155-kd and the 140-kd proteins, while 25 were reactive with the 155-kd protein alone. No samples were found to be positive for the 140-kd protein alone.

We sought to confirm that the 155-kd antigen recognized by anti-155/140 antibodies was TIF-1γ, as was preliminarily reported for anti-p155 antibodies (14). K562 cell lysates were incubated with protein A–Sepharose beads preincubated with sera reactive with the 155-kd and 140-kd antigens and those reactive with the 155-kd antigen alone, and precipitated proteins were probed with polyclonal antibodies to TIF-1γ in Western blotting. Polyclonal anti–TIF-1γ antibodies reacted with

both of the immunoprecipitates generated with anti-155/140–positive sera and anti-155–positive sera (Figure 1B). To further confirm that these sera recognized the same antigens, K562 cell extracts were first absorbed using polyclonal antibodies to TIF-1γ, and then immunoprecipitation was performed using patient sera that were reactive with both the 155-kd and 140-kd antigens or with the 155-kd antigen alone. The polyclonal antibodies depleted the 155-kd band (Figure 1C), demonstrating that both anti-p155 antibodies and anti-155/140 antibodies reacted with TIF-1γ. Furthermore, anti-155/140 sera reacted strongly with recombinant TIF-1γ protein in Western blotting (Figure 1D). Therefore, it was formally demonstrated that "anti-p155" and "anti-155/140" antibodies are the same in that they both recognize TIF-1γ as the 155-kd antigen.

The 140-kd antigen is TIF-1α. Next, we sought to identify the 140-kd protein targeted by anti-155/140 antibodies. On 7% polyacrylamide gels, the 140-kd protein appeared different from MDA-5, which is targeted by anti-CADM140 antibodies, and NXP-2 (also known as MORC3), which is targeted by anti-MJ (anti-p140) antibodies (Figure 1E). On the other hand, another TIF-1 family protein, TIF-1α, migrated at a molecular weight identical to the 140-kd antigen precipitated with anti-155/140 antibodies (Figure 1E). Therefore, we examined whether the 140-kd autoantigen targeted by anti-155/140 antibodies was TIF-1α. First, K562 cell lysates were incubated with protein A–Sepharose beads preincubated with anti-155/140–positive sera, and precipitated proteins were probed with polyclonal antibodies to TIF-1α in Western blotting. While control samples from healthy subjects did not show any band, those from anti-155/140–positive sera developed a strong 140-kd band that was recognized by polyclonal anti–TIF-1α antibodies (Figure 1F). Also, in the immunodepletion assay, polyclonal antibodies to TIF-1α depleted the 140-kd band that was recognized by anti-155/140–positive sera (Figure 1C). Moreover, when recombinant GST-tagged full-length human TIF-1α protein was subjected to SDS-PAGE and Western blotting with anti-155/140–positive sera, the sera positive for the antibodies reacted with the recombinant protein (Figure 1G). These sera did not react with GST alone (results not shown). Therefore, the 140-kd antigen of anti-155/140 antibodies was identified as TIF-1α.

A portion of anti-155/140–positive sera react with TIF-1β. TIF-1β also belongs to the TIF-1 family. Therefore, we examined whether TIF-1β was another target recognized by anti-155/140–positive sera. When immunoprecipitated with polyclonal anti–TIF-1β antibodies

and subjected to SDS-PAGE, TIF-1β migrated at ~100 kd and appeared as a thick band (Figure 2A). Therefore, we first screened anti-155/140–positive serum samples that also precipitated proteins at ~100 kd. Of 77 serum samples that were positive for anti-155/140 antibodies, 6 samples showed a similar thick band that was identical to the TIF-1β band immunoprecipitated with polyclonal anti–TIF-1β antibodies and that did not match previously identified autoantigens including PL-12 (Figure 2A). We thus investigated whether this immunoprecipitated protein was indeed TIF-1β.

K562 cell lysates were incubated with protein A–Sepharose beads preincubated with serum samples positive for this 100-kd protein, and precipitated proteins were assessed in Western blotting by probing with polyclonal antibodies to TIF-1β. While samples immunoprecipitated with serum from healthy subjects did not show any band, those immunoprecipitated with anti–100-kd–positive sera developed a strong 100-kd band that was recognized by polyclonal anti–TIF-1β antibodies (Figure 2B). This was also confirmed by immunodepletion assay, in which polyclonal antibodies to TIF-1β decreased the 100-kd band (Figure 2C). Moreover, anti–100-kd sera reacted with recombinant GST-tagged human TIF-1β protein, but not with GST alone, in Western blotting (Figure 2D). Therefore, a portion of the patients with anti-155/140 antibodies also possessed autoantibodies that targeted TIF-1β. Taken together, the results show that the TIF-1 family proteins TIF-1α, TIF-1β, and TIF-1γ can be targeted by anti-155/140–positive sera.

To assess the possibility that anti–TIF-1β antibodies appeared independently of anti–TIF-1α or anti–TIF-1γ, we reviewed the results from the 456 patients with DM as well as from the 62 patients with PM, 108 with SLE, and 433 with SSc, and we found an additional serum sample from a 36-year-old woman with clinically amyopathic DM that reacted with the 100-kd protein but not with the 155-kd and 140-kd proteins (Figure 2A). The reactivity with TIF-1β in this sample was also confirmed by the assay using immunoprecipitation and Western blotting with polyclonal anti–TIF-1β antibodies as well as Western blotting using recombinant protein (results not shown). Serum samples from patients with other diseases did not react with the 100-kd protein.

In summary, 78 of 456 DM sera (17%) were positive for at least 1 anti–TIF-1 antibody (anti–TIF-1α, anti–TIF-1β, or anti–TIF-1γ). Among these 78 sera, reactivity with all 3 antibodies was observed in 4 sera (5.1%), reactivity with anti–TIF-1α and anti–TIF-1γ in 48 sera (62%), reactivity with anti–TIF-1β and anti–
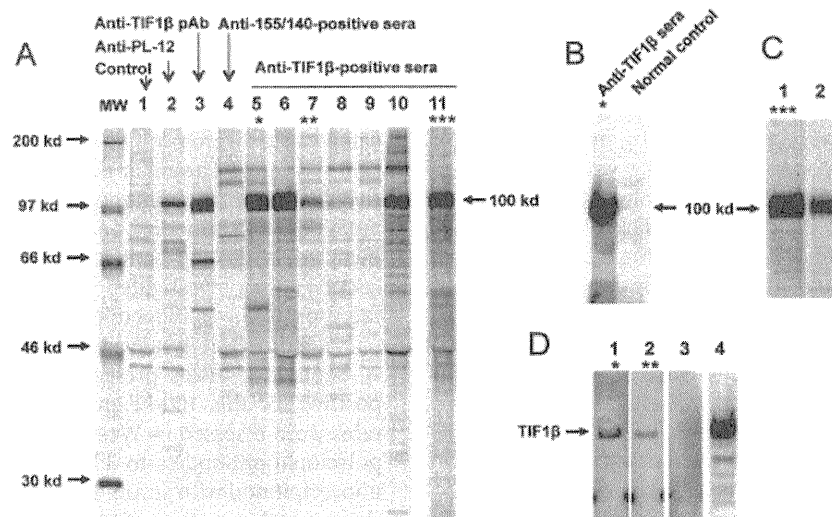
Figure 2. A, Immunoprecipitation of the 100-kd autoantigen from ^{35}S-methionine–labeled K562 cell extracts is shown, as described in Figure 1A. Lane 1, Normal human serum; lane 2, prototype serum positive for anti–PL-12 antibodies; lane 3, sample immunoprecipitated with polyclonal antibodies to TIF-1β (the position of the 100-kd TIF-1β antigen is indicated); lane 4, prototype serum positive for anti-155/140 antibodies; lanes 5–11, serum samples from DM patients positive for anti–TIF-1β antibodies (lane 11 shows a serum sample from a 36-year-old woman with clinically amyopathic DM; see Results). **B,** K562 cell extracts were immunoprecipitated with anti–TIF-1β–positive sera or normal control sera and were subjected to SDS-PAGE, transferred onto nitrocellulose membranes, and probed with anti–TIF-1β polyclonal antibodies. The molecular weight of TIF-1β (100 kd) is indicated. **C,** Immunodepletion analysis of anti–TIF-1β antibodies is shown. TIF-1β was immunodepleted from ^{35}S-methionine–labeled K562 cell extracts using anti–TIF-1β polyclonal antibodies (lane 2) and then immunoprecipitated with anti–100-kd–positive sera. Control IgG is shown in lane 1. **D,** Recombinant TIF-1β protein was subjected to Western blotting with anti–TIF-1β–positive sera. Lanes 1 and 2, Anti–TIF-1β–positive sera; lane 3, normal control sera; lane 4, anti–TIF-1β polyclonal antibodies. **Asterisks** indicate the same serum samples. See Figure 1 for definitions.

TIF-1γ in 2 sera (2.6%), reactivity with anti–TIF-1γ alone in 23 sera (29%), and reactivity with anti–TIF-1β alone in 1 serum sample (1.3%) (Figure 3A). No sera reacted with anti–TIF-1α alone or with anti–TIF-1α and anti–TIF-1β without reacting with anti–TIF-1γ. Thus, TIF-1γ was the most commonly targeted protein, followed by TIF-1α and TIF-1β. Collectively, these findings demonstrated that anti-155/140–positive sera target all 3 TIF-1 family proteins with varied patterns of reactivity.

Clinical associations. The clinical association of anti–TIF-1α, anti–TIF-1β, and/or anti–TIF-1γ (anti–TIF-1α/β/γ) antibodies was analyzed in the 78 patients. Among them, 74 patients were age >15 years, and 4 patients were age <15 years (Figure 3B). Thus, 17% of adult DM patients (74 of 445) and 36% of juvenile DM patients (4 of 11) were positive for anti–TIF-1α/β/γ antibodies. This was consistent with reports from the US and Europe that anti-155/140 antibodies are a major serologic subset both in juvenile DM and in adult cancer-associated DM (14,15,22,23). Most of the adult patients were age >45 years at onset, although it was

notable that there was another small peak of "young adults" between ages 25 and 39 years. Anti–TIF-1α/β/γ–positive adult DM patients had malignant disease at a rate of 65% (48 of 74), while none of the young adult patients had a history of malignancy. Thus, although this finding was not statistically significant, anti–TIF-1α/β/γ antibodies may underlie a subset of "young adult DM" that is not associated with malignancy. In contrast, among 64 patients age >40 years, 48 had malignancy (75%). In particular, patients age >60 years had a frequency of malignancy as high as 86% (30 of 35). The cancer sites in 48 patients are shown in Table 1.

DM patients present either the classic DM phenotype (i.e., with muscle involvement) or the clinically amyopathic DM phenotype. In the 78 patients with anti–TIF-1α/β/γ antibodies, 53 had classic DM (68%), while 25 had clinically amyopathic DM. All patients with juvenile DM were classified as having classic DM. In contrast, among the 8 young adult DM patients positive for anti–TIF-1α/β/γ antibodies, 6 had no symptoms of muscle involvement and were classified as having clini-
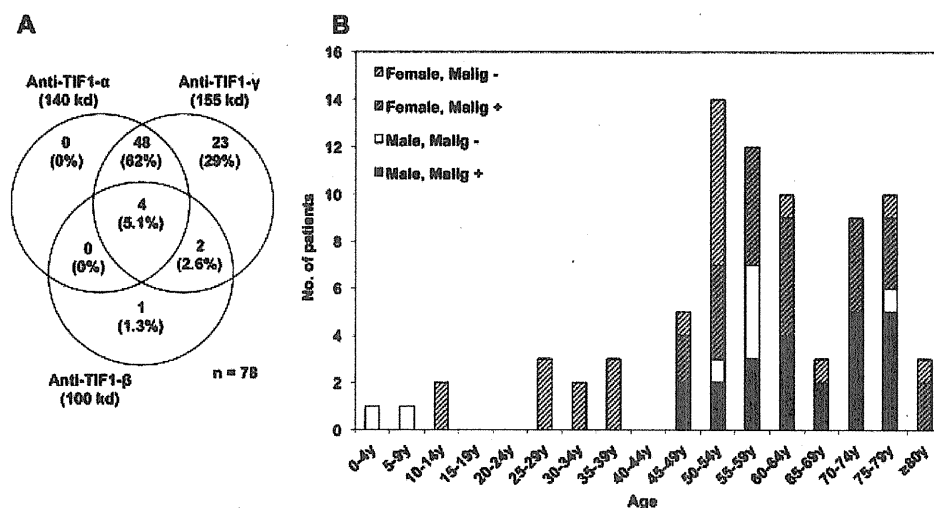
Figure 3. A, Numbers and percent of anti–TIF-1α, anti–TIF-1β, and anti–TIF-1γ antibodies in 78 DM patients. B, Distribution of the age at onset and the presence or absence of malignancy in 78 DM patients positive for anti–TIF-1α, anti–TIF-1β, and anti–TIF-1γ antibodies. Bars show the numbers of female and male patients with or without malignancy (Malig+ and Malig−, respectively). See Figure 1 for other definitions.

cally amyopathic DM. Another young adult patient was initially diagnosed as having clinically amyopathic DM but developed mild myositis 6 months later. The rates of malignancies in patients with adult classic DM and those

Table 1. Sites/type of malignancies and autoantibody reactivity to TIF-1 proteins in the 48 dermatomyositis patients with cancer*

	Total (anti–TIF-1γ positive) (n = 48)†	Anti–TIF-1α positive (n = 36)	Anti–TIF-1β positive (n = 2)†
Lung	14	10	0
Stomach	11	8	0
Colon and rectum	4	4	0
Ovary	4	4	2
Breast	4	3	0
Thymus	3	2	0
Gall bladder and bile duct	2	1	0
Uterus	2	1	0
Prostate	1	1	0
Pancreas	1	1	0
Epipharynx	1	1	0
Lymphoma	1	1	0
Renal pelvis	1	1	0
Bladder	1	1	0
Thyroid	1	0	0
Unknown origin	1	0	0

* Values are the number of patients. Patients with cancer at multiple sites are included in the numbers for each involved site.
† All patients having malignancy were positive for anti–transcription intermediary factor 1γ (anti–TIF-1γ) antibodies.
‡ Both patients were also positive for anti–TIF-1α.

with clinically amyopathic DM were 69% (34 of 49) and 56% (14 of 25), respectively. Thus, the rate of malignancy was slightly higher in patients with classic DM, although there was no significant difference. Interstitial lung disease was observed in only 3 patients (3.8%).

When the correlation with antibody reactivity was assessed, patients positive for both anti–TIF-1α and anti–TIF-1γ antibodies were found to have a 73% rate of malignancy (36 of 49), while those positive for anti–TIF-1γ antibodies alone had a 50% rate of malignancy (12 of 24) (Table 2). Thus, the incidence of malignancy was significantly higher in those with anti–TIF-1α and anti–TIF-1γ antibodies than in those with anti–TIF-1γ antibodies alone (P < 0.05). There was no specific association between cancer type or site and reactivity with TIF-1α and TIF-1γ. Additionally, among the 7 patients who were positive for anti–TIF-1β antibodies, 2 were diagnosed as having malignancies, both of which were ovarian cancer. Patients positive for both anti–TIF-1α and anti–TIF-1γ antibodies had internal malignancy and truncal erythema more frequently than those positive for anti–TIF-1γ antibodies alone (Table 2).

Longitudinal changes in serum antibody titers were also assessed in 8 patients positive for anti–TIF-1α and anti–TIF-1γ antibodies. After treatment, the titer of anti–TIF-1γ antibodies as measured by ELISA had decreased in all patients (Figure 4), although they remained positive in immunoprecipitation assays (data

Table 2. Demographic, clinical, and laboratory features in the adult dermatomyositis patients with both anti–TIF-1α and anti–TIF-1γ antibodies and in those with anti–TIF-1γ antibodies alone*

	Anti–TIF-1α and anti–TIF-1γ antibodies (n = 49)	Anti–TIF-1γ antibodies alone (n = 24)	P
Age at onset, mean (range) years	62 (29–89)	57 (27–75)	NS
No. men/no. women	22/27	9/15	NS
Skin eruptions			
Heliotrope rash	62	67	NS
Gottron's papules	82	83	NS
Perionychia erythema	62	50	NS
Nailfold punctate hemorrhage	38	39	NS
Truncal erythema	77	33	<0.01
Calcinosis	0	6	NS
Ulceration	3	17	NS
Clinical features			
Muscle weakness	75	61	NS
Raynaud's phenomenon	10	0	NS
Arthritis	3	6	NS
Fever	18	11	NS
Organ involvement			
Interstitial lung disease	2	8	NS
Internal malignancy	73	50	<0.05
Laboratory findings			
Elevated CK	69	56	NS
Highest CK level, mean (range) IU/liter	1,456 (55–8,670)	850 (40–2,805)	NS

* Except where indicated otherwise, values are the percent of patients. Anti–TIF-1 = anti–transcription intermediary factor 1; NS = not significant; CK = creatine kinase.
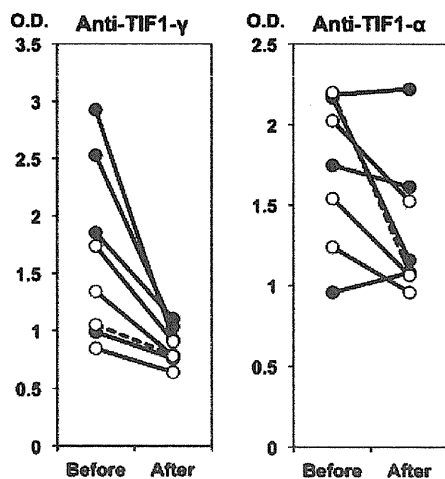

Figure 4. Relative titer of anti–TIF-1γ and anti–TIF-1α antibodies in sera from 8 DM patients before and after treatment. Recombinant TIF-1γ and TIF-1α proteins were coated onto microtiter plates, and antibody binding in the serum samples obtained at the first visit (before) and when patients underwent treatment and the disease was inactive (after) was evaluated by enzyme-linked immunosorbent assay. Solid circles indicate DM patients with malignancy; open circles indicate DM patients without malignancy; solid lines indicate adult patients with DM; dashed line indicates a patient with juvenile DM. OD = optical density (see Figure 1 for other definitions).

not shown). In contrast, the titer of anti–TIF-1α antibodies decreased in 6 patients after treatment, while 2 patients showed a slight increase in antibody titer (Figure 4).

DISCUSSION

In the current study, we have confirmed that the 155-kd protein recognized by anti-155/140 antibodies is TIF-1γ, and we have demonstrated that the 140-kd antigen is TIF-1α. Moreover, a portion of the patients also had autoantibodies directed to TIF-1β. Therefore, the TIF-1 family of proteins is targeted by anti-155/140 antibodies. Also, it was formally confirmed that anti-p155 and anti-155/140 antibodies both react with TIF-1γ and thus are indeed the same. This study also assessed the largest number of anti–TIF-1α/β/γ–positive patients (n = 78) to date and demonstrated that 65% of adult patients had cancer. It is also noteworthy that anti–TIF-1α/β/γ antibodies underlie a distinct subset of "young adult DM" without malignancy, in addition to juvenile DM and adult malignancy-associated DM.

While a few non-DM patients have been documented to have anti–TIF-1α and/or anti–TIF-1γ (anti–TIF-1α/γ) antibodies, including 1 patient with SLE (14)

and 1 patient with PM (24), anti–TIF-1α/γ antibodies are considered highly specific for DM. Anti–TIF-1α/γ antibodies have been detected in 18–23% of the adult DM patients in the US and European populations (14,22,24). In the current study, the prevalence of anti–TIF-1α/γ antibodies was 16%. This was higher than in our previous study, in which we observed anti–TIF-1α/γ positivity to be 7% (25 of 376) (21). This is mainly because our previous study only included patients having autoantibodies reactive with both TIF-1α and TIF-1γ. Nonetheless, the positivity may still be slightly lower than that reported in Caucasian populations. This may be due to ethnic differences since, for example, anti-CADM140 antibodies appear more frequent in Asian populations than in Caucasian populations (11,25,26).

The association of anti–TIF-1α/γ antibodies with cancer has been described in a number of reports. The incidence of cancer in anti–TIF-1α/γ–positive patients with adult DM is 42–75% (14,15,21,22,24,27). On the other hand, positivity for anti–TIF-1α/γ antibodies in cancer-associated DM is 43–75%. Another subset in which anti–TIF-1α/γ antibodies are frequently detected is juvenile DM. The frequency of anti–TIF-1α/γ antibodies in juvenile DM is 23–29% (14,23). The current study also confirmed that anti–TIF-1α/γ antibodies are frequently present in juvenile DM in a Japanese population. Moreover, use of a large population in our study had the merit of detecting a "young adult" population of DM patients who were positive for these antibodies, in addition to patients with juvenile DM, although this finding was not statistically significant due to the small number. These patients were age <40 years, female, and predominantly categorized as having clinically amyopathic DM. None of these patients had a history of malignancy. Nonetheless, further accumulation and followup of cases are needed to clarify whether these patients are at risk of malignancy, and whether this finding is also true across racial groups.

This report is the first to describe the presence of autoantibodies against TIF-1β in DM. A protein array analysis revealed that anti–TIF-1β antibodies were detected in 8 of 43 patients with colorectal cancer and 1 of 40 controls without cancer (28). In our study, 7 DM patients were positive for anti–TIF-1β antibodies. Among them, 6 were also positive for anti–TIF-1α/γ antibodies, while 1 was positive for anti–TIF-1β antibodies alone. Two patients were classified as having cancer-associated DM (ovarian cancer), while 2 other patients were classified as having "young adult" DM without cancer. No patients with juvenile DM were positive for anti–TIF-1β antibodies. Nonetheless, this

finding may be due to a relatively small number of patients with juvenile DM.

The TIF-1 family, a subgroup of the tripartite motif–containing (TRIM) proteins, consists of at least 3 members: TIF-1α (TRIM24), TIF-1β (KAP1, TRIM28), and TIF-1γ (TRIM33). Additionally, TIF-1δ has been identified in mice (29), while its function remains relatively unknown. Studies have revealed intriguing roles of TIF-1 proteins in carcinogenesis. TIF-1α ubiquitinates the tumor suppressor gene p53 (30) and also activates estrogen-dependent genes associated with cellular proliferation and tumor development (31). The depletion of TIF-1α expression in human breast cancer cells causes spontaneous apoptosis (30), and aberrant overexpression of TIF-1α in breast cancer patients is frequent and correlates with poor survival (31). In contrast, in liver, TIF-1α is shown to act as a functional tumor suppressor gene by inhibiting the retinoic acid pathway in mice (32). TIF-1β has an antiapoptotic effect by inhibiting p53 acetylation and promoting p53 ubiquitination (33), and is overexpressed in gastric cancer (34). TIF-1γ, which appears to contribute to transforming growth factor β signaling, exerts a protective role in pancreatic carcinogenesis in mice by cooperating with $Kras^{G12D}$ (35).

Collectively, these findings demonstrate that TIF-1 proteins play pivotal positive and/or negative roles in carcinogenesis, suggesting the possibility that the autoantibodies to these proteins develop during antitumor immune responses that contribute to the development of cancer-associated DM. Indeed, Casciola-Rosen and colleagues have demonstrated that myositis autoantigen expression is markedly increased in cancers known to be associated with myositis but not in their related normal tissues, and have proposed that autoimmune response directed against cancer cross-reacts with regenerating muscle cells, enabling a feed-forward loop of tissue damage and antigen selection in cancer-associated myositis (36). With regard to the close relationship of TIF-1 proteins with p53, it has been well appreciated that autoantibodies to p53 are detected in patients with a wide variety of cancers (37). The close association of TRIM proteins with interferon (IFN)–mediated immunity is also noteworthy (38,39). Large numbers of TRIM proteins are up-regulated by IFN, and some are also reported to regulate IFN expression in turn. Since IFN is implicated in the pathogenesis of DM, it is intriguing to hypothesize that TIF-1 proteins serve as a bridge between cancer and IFN-mediated immunity.

In summary, the current study revealed that anti-155/140 antibodies that are frequently detected in

patients with cancer-associated DM target the TIF-1 family members TIF-1α, TIF-1β, and TIF-1γ. While TIF-1γ is the most commonly recognized antigen, antibodies to TIF-1α are also frequently detected. A small number of patients exhibited reactivity with TIF-1β. Since these TIF-1 proteins are highly homologous, it is plausible to hypothesize that TIF-1γ is the original target and that other antigens are recognized by cross-reactivity, especially based on the observation that most sera had reactivity with TIF-1γ. In immunoprecipitation assays using the sera that were preabsorbed with recombinant TIF-1γ protein, the reactivity with TIF-1α was substantially reduced in many cases (data not shown), supporting this notion that the autoantibodies predominantly target homologous sequences. In contrast, as shown in Figure 4, the directionality of the change in titer of anti–TIF-1α and anti–TIF-1γ antibodies was sometimes discordant, suggesting that not all antibodies are directed to the homologous sequences. Therefore, precise determination of the epitopes will be needed in the future. The TIF-1 family plays pivotal roles in oncogenesis, including p53 regulation, and overexpression of these proteins in tumor tissues has been reported, suggesting that autoantibodies to TIF-1 proteins may result from a misdirected antitumor response. Different reactivity to TIF-1 proteins in individual patients may be dependent on the tissue and/or types of the tumors.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Fujimoto had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Study conception and design. Fujimoto.
Acquisition of data. Fujimoto, Hamaguchi, Kaji, Matsushita, Ichimura, Kodera, Ishiguro, Ueda-Hayakawa, Asano, Ogawa, Fujikawa, Miyagi, Mabuchi, Hirose, Akimoto, Hatta, Tsutsui, Higashi, Igarashi, Seishima, Hasegawa, Takehara.
Analysis and interpretation of data. Fujimoto, Hamaguchi, Kaji, Matsushita, Hasegawa, Takehara.

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Clinical Correlations With Dermatomyositis-Specific Autoantibodies in Adult Japanese Patients With Dermatomyositis

A Multicenter Cross-sectional Study

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Objective: To clarify the association of clinical and prognostic features with dermatomyositis (DM)-specific autoantibodies (Abs) in adult Japanese patients with DM.

Design: Retrospective study.

Setting: Kanazawa University Graduate School of Medical Science Department of Dermatology and collaborating medical centers.

Patients: A total of 376 consecutive adult Japanese patients with DM who visited our hospital or collaborating medical centers between 2003 and 2008.

Main Outcome Measures: Clinical and laboratory characteristics of adult Japanese patients with DM and DM-specific Abs that include Abs against Mi-2, 155/140, and CADM-140.

Results: In patients with DM, anti–Mi-2, anti–155/140, and anti–CADM-140 were detected in 9 (2%), 25 (7%), and 43 (11%), respectively. These DM-specific Abs were mutually exclusive and were detected in none of 34 patients with polymyositis, 326 with systemic sclerosis, and 97 with systemic lupus erythematosus. Anti–Mi-2 was associated with classical DM without interstitial lung disease or malignancy, whereas anti–155/140 was associated with malignancy. Patients with anti–CADM-140 frequently had clinically amyopathic DM and rapidly progressive interstitial lung disease. Cumulative survival rates were more favorable in patients with anti–Mi-2 compared with those with anti–155/140 or anti–CADM-140 ($P<.01$ for both comparisons). Nearly all deaths occurred within 1 year after diagnosis in patients with anti–CADM-140.

Conclusion: Dermatomyositis-specific Abs define clinically distinct subsets and are useful for predicting clinical outcomes in patients with DM.

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P OLYMYOSITIS (PM) AND DERMATOMYOSITIS (DM) represent a group of chronic inflammatory disorders characterized by myogenic changes, skin eruptions, or both. Clinical features are heterogeneous, with various degrees of skin manifestations, myositis, and pulmonary involvement, which

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considerably determine the severity and prognosis.[1] Although the causes of these disorders remain unclear, autoimmunity is considered to have a critical role because the presence of diagnostic autoantibodies (Abs),

known as myositis-related Abs, is a prominent feature.[2] A variety of serum Abs are detected in patients with PM/DM, including Abs reactive with aminoacyltransfer RNA synthetase (ARS),[3] signal recognition particle,[4] and Mi-2.[5] These Abs are associated with clinically distinct subsets of PM/DM, that is, anti-ARS with interstitial lung disease (ILD), arthritis, Raynaud phenomenon, and mechanic hand[6,7]; anti–signal recognition particle with acute-onset severe refractory PM[8-10]; and anti–Mi-2 with typical DM with a lower risk of ILD and internal malignancy and good response to treatment.[11-13] In addition, anti–PM-Scl, anti-Ku, and anti-U1RNP Abs are associated with myositis overlap syndrome.[14] Therefore, identification of myositis-related Abs is use-

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ful in defining clinically homogenous patient subsets, in predicting prognosis, and in clarifying the pathogenesis. Recently, 2 myositis-related Abs, anti–155/140[15,16] and anti–CADM-140,[17] have been reported. Subsequent studies have revealed corresponding autoantigens: transcriptional intermediary factor 1-γ for anti–155/140[18] and melanoma differentiation–associated gene 5 for anti–CADM-140.[19] Anti–155/140 Abs are reported to represent malignancy-associated or juvenile DM,[15,20] and anti–CADM-140 Abs are reported to be associated with amyopathic DM and rapidly progressive ILD (RP-ILD).[17,19] Although precise clinical details of these 2 Abs need to be clarified further, anti–155/140, anti–CADM-140, and anti–Mi-2 Abs are considered to be highly specific for DM.

To our knowledge, this is the first large comprehensive study that includes all currently available myositis-related Abs intended for a variety of adult Japanese patients with DM. We focused particularly on anti–Mi-2, anti–155/140, and anti–CADM-140 and attempted to investigate a correlation between these 3 DM-specific Abs and clinical features and prognosis in detail.

METHODS

PATIENTS AND SERUM SAMPLES

Serum samples were obtained from 376 adult Japanese patients with DM who were observed in the Department of Dermatology, Kanazawa University, Kanazawa, Japan, and collaborating medical centers between January 1, 2003, and December 31, 2008. Of the 376 patients with DM, 325 fulfilled the criteria of Bohan and Peter.[21,22] The remaining 51 patients fulfilled the criteria of Sontheimer[23] because of the absence of clinical muscle symptoms and the presence of subsistent clinical DM skin eruptions. Clinically amyopathic DM included patients with amyopathic DM and patients with hypomyopathic DM. Patients with hypomyopathic DM had DM rash and subclinical evidence of myositis on electrophysiologic, radiologic, or laboratory evaluation.[24] Thirteen patients (5 with anti–155/140 Abs and 8 with anti–CADM-140 Abs) who were observed at Nagasaki University[25] were included in this study. As controls, serum samples from 34 patients with PM, 326 with systemic sclerosis, and 97 with systemic lupus erythematosus who were observed during the same period were also assessed. The diagnosis of PM was based on the criteria of Bohan and Peter.[21,22] All the patients with systemic lupus erythematosus or systemic sclerosis fulfilled the American Rheumatism Association criteria.[26,27] A PM/DM overlap was diagnosed by the coexistence of systemic lupus erythematosus or systemic sclerosis in addition to PM or DM.

Clinical information was collected retrospectively from all the patients by reviewing their clinical medical records. Initial symptoms were defined as clinical presentation at the first clinic visit. Muscle involvement in an initial symptom included clinical signs of muscle disease or abnormality evaluated using electrophysiologic, radiologic, or laboratory tests. The patients were diagnosed as having ILD according to the results of chest radiography, chest computed tomography, and pulmonary function tests, which included the percentage predicted values for forced vital capacity and diffusing capacity for carbon monoxide. A subset of patients with RP-ILD was defined as those with progressive dyspnea and progressive hypoxemia and a worsening of interstitial changes on the chest radiograph within 1 month from the onset of respiratory symptoms. Malignancy that included internal and hematologic malignancy in patients with

DM was defined using criteria described previously.[11] No patient with DM had a history of malignant disease. The protocol was approved by the Kanazawa University Graduate School of Medical Science and Kanazawa University Hospital.

IMMUNOPRECIPITATION

Immunoprecipitation (IP) assays were performed using extracts of the leukemia cell line K562.[17] A total of 10 μL of the patient's serum was bound to 2 mg of protein A–Sepharose beads (Amersham Biosciences, Piscataway, New Jersey) in 500 μL of IP buffer (10mM Tris hydrochloride, pH 8.0; 50mM sodium chloride; and 0.1% Nonidet P-40 [Caledon Laboratories Ltd, Georgetown, Ontario, Canada]) and was incubated for 2 hours at 4°C and then washed 5 times with IP buffer. Autoantibody-coated Sepharose beads were mixed with 100 μL of ^{35}S-methionine–labeled K562 cell extracts derived from 10^6 cells and rotated at 4°C for 2 hours. After 5 washes, the beads were resuspended in sodium dodecyl sulfate sample buffer, and the polypeptides were fractionated by 7.5% sodium dodecyl sulfate–polyacrylamide gel electrophoresis followed by autoradiography. Anti–Mi-2 immunoprecipitated polypeptides of 200 to 240, 150, and 65 to 75 kDa, and anti–155/140 immunoprecipitated 155- and 140-kDa proteins. Anti–Mi-2, anti–155/140, and anti–CADM-140 were considered positive if serum samples produced precipitin lines with immunologic identity to reference sera.[13,15,17]

IDENTIFICATION OF ANTI–CADM-140

The presence of anti–CADM-140 was confirmed in serum samples that immunoprecipitated a protein with a molecular weight of 140 kDa by IP assay by immunoblots and enzyme-linked immunosorbent assay using recombinant melanoma differentiation–associated gene 5 as an antigen.[19] This procedure aimed to exclude several other Abs, such as anti–NXP-2 (previously termed MJ),[28] that target a protein of approximately 140 kDa.

STATISTICAL ANALYSIS

The Fisher exact probability test was used for comparison of frequencies, and 1-factor analysis of variance was used for multiple comparisons. $P < .05$ was considered statistically significant. All data are reported as mean (SD).

RESULTS

DISEASE SPECIFICITY OF THE MYOSITIS-RELATED Abs

Figure 1 shows representative results of an IP assay. A total of 47 serum samples from patients with DM immunoprecipitated a protein with a molecular weight of approximately 140 kDa. Of these samples, 43 (91%) were reactive with melanoma differentiation–associated gene 5 by immunoblots and enzyme-linked immunosorbent assay, confirming the presence of anti–CADM-140. The frequencies of myositis-related Abs in patients with PM, DM, systemic sclerosis, and systemic lupus erythematosus are summarized in **Table 1**. Anti–Mi-2 antibodies were found in 2% of serum samples from patients with DM, anti–155/140 in 7%, and anti–CADM-140 in 11%, but none of these 3 DM-related Abs was detected in patients with PM or other connective tissue diseases. In addition, they did not coexist. These 3 Abs accounted for 21% of all patients with DM.
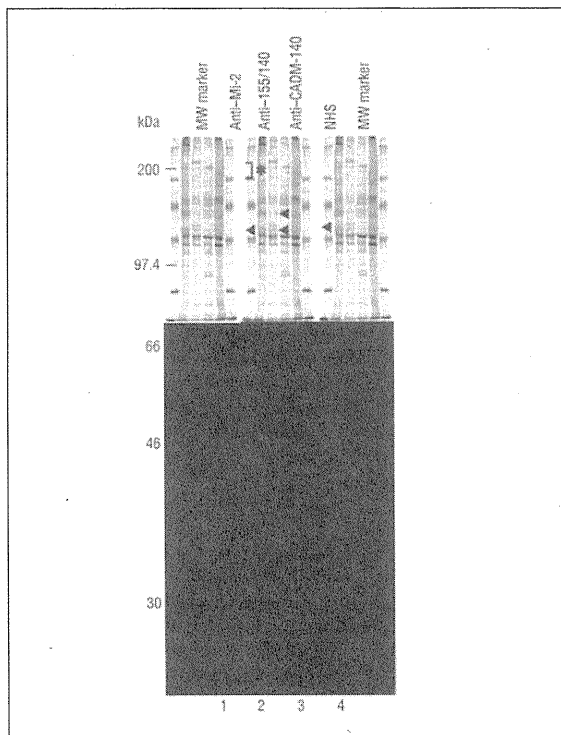
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Figure 1. Immunoprecipitation assay of autoantibodies related to dermatomyositis. Immunoprecipitation of ^{35}S-methionine–labeled K562 cell extracts was performed on serum samples from patients with dermatomyositis (lanes 1-3) and on normal human serum (NHS) (lane 4), separated on 7.5% sodium dodecyl sulfate–polyacrylamide gel electrophoresis, and analyzed by autoradiography. The molecular weight (MW) marker lane includes protein bands corresponding to 200, 97.4, 66, 46, and 30 kDa. Arrowheads indicate Mi-2 (lane 1), 155/140 (lane 2), and CADM-140 (lane 3) proteins. *Two hundred– to 240-kDa proteins of Mi-2.

Table 1. Frequency of Myositis-Related Autoantibodies in Patients With Connective Tissue Diseases

	Patients, No. (%)				
Autoantibodies	DM (n=376)	PM (n=34)	PM/DM Overlap (n=21)	SSc (n=326)	SLE (n=97)
Anti–Jo-1	21 (6)	4 (12)	0	1 (0.3)	0
Anti–ARS excluding anti–Jo-1	49 (13)	6 (18)	0	7 (2)	1 (1)
Anti-SRP	7 (2)	2 (6)	0	1 (0.3)	0
Anti–Mi-2	9 (2)	0	0	0	0
Anti–155/140	25 (7)	0	0	0	0
Anti–CADM-140	43 (11)	0	0	0	0
Anti–U1RNP	11 (3)	1 (3)	8 (38)	20 (6)	19 (20)
Anti–PM-Scl	0	0	0	0	0
Anti–Ku	2 (0.5)	3 (9)	6 (29)	3 (1)	0

Abbreviations: anti-ARS, anti–aminoacyltransfer RNA synthetase; anti-SRP, anti–signal recognition particle; DM, dermatomyositis; PM, polymyositis; SLE, systemic lupus erythematosus; SSc, systemic sclerosis.

Table 2. Malignancy and Interstitial Lung Disease in Patients With DM Without Autoantibodies to Mi-2, 155/140, or CADM-140 and Patients With DM With These 3 Autoantibodies[a]

	Patients, No. (%)			
Variable	Anti–Mi-2 Positive (n=9)	Anti–155/140 Positive (n=25)	Anti–CADM-140 Positive (n=43)	Others (n=299)
Interstitial lung disease	1 (11)	3 (12)	40 (93)	105 (35)
Malignancy	0	17 (68)	4 (9)	18 (6)

Abbreviation: DM, dermatomyositis.
[a] $P<.001$ for all.

COMPARISON OF CLINICAL FEATURES AMONG PATIENTS WITH DM WITH DM-SPECIFIC Abs

First, we compared the rates of malignancy and ILD in the 77 patients with the 3 DM-specific Abs and the 299 patients with DM who did not have any of the 3 DM-specific Abs (**Table 2**). Interstitial lung disease was seen most frequently in patients with anti–CADM-140 Abs, and the incidence of malignancy was highest in patients with anti–155/140 Abs ($P<.001$ for all comparisons).

Next, we compared demographic, clinical, and laboratory data in each DM-specific Ab-based subgroup (**Table 3**). Patients with anti–CADM-140 had the lowest prevalence of DM but the highest prevalence of clinically amyopathic DM ($P<.001$ and $P<.001$, respectively, for all comparisons). Regarding initial symptoms, although muscle or muscle and skin involvement is less common in clinically amyopathic DM, the addition of ILD led to a higher prevalence of combined muscle, skin, and lung disease in the anti–CADM-140 subset ($P<.04$ for all comparisons). For clinical features, fever and arthritis were most frequently seen in patients with anti–CADM-140 ($P<.001$ and $P=.02$, respectively, for all comparisons). Patients with anti–CADM-140 had ILD at the highest rates ($P<.001$ for all comparisons), whereas ma-

lignancy was most frequently seen in patients with anti–155/140 ($P<.001$ for all comparisons). Malignancies were observed in 17 of 25 patients with anti–155/140 Abs, and 3 of those had double malignancy: 7 patients with lung cancer, 3 with breast cancer, 2 with colon cancer, 2 with gastric cancer, and a single case of prostate, biliary tract, pancreas, ovarian, and nasopharyngeal cancer and non-Hodgkin lymphoma. Ten of 17 patients with malignancy simultaneously developed DM, and 6 of 17 had malignancy before the development of DM.

Regarding skin eruptions, punctate hemorrhages on the perionychium were most frequently seen in patients with anti–Mi-2 ($P=.04$ for all comparisons). In patients with anti–155/140, punctate hemorrhages on the perionychium were more frequently seen in those without malignancy than in those with malignancy ($P=.007$) (**Table 4**). The frequency of truncal erythema in patients with anti–CADM-140 was lowest among the 3 subgroups ($P=.001$ for all comparisons). On the other hand, patients with anti–CADM-140 had skin ulcers most frequently among the 3 subgroups ($P<.008$ for all comparisons). However, the presence of skin ulcers was not a prognostic marker in patients with anti–CADM-140 (**Table 5**).

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Table 3. Demographic, Clinical, and Laboratory Features in 77 Japanese Patients With DM According to the Presence of 3 DM-Specific Autoantibodies

Variable	Anti–Mi-2 Positive (n=9)	Anti–155/140 Positive (n=25)	Anti–CADM-140 Positive (n=43)	P Value
Age at onset, median (range), y	45 (16-66)	62 (31-79)	53 (15-76)	.005
Sex, M/F, No.	6/3	11/14	9/34	.01
Diagnosis, %				
Classical DM	100	72	23	<.001
Clinically amyopathic DM	0	28	77	<.001
Initial symptom, %				
Skin alone	44	40	35	.80
Muscle alone	11	4	0	.14
Lung alone	0	0	5	.44
Skin and muscle	44	44	12	.005
Skin and lung	0	4	21	.80
Muscle and lung	0	0	0	...
Skin, muscle, and lung	0	8	28	.04
Clinical features, %				
Fever	22	24	74	<.001
Raynaud phenomenon	0	12	28	.12
Muscle weakness	100	72	23	.02
Arthritis	11	8	42	.02
Interstitial lung disease	11	12	93	<.001
Malignancy	0	68	9	<.001
Skin eruptions, %				
Heliotrope rash	67	72	56	.37
Facial erythema other than heliotrope rash	56	88	51	.12
Gottron sign (hand)	89	96	86	.41
Gottron sign (elbow or knee)	56	76	77	.32
Periungual erythema	89	76	72	.67
Punctate hemorrhages on the perionychium	89	44	37	.04
Truncal erythema	89	88	35	<.001
Skin ulcers	0	4	30	.008
Cutaneous calcification	11	0	2	.29
Laboratory findings, mean (SD)				
CK, U/L	5283 (3649)	1364 (2263)	425 (1667)	<.001
LDH, U/L	814 (439)	564 (726)	547 (241)	.38
KL-6, U/mL	378 (1553)	476 (527)	2122 (1923)	<.001

Abbreviations: CK, creatine kinase; DM, dermatomyositis; ellipsis, not applicable; LDH, lactate dehydrogenase.
SI conversion factors: To convert CK and LDH to microkatals per liter, multiply by 0.0167.

Table 4. Clinical and Laboratory Features in 25 Anti–155/140 Antibody–Positive Patients With Dermatomyositis by the Presence or Absence of Malignancy

Variable	Malignancy Present (n=17)	Malignancy Absent (n=8)	P Value
Age at onset, median (range), y	68 (54-79)	49 (31-63)	<.001
Follow-up, mean, y	1.8	3.5	.06
Sex, M/F, No.	10/7	1/7	.04
Clinical features, %			
Muscle involvement[a]	71	100	.14
Arthritis	12	0	>.99
Interstitial lung disease	12	13	>.99
Skin eruptions, %			
Punctate hemorrhages on the perionychium	24	88	.007

[a] Muscle involvement included muscle weakness and subclinical myositis.

Regarding laboratory findings, maximum serum creatine kinase levels were significantly lower in patients with anti–CADM-140 than in those with anti–Mi-2 and anti–155/140 (P<.001 for all comparisons). KL-6 is a mucin-like high-molecular weight glycoprotein that is strongly expressed on type II alveolar pneumocytes and bronchiolar epithelial cells. Serum KL-6 levels are associated with the activity and severity of ILD.[29] Serum KL-6 levels were higher in patients with anti–CADM-140 than in those with anti–Mi-2 and anti–155/140 (P<.001 for all comparisons).

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TREATMENT AND PROGNOSIS IN PATIENTS WITH DM WITH DM-SPECIFIC Abs

The treatment regimens and prognosis of individual patients with anti–Mi-2, anti–155/140, and anti–CADM-140 are summarized in eTable 1, eTable 2, and eTable 3, respectively (available at http://www.archdermatol.com). Although most patients with anti–Mi-2 responded well to the initial therapy, 6 of 9 patients had a recurrence of muscle or skin involvement during follow-up. In 15 of 17 patients (88%) with anti–155/140 who had malignancy, treatment for malignancy did not improve the symptoms of DM. Effective initial and additional treatments in patients with anti–CADM-140 were not elucidated in this study (Table 5).

We assessed the survival rates between disease subsets (**Figure 2**). Overall cumulative survival from the time of DM diagnosis in all 77 patients with DM was 65% at 5 years. Survival in patients with anti–155/140 and those with anti–CADM-140 was 68% and 56% at 5 years, respectively. Both cumulative survival rates were significantly decreased compared with those for patients with anti–Mi-2 ($P=.01$ for both). The cumulative rates were not identical between patients with anti–155/140 and those with anti–CADM-140 because RP-ILD in patients with anti–CADM-140 often developed rapidly in a short period after onset of the disease.

The prognosis of patients with anti–Mi-2 was favorable: no patients had malignancy and only 1 had mild ILD (Table 3). Although 8 patients (32%) with anti–155/140 died mainly from progression of malignancy during follow-up, the prognosis of anti–155/140–positive patients without malignancy was favorable. No significant trend was observed concerning the type of malignancy in anti–155/140–positive patients. Although patients with anti–CADM-140 whose prognosis was poor had significantly increased serum KL-6 levels ($P=.04$), no apparent negative prognostic factors were noted in those with anti–CADM-140 who died (Table 5).

CAUSE OF DEATH

Twenty-seven of 77 patients with DM died during follow-up. Of the 25 patients with anti–155/140, 7 died of malignancy and 1 died of bacterial pneumonia. Of the 43 anti–CADM-140–positive patients, 16 died of ILD and 1 died of *Pneumocystis jiroveci* pneumonia. One anti–CADM-140–positive patient died of disseminated intravascular coagulation. Thus, the major cause of death in patients with DM was associated with DM-related internal organ involvement. This is consistent with previous reports that malignancy and ILD are the major causes of death in patients with DM.

COMMENT

In this study, we compared clinical features and prognosis in adult Japanese patients with DM based on their DM-specific Abs. This study includes 3 major findings. First, to our knowledge, this is the first study to investigate the association of clinical features with 3 DM-specific Abs in adult Japanese patients with DM on a large scale. Second, the 3 DM-specific Abs are mutually exclusive and do not coexist. Third, each of these DM-specific Abs defines a clinically distinct phenotype and may work as a predictor of clinical complications and

Table 5. Clinical and Laboratory Features in 43 Anti–CADM-140 Autoantibody–Positive Patients With Dermatomyositis by Prognosis

Variable	Dead (n=19)	Alive (n=24)	P Value
Age at onset, median (range), y	58 (30-76)	50 (15-74)	.06
Sex, M/F, No.	4/15	5/19	>.99
Clinical features, %			
Fever	74	75	>.99
Muscle involvement[a]	26	67	.01
Arthritis	32	50	.34
Interstitial lung disease	100	88	.24
Pneumomediastinum	32	13	.26
Type of ILD, %			
Rapidly progressive	89	54	.02
Classical	11	33	.14
Skin eruptions, %			
Heliotrope rash	42	63	.35
Facial erythema other than heliotrope rash	32	67	.06
Punctate hemorrhages on the perionychium	26	46	.19
Truncal erythema	16	46	.09
Skin ulcers	16	42	.10
Laboratory findings, mean (SD)			
KL-6, U/mL	2656 (1989)	1703 (1803)	.04
Initial treatment, %			
Prednisolone only	26	58	.06
Prednisolone and methylprednisolone pulse	63	29	.13
Additional treatment, %			
Methylprednisolone pulse	74	50	.06
Cyclophosphamide	53	42	.55
Cyclosporine	79	54	.12

Abbreviation: ILD, interstitial lung disease.
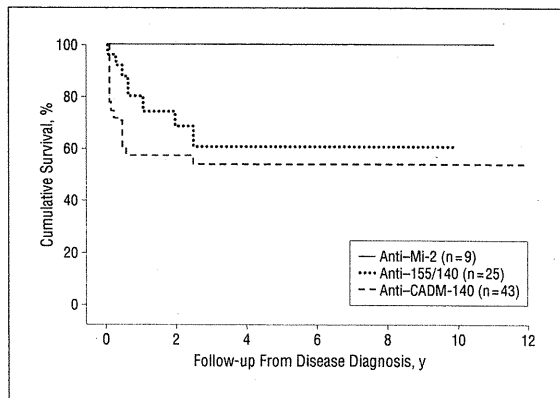[a] Muscle involvement included muscle weakness and subclinical myositis.

Figure 2. Cumulative survival rates from the time of diagnosis in 77 Japanese patients with dermatomyositis with serum anti–Mi-2, anti–155/140, and anti–CADM-140 autoantibodies. Cumulative survival rates were compared using log-rank tests.

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prognosis. Thus, classifying patients with DM based on their serum Ab profiles seems to be beneficial for focusing on their clinical features.

In an association of myositis-related Abs with the connective tissue diseases, PM/DM overlap was associated with anti-U1RNP and anti-Ku, and Abs detected in PM were predominantly anti-ARS and anti–signal recognition particle, as previously reported.[4,30,31] We also confirmed that anti–Mi-2, anti–155/140, and anti–CADM-140 were specific to DM in adult Japanese connective tissue diseases, and this finding was consistent with previous studies[5,16,20,30] assessing other ethnic groups.

Clinical characteristics of patients with anti–Mi-2 in this study were generally consistent with previous studies that anti–Mi-2 is associated with typical cutaneous lesions and mild to moderate muscle involvement and responds well to corticosteroid treatment.[5,11-13] However, recurrence of skin or muscle involvement might not be as rare as expected. Thus, although the overall prognosis is favorable, it is important to keep in mind that intractable myositis and rashes can occur, and careful observation is needed for monitoring flare-ups of the disease.

In this study, malignancy preceded DM in 6 patients, and the 2 conditions were simultaneously diagnosed in 10 patients. Malignancy was found after the diagnosis of DM in only 1 patient. This contrasts somewhat with previous studies[32,33] reporting that the diagnosis of DM is made before the development of malignancy in at least half of patients. Regarding patients with anti–155/140 Abs, Chinoy et al[34] reported that malignancy preceded the onset of DM in only 1 of 8 patients. Two patients had DM and malignancies at the same time. The remaining 5 patients developed malignancies shortly after the diagnosis of DM. Although we currently cannot explain why the discrepancy that the diagnosis of malignancy preceded the onset of DM in a large portion of patients in our study, the discrepancy may have resulted from the timing of screening for malignancy because the interval between detection of the 2 conditions was short in most cases. Also, the discrepancy about the association of the diagnoses of malignancy with the onset of DM may be affected by center-based bias in collecting samples. Alternatively, ethnicity might account for the present data. More studies are required to confirm the relationship between the onset of DM and the development of malignancy.

Previous studies[15,16,20] have described at least 2 different subsets in patients with anti–155/140: adult malignancy-associated DM and juvenile DM. Although anti–155/140 was associated with malignancy, 32% of patients (8 of 25) did not have malignancy in this study. Although most patients with malignancy were elderly and male, clinical features were generally similar between patients with malignancy and those without except for punctate hemorrhages on the perionychium. In addition, clinical features in anti–155/140–positive adult patients without malignancy were similar to those seen in juvenile patients in that they had more extensive skin involvement, such as Gottron papules, over a wider distribution.[16,20] It is unclear why transcriptional intermediary factor 1-γ is common as a major autoantigen in these 2 groups (adult malignancy and juvenile). Gunawardena

et al[30] proposed the possibility that some perturbation of transcriptional intermediary factor 1-γ in proliferating cells combined with a more efficient anticancer response by a younger immune system may be important. Of interest is that patients with malignancy had a lower frequency of punctate hemorrhages on the perionychium compared with those without malignancy. Peripheral circulatory disturbances, including vasculopathy and microcirculation injury, are considered to be a hallmark of autoimmune connective tissue diseases. The lower frequency of punctate hemorrhages on the perionychium in patients with malignancy might be explained by the different mechanisms in developing skin and by muscle involvement between those with malignancy and those without, although both groups had the same Abs against transcriptional intermediary factor 1-γ. Further investigation is needed to reveal the pathogenesis of these 2 subsets.

Interstitial lung disease is a crucial complication for patients with DM because the prognosis of ILD in DM varies. The severity, clinical course, and prognosis of ILD in DM vary, and ethnicity seems to affect the clinical presentation. In the United States, the frequency of ILD in patients with clinically amyopathic DM is low, and the prognosis is favorable if they do not have a malignancy.[35-37] On the other hand, patients developing RP-ILD have been frequently reported not only in Japanese individuals but also in Chinese individuals and those of other Asian ethnicity. For example, Lee et al[38] reported 2 cases of idiopathic inflammatory myopathy with diffuse alveolar damage. Ye et al[39] also reported that 21 of 28 patients with clinically amyopathic DM had ILD, and, even in classical DM, 50% of patients had ILD. The Asian population might be sensitive to lung damage accompanied by genetically susceptible factors because severe acute respiratory syndrome caused by a coronavirus prevailed predominantly in eastern Asia.[40]

It is considered that the Abs present are associated with a type of ILD. Interstitial lung disease in anti-ARS–positive patients is characterized by the chronic course of the disease and elevation of the diaphragm.[41] Detection of anti–CADM-140 is extremely important because patients with anti–CADM-140 can frequently develop RP-ILD. Therefore, predictors of poor prognosis in this subgroup are needed. Skin ulcers, arthralgia or arthritis, lower arterial PO_2, and higher lactate dehydrogenase levels are considered to be risk factors for poor prognosis.[39] It is also reported that anti–CADM-140–positive patients with RP-ILD have rashes typically seen in DM.[24] In other studies,[42,43] spontaneous pneumomediastinum or pneumothorax is a severe complication and may indicate poor prognosis. However, the clinical phenotype was otherwise similar between patients with a poor prognosis and those with a favorable prognosis in this study.

To establish the treatment for RP-ILD is another urgent issue that needs a solution. Cyclophosphamide and cyclosporine are recommended in the early phase of the disease.[41,44] In contrast, Lee et al[38] reported that 4 patients with RP-ILD received 1 course of intravenous cyclophosphamide therapy and additional cytotoxic agents, such as azathioprine, cyclosporine, and methotrexate, but none responded. In this study, we could not elucidate definite predictors of poor prognosis and recommended

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treatment. Thus, it might be required to attempt the maximum possible combination of immunosuppressive treatments when patients with anti–CADM-140 present signs of developing RP-ILD.

In conclusion, classifying patients with DM according to their DM-specific Abs may guide the physician to focus on particular manifestations with high risk during follow-up of individual patients. However, the detection of DM-specific Abs is limited only to certain facilities because it requires a complicated technique. Establishment of a system to screen DM-specific Abs, such as an enzyme-linked immunosorbent assay, is needed. We acknowledge several limitations of this study. First, it included a relatively small number of patients with PM as a control because most enrolling institutions were dermatology departments. Second, we did not include juvenile patients with DM and other juvenile patients with connective tissue diseases. Third, anti–NXP-2 Abs were not included in this study because they are extremely rare in Japanese patients with DM. In addition, most of the facilities enrolled in this study were referral centers. Therefore, the possibility of center-based bias in collecting samples cannot be ruled out. More studies are needed for a better general understanding of patients with DM-specific Abs.

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RHEUMATOLOGY

Original article

Association between nail-fold capillary findings and disease activity in dermatomyositis

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Abstract

Objective. Although findings of nail-fold capillary changes and reduced red blood cell velocity in SSc patients are well established, studies in adult-onset DM patients are scarce. Our objective was to assess the changes and red blood cell velocity in finger nail-fold capillaries using nail-fold video capillaroscopy (NVC) in patients with adult-onset DM.

Methods. This study included 50 patients with adult-onset DM and 20 healthy subjects. A semi-quantitative rating scale was used to score capillaroscopy changes. Red blood cell velocity was evaluated using frame-to-frame determination of the position of capillary plasma gaps.

Results. Thirty-seven (74%) patients showed the scleroderma NVC pattern. Patients with the scleroderma pattern exhibited elevated serum creatine kinase levels more frequently and increased visual analogue scale of muscle disease activity. Scores of loss of capillaries were associated with muscle and global disease activity, whereas scores of haemorrhages were associated with skin disease activity. However, NVC findings were not significantly associated with lung involvement. The scores of irregularly enlarged capillaries, haemorrhages and loss of capillaries were reduced after stabilization of disease activity by treatment. The mean red blood cell velocity was not significantly reduced in DM patients compared with healthy controls and was not changed by treatment.

Conclusion. Our results suggest that changes in nail-fold capillaries reflect disease activity in DM. Furthermore, the differences found in red blood cell velocity may reflect somewhat distinct microcirculation injuries in DM and SSc.

Key words: Capillaroscopy, Dermatomyositis, Disease activity, Red blood cell velocity, Systemic sclerosis.

Introduction

DM is an autoimmune connective tissue disease in which characteristic patterns of inflammatory injury occur in striated muscle. PM is a similar disease, but DM can be distinguished from PM via the presence of cutaneous features such as heliotrope rash or Gottron's papule/sign. Interstitial pneumonia and internal malignancy are

examples of organ complications that affect the prognosis of DM. Pulmonary involvement is frequently seen in patients with anti-aminoacyl tRNA synthetase (ARS) antibodies [1] or anti-clinically amyopathic DM (CADM)-140 antibody [2]. In contrast, a recent study has reported that autoantibodies reactive with 155 kDa (and 140 kDa) nuclear proteins (anti-p155 antibody/anti-155/140 antibody) are associated with malignancy in patients with DM [3, 4]. DM patients with anti-Mi-2 antibodies typically present with relatively mild symptoms and have a good prognosis [5].

A vast amount of well-documented data exist regarding nail-fold capillaroscopy and nail-fold video capillaroscopy (NVC) in the diagnosis and follow-up of microvascular damage in patients with RP or scleroderma spectrum disorder (SSD), SSc and its related diseases [6–11]. Furthermore, NVC changes are just as prevalent and prominent in DM as in SSD [12, 13]. However, such findings

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are not frequently detected in patients with other connective tissue diseases. Video recordings of blood capillary flow also allow measurement of capillary red blood cell velocity. We recently reported that red blood cell velocity is remarkably reduced in SSc patients compared with healthy individuals [14]. Additionally, reduced peripheral blood flow has been demonstrated using laser-Doppler perfusion imaging [15, 16].

Despite numerous reports regarding NVC findings in SSc, there are relatively few NVC examinations in DM, and this is especially true for adult-onset DM [17–20] compared with JDM [21–23]. In addition, the clinical relevance of NVC findings in DM has not yet been established, as there are inconsistencies among reports, and quantitative analyses have not yet been adequately assessed. Furthermore, red blood cell velocity has not been evaluated, except for our previous report of a small cohort [14] of patients with DM. In this study, we assessed changes in nail-fold capillaroscopy and red blood cell velocity in patients with DM.

Materials and methods

Patients and clinical assessment

Fifty Japanese patients with DM [39 females and 11 males; mean (s.d.) age, 54.7 (14.8) years] who visited Kanazawa University Hospital between 7 October 2007 and 31 July 2010 were included in this study. Forty patients fulfilled the criteria of Bohan and Peter [24, 25], while the remaining 10 did not fulfil the criteria, but fulfilled Sontheimer's criteria [26], due to the absence of clinical muscle symptoms and presence of subsistent clinical skin eruptions. No patients met the criteria for other rheumatic diseases such as SSc. Mean disease duration was 3.9 (5.1) years. At the time of evaluation, 72% of the patients were already receiving oral predonisolone (PSL) therapy and 26% of the patients were treated with immunosuppressive drugs, including CYC, CSA and tacrolimus, in addition to PSL. A total of 20 healthy subjects [15 females and 5 males; mean (s.d.) age, 51.4 (17.2) years] were also evaluated.

Complete medical histories, physical examinations and laboratory tests were conducted for all patients during the first visit, with limited evaluations during follow-up visits. The patients were diagnosed as having interstitial lung disease (ILD) according to the results of chest radiography, chest CT and pulmonary function testing. Serum Krebs von den Lungen-6 (KL-6) levels as a serum marker of ILD were determined by ELISA as described previously [27]. Presence of internal malignancy was carefully examined using CT, gastrointestinal fibrescope, gallium scintigraphy and other procedures according to need. The local ethics committee (Kanazawa University Hospital, Ishikawa, Japan) approved this study protocol. Informed consent was obtained from each patient.

Immunoprecipitation assays were performed to identify autoantibodies using extracts of the leukaemia cell line K562, as previously described [2]. Using this method, 25.5% of patients were positive for anti-ARS antibody,

18.6% were positive for anti-155/140 antibody and 9.3% were positive for anti-Mi-2 antibody. Anti-SS-A antibody was detected in two patients and anti-NOR 90 antibody, anti-SRP antibody, anti-U1 RNP antibody and anti-Wa antibody were detected in one patient each.

To assess disease activity based on individual organ systems, the Myositis Disease Activity Assessment VAS (MYOACT) portion of the Myositis Disease Activity Tool [28] was used. The MYOACT assessment utilizes separate 100-mm visual analogue scales (VASs) to gauge the physician's evaluation of disease activity in several discrete domains. Involvement of all non-muscle organ systems (constitutional, cardiac, pulmonary, gastrointestinal, skeletal and cutaneous) was also evaluated using the composite extra-skeletal muscle VAS score. An additional VAS measure, the global VAS score, was used to rate overall disease activity.

NVC pattern classification

We assessed NVC findings using a video capillaroscopy system (CP-1000; Chunichi Denshi, Nagoya, Japan), as previously reported [14]. Classification of NVC patterns was performed according to the criteria of Maricq and colleagues [29, 30]. Diagnostic capillaroscopy patterns were grouped into the following categories, as previously described [31]: (i) normal pattern: homogeneous capillary distribution in the nail-fold plexus without capillary loss (normal medium density: linear 30 capillaries per 5 mm) and no morphological alterations; (ii) scleroderma pattern (defined according to Maricq and colleagues [29, 30], with modifications according to Bergman *et al.* [32]): two or more of the following abnormalities: enlarged capillaries, haemorrhages (more than two punctate haemorrhages per finger, or confluent haemorrhage areas), disorganization of the normal capillary distribution, moderate or extensive capillary loss (i.e. avascular areas) and tortuous, crossed and/or ramified capillaries; and (iii) non-specific pattern: lack of complete scleroderma pattern criteria. Since it was often difficult to distinguish between the normal and non-specific patterns, these patterns were combined into a normal/non-specific group in this study.

The scleroderma patterns were further classified as previously reported [33], as follows: (i) early NVC pattern: few enlarged/giant capillaries, few capillary haemorrhages, relatively well-preserved capillary distribution and no evident loss of capillaries; (ii) active NVC pattern: frequent giant capillaries, frequent capillary haemorrhages, moderate loss of capillaries with some avascular areas, mild disorganization of the capillary architecture and absent or ramified capillaries; and (iii) late NVC pattern: irregular capillary enlargement, few or absent giant capillaries, absence of haemorrhages, severe loss of capillaries with large avascular areas, severe disorganization of the normal capillary array and frequent ramified/bushy capillaries.

Scoring of NVC findings

A semi-quantitative rating scale to score six capillary parameters (irregularly enlarged capillaries, giant capillaries, haemorrhages, loss of capillaries, disorganization of the

vascular array and capillary ramifications) was adopted (0 = no changes, 1 = <33% capillary alterations/reduction, 2 = 33-66% capillary alterations/reduction, 3 = >66% capillary alterations/reduction, per linear mm) according to previous studies [11].

Measurement of red blood cell velocity

We measured red blood cell velocity using the video capillaroscopy system, as previously reported [34]. Mean blood cell velocity was calculated by averaging the results from three capillaries, excluding giant or ramified capillaries, in each ring finger. The examination was performed blindly by the same operator, without knowledge of patients' clinical conditions or characteristics. Red blood cell speed in nail-fold capillaries was evaluated by frame-by-frame analysis of video data, as described previously [35]: displacement of the edge of the plasma gap was measured on a frame-to-frame basis as it moved in the capillary limb. The velocity was then calculated by relating this displacement to the framing rate (fixed at 30 frames/s) [35].

Statistical analysis

Statistical analyses were performed using JMP® 7.01 Statistical Discovery Software (SAS institute, Cary, NC, USA). Chi-squared test and t-test were used for comparing the frequency and the mean value, respectively. Spearman's rank correlation coefficient was used to examine the relationship between two continuous variables. Bonferroni correction was performed for multiple comparisons. A $P < 0.05$ after Bonferroni correction was considered indicative of statistical significance. All data are shown as mean (s.d.), unless otherwise indicated.

Results

NVC pattern classification

Among patients with DM, 74% displayed the NVC scleroderma pattern. Among these patients, the early NVC pattern was observed in 12 (24.0%) patients, the active NVC pattern was noted in 23 (46.0%) patients and the late NVC pattern was recognized in 2 (4.0%) patients. None of the 20 normal control subjects demonstrated a scleroderma pattern.

Association between scleroderma pattern and clinical/laboratory findings

We examined the association between NVC changes and clinical or laboratory features. Patients with the scleroderma pattern had shorter disease duration than patients with the normal NVC pattern, but the difference was not significant (Table 1). The frequencies of muscle weakness, Gottron's sign and heliotrope rash were higher in patients with the scleroderma pattern than in patients with the normal NVC pattern, but these differences were not significant. In addition, patients with the scleroderma pattern displayed internal malignancies more frequently than patients without the pattern, although this difference was not significant. The presence or duration of RP was not

TABLE 1 Association between scleroderma pattern and clinical or laboratory findings

Clinical or laboratory finding	Scleroderma pattern (n = 37)	Normal/ non-specific pattern (n = 13)	P-value
Age, mean (s.d.), years	55.9 (14.5)	51.3 (16.0)	1.0
Sex (male : female)	6 : 31	5 : 8	0.30
Disease duration, mean (s.d.), months	37.0 (62.0)	75.8 (50.7)	0.14
Symptoms, %			
Muscle weakness	48.6	30.8	1.0
Gottron's sign	54.1	38.5	0.30
Heliotrope rash	24.3	7.7	0.60
ILDs	43.2	53.8	1.0
Internal malignancy	24.3	7.7	0.22
RP	21.6	15.4	1.0
Laboratory findings, %			
Elevated CK	40.5	0	0.0061**
Elevated KL-6	37.8	61.5	0.14
Autoantibodies, %			
Anti-ARS antibody	29.7	15.4	0.93
Anti-155/140 antibody	24.3	0	0.15
Anti-Mi-2 antibody	5.4	15.4	0.75
Medications, %			
PSL	62.2	100	0.090
CYC	2.7	0	1.0
CSA	8.1	53.8	0.090
Tacrolimus	2.7	0	1.0
MTX	0	7.7	1.0
IVIG	2.7	15.4	1.0

$**P < 0.01$.

associated with scleroderma pattern. Examination of laboratory findings revealed that patients with the scleroderma pattern displayed elevated serum creatine kinase (CK) levels more frequently than patients without the scleroderma pattern ($P < 0.01$). Although patients with anti-155/140 antibody had the scleroderma pattern more frequently compared with patients without the antibody, the difference was not statistically significant. The frequency of oral PSL and CSA use was lower in patients with the scleroderma pattern than in patients without it, but the difference was not significant. Other than these results, there were no associations between the NVC scleroderma pattern and clinical or laboratory features in patients with DM. Thus, the scleroderma pattern was most associated with elevated serum CK levels in patients with DM.

Association between scleroderma pattern and disease activity

To assess disease activity based on individual organ systems, the MYOACT portion of the Myositis Disease Activity Assessment Tool was used. The VAS scales of muscle disease activity were significantly higher in patients with the scleroderma pattern than in patients

without it (*P* < 0.01, Table 2). We examined six aspects of global extra-skeletal muscle disease activity. The total global extra-skeletal muscle disease activity, constitutional disease activity, cutaneous disease activity and skeletal disease activity were higher in patients with the scleroderma pattern than in patients without it, although these differences were not significant. Pulmonary activity was comparable between patients with the scleroderma pattern and patients with the normal/non-specific pattern. Global disease activity, defined as muscle disease activity merged with global extra-skeletal muscle disease activity, was higher in patients with the scleroderma pattern than in patients without it, but the difference was not significant. Thus, the scleroderma pattern was significantly associated with muscle disease activity.

Association between the score of NVC changes and disease activity scales

Among six capillaroscopic parameters, the score of capillary ramifications was excluded in this analysis, since the number of patients with this abnormality was small (12%). The scores for loss of capillaries were significantly associated with the scales of muscle disease activity (*r* = 0.34; *P* < 0.05) and global disease activity (*r* = 0.37; *P* < 0.05, Table 3). On the other hand, the scores of haemorrhages

were significantly associated with the scales of cutaneous disease activity (*r* = 0.41; *P* < 0.01, Table 3). However, no parameters were associated with pulmonary disease activity. Thus, there are some specific associations between the scores of NVC changes and muscle, cutaneous and global disease activity scales.

Red blood cell velocity

Mean red blood cell velocity was 0.800 (0.164) mm/s in healthy volunteers (Fig. 1). Patients with DM had a reduced blood velocity of 0.663 (0.204) mm/s, which was 82.9% that of healthy controls; however, this difference was not statistically significant. In addition, the blood velocity values were not significantly different between NVC patterns (early, active, late and normal/non-specific). No significant association between blood velocity and clinical features was found in patients with DM (data not shown). Although there are several patients who showed reduced red blood cell velocity, no specific clinical features were detected in these patients. Thus, red blood cell velocity was not significantly changed in patients with DM.

NVC changes during the longitudinal study

Twelve patients who had a first visit due to active DM were followed up until the disease was stabilized by treatment with immunosuppressive agents, a period of time that averaged 9.2 (8.4) months. The profile of these patients is shown in Table 4. The mean scale of global disease activity was 33.3 (16.1) at their first visit, which was significantly reduced to 11.9 (9.4) after stabilization by treatment (*P* < 0.001). Of six capillaroscopic parameters, the score of capillary ramifications was excluded from this analysis, since only one patient had this abnormality. The scores of irregularly enlarged capillaries [1.33 (0.89) →0.17 (0.58) mm/s], haemorrhages [1.83 (1.19)→0.17 (0.39) mm/s] and loss of capillaries [0.58 (0.72)→0.08 (0.29) mm/s] were significantly reduced after stabilization of disease (*P* < 0.01), whereas the scores of giant capillaries [1.92 (0.67)→1.17 (0.39) mm/s] and disorganization of the vascular array [1.17 (1.19)→0.75 (0.87) mm/s] were not significantly changed. Five representative cases are shown in Fig. 2. These pictures demonstrate that irregularly enlarged capillaries, haemorrhages and loss of capillaries are reduced or disappear, and gradually regenerate, after stabilization of disease activity. In contrast, red blood cell

TABLE 2 Association between scleroderma pattern and myositis disease activity scale

Myositis disease activity scale	Scleroderma pattern (*n* = 37)	Normal/ non-specific pattern (*n* = 13)	*P*-value
Muscle disease activity	20.7 (24.7)	4.6 (7.8)	0.0030*
Global extra-skeletal muscle disease activity	13.7 (7.7)	7.9 (6.7)	0.060
Constitutional disease activity	18.2 (17.0)	6.2 (8.7)	0.29
Cutaneous disease activity	37.0 (23.5)	18.2 (17.8)	0.17
Skeletal disease activity	3.5 (9.2)	0.8 (2.8)	1.0
Gastrointestinal disease activity	2.4 (11.6)	2.3 (6.0)	1.0
Pulmonary disease activity	17.8 (24.2)	20.0 (24.5)	1.0
Cardiac disease activity	3.2 (6.7)	0	1.0
Global disease activity	23.9 (16.6)	11.4 (11.9)	0.054

*P < 0.01. Data are shown as mean (s.d.).

TABLE 3 Association between the score of NVC change and disease activity scale

Myositis disease activity scale	Irregularly enlarged capillaries, *r*	Giant capillaries, *r*	Haemorrhages, *r*	Loss of capillaries, *r*	Disorganization of the vascular array, *r*
Muscle disease activity	0.08	0.11	0.21	0.34*	0.28
Cutaneous disease activity	0.29	0.22	0.41**	0.21	0.30
Pulmonary disease activity	0.03	0.16	−0.15	0.003	0.04
Global disease activity	0.15	0.26	0.19	0.37*	0.33

*P < 0.05, ***P* < 0.01.