CASE REPORT

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Multiple lymphadenopathy as an initial sign of extramammary Paget disease

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Conflicts of interest
None declared.

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Summary

Extramammary Paget disease (EMPD) often develops in external genitalia. Paget cells can, however, adopt an invasive phenotype and metastasize to regional lymph nodes and beyond, leading to poor patient outcomes. Based on this clinical observation, multiple lymphadenopathy may represent an initial sign of EMPD. To address the potential significance of multiple lymph node swelling in EMPD, we report two patients with cutaneous primary EMPD who showed multiple lymphadenopathy as an initial sign during the clinical course of the disease as well as tumour metastasis. Significantly, marked lymphatic vessel growth was observed in regional lymph nodes that underwent massive tumour cell invasion. Therefore, nodal lymphangiogenesis may promote tumour cell invasion and metastasis to distant organs, including the lymph nodes, emphasizing the clinical relevance of multiple lymphadenopathy.

Extramammary Paget disease (EMPD) is a rare skin tumour that possibly arises from apocrine ductal cells.[1] Histological features of EMPD represent adenocarcinoma in the skin, and most cases are found as carcinoma *in situ*, indicating a good prognosis. However, invasive Paget disease often develops metastases to regional lymph nodes and/or distant organs, resulting in reduced patient survival. Recently, tumour-associated lymphangiogenesis was found within primary sites of EMPD and other malignant neoplasms such as cutaneous melanoma.[2–4] More importantly, lymphangiogenesis is strongly induced in sentinel and/or regional lymph nodes targeted by metastatic EMPD, suggesting that nodal lymphangiogenesis may facilitate tumour spread via lymphatic vessels. However, the significance of nodal lymphangiogenesis to clinical outcomes is not yet established. We thus report two cases of EMPD in which multiple lymphadenopathy was an initial sign of the disease, one that preceded detection of primary sites in the skin.

Case reports

Patient 1

A 53-year-old man undergoing a routine health check was found to show elevated serum levels of carcinoembryonic antigen (9·6 ng mL^{-1}; normal ≤ 3·4). Positron emission tomography combined with computed tomography (CT) revealed 2-(^{18}F)fluoro-2-deoxy-D-glucose accumulation in abdominal para-aortic and left inguinal lymph nodes (Fig. 1a, arrowheads). A CT image at the femoral joint level revealed swollen lymph nodes in the left inguinal region (Fig. 1b, circled), suggesting the presence of multiple lymph node metastases by a malignant neoplasm. An initial biopsy of a massive inguinal lymph node was obtained. Haematoxylin and eosin (H&E) staining of biopsy material indicated metastatic adenocarcinoma in the lymph node (Fig. 1c). However, general examination failed to identify the exact origin of metastatic tumours. Therefore, an additional examination was performed at a dermatology unit. The patient exhibited a demarcated depigmented macule in the perineum with focal erosions (Fig. 2a), a feature typical of EMPD. The patient was diagnosed with metastatic EMPD, and surgical resection was performed to remove the cutaneous primary lesion and regional lymph nodes. In addition to surgical resection, systemic chemotherapy was initiated with docetaxel combined with trastuzumab, a neutralizing antibody targeting the HER2 receptor, which is expressed by Paget cells (Fig. 2i). However, during treatment the patient developed distant organ metastases, including to liver, and died after surviving 24 months since the initial signs of lymphadenopathy emerged.

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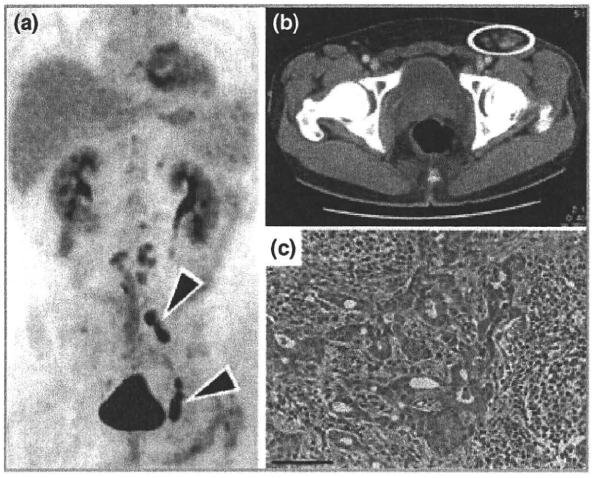

Fig 1. Multiple lymphadenopathies are seen in patient 1 with extramammary Paget disease. (a) A positron emission tomography–computed tomography (CT) image of the abdomen shows 2-(^{18}F)fluoro-2-deoxy-D-glucose accumulation among para-aortic and left inguinal lesions (arrowheads), indicating multiple metastases from the lymph nodes in patient 1. (b) A CT image at the femoral joint level shows multiple swollen lymph nodes in the left inguinal region (circle). (c) Haematoxylin and eosin staining of the inguinal lymph node from biopsy material shows metastatic foci representing adenocarcinoma of unknown origin. Scale bar = 100 μm.

Patient 2

A 49-year-old man was referred for consultation because of general fatigue and right inguinal lymph node swelling, 2 cm in diameter. Initially, malignant lymphoma was suspected due to multiple lymphadenopathy detected by whole-body CT (Fig. 3a–c). Physical examination of the groin revealed well-demarcated, pinkish erythematous plaques with dotty scale and erosion on the scrotum (Fig. 3d). Histological examination of the cutaneous primary lesion revealed invasive EMPD. Pathological analysis identified nodal lymphangiogenesis and tumour cell invasion of lymphatic sinusoids within inguinal lymph nodes. Such multiple lymphadenopathy was determined to be due to lymph node metastases. Although systemic chemotherapy with 5-fluorouracil and cisplatin was administered in addition to trastuzumab treatment, the patient died of tumour progression 39 months after the primary consultation.

Materials and methods

Primary tumours or regional lymph nodes from patients were fixed in buffered formalin or embedded in OCT compound (Sakura Finetek, Torrance, CA, U.S.A.) and snap-frozen. Sections (5 μm) were immunostained as described,[2] using the primary antibodies: NZ-1 for podoplanin, OV-TL 12/30 for

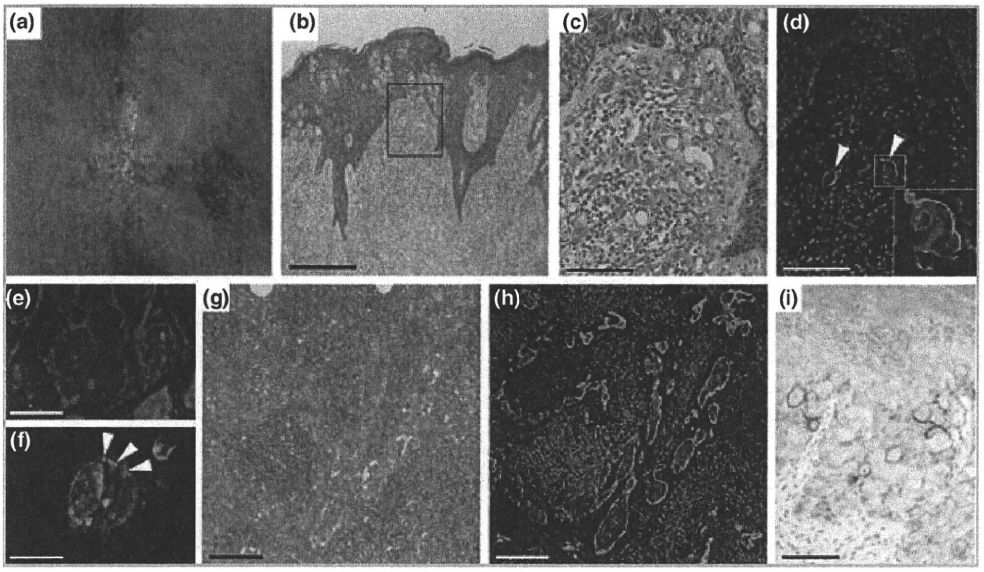

Fig 2. Noninvasive appearance and aggressive features of highly metastatic extramammary Paget disease. (a) Macroscopic appearance of the primary lesion in patient 1. Moderate and demarcated erythema was found with focal erosion in the perineum. (b) Haematoxylin and eosin (H&E) staining of tissue from the primary site shows epidermal hyperplasia with tumour cells spreading within the epidermis in a pagetoid pattern. (c) High-power analysis of boxed region in (b) shows that Paget cells invade the papillary dermis. (d) Double immunofluorescence staining of a serial section shows lymphatic invasion by cytokeratin 7-positive Paget cells (red) within podoplanin-positive tumour-associated lymphatic vessels (green, arrowheads). Inset shows a higher-power magnification of lymphatic invasion. (e, f) Double immunofluorescence for cytokeratin 7 (red) and N-cadherin (green) demonstrates that Paget cells in carcinoma *in situ* do not express N-cadherin (e), whereas invasive Paget cells show N-cadherin positivity on the cell surface (f, arrowheads). (g) H&E staining shows metastatic foci in regional/inguinal lymph nodes obtained from surgical resection. (h) Double staining for cytokeratin 7 (red) and podoplanin (green) indicates marked induction of sinusoidal lymphatic vessel growth, suggesting that Paget cells invade and metastasize via lymphatic vessels. Nuclei are stained blue with 4′,6-diamidino-2-phenylindole. (i) Immunohistochemical staining for HER2 shows that Paget cells express the receptor tyrosine kinase on their cell surface. Scale bars = 500 μm (b); 100 μm (c, d); 10 μm (e, f); 200 μm (g, h), 50 μm (i).

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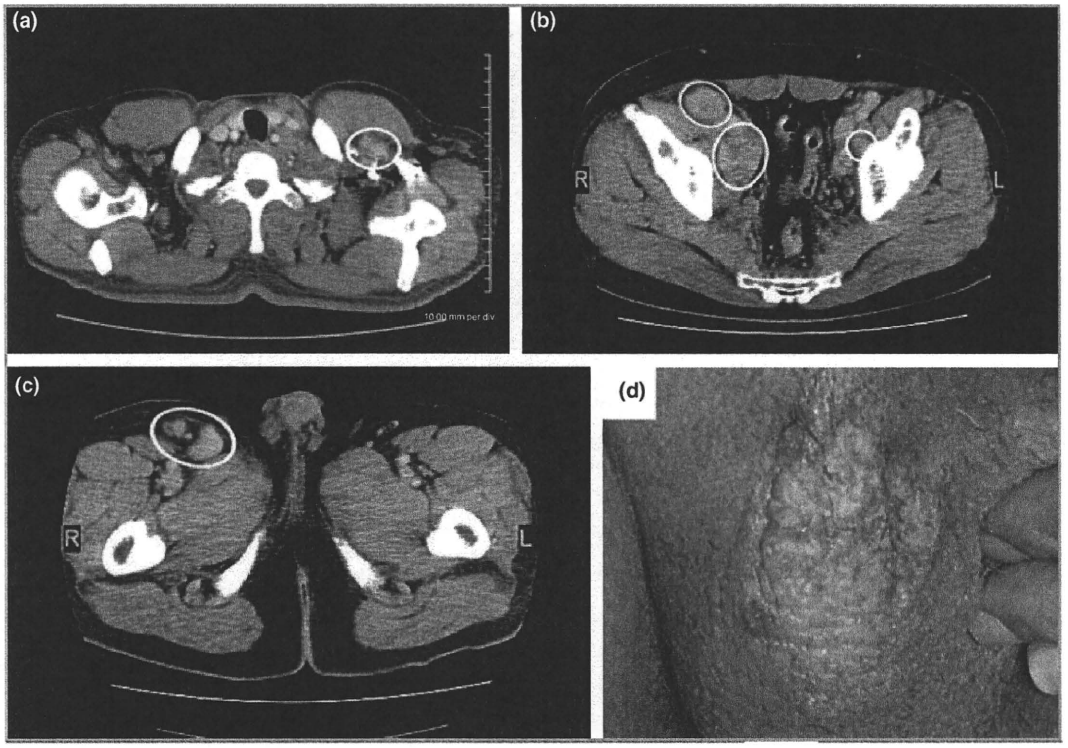

Fig 3. Multiple lymphadenopathy and clinical features in patient 2 with extramammary Paget disease. A whole-body computed tomographic image shows multiple swollen lymph nodes in the left supraclavicular (a), the bilateral external iliac (b) and the right inguinal regions (c), all indicated by circles. (d) Macroscopic appearance of cutaneous primary lesion shows demarcated, erythematous plaques with small erosions on the scrotum.

cytokeratin 7 (Dako, Carpinteria, CA, U.S.A.), 13A9 for N-cadherin (Millipore, Billerica, MA, U.S.A.) and EP1045Y for HER2 (Epitomics, Burlingame, CA, U.S.A.). The respective secondary antibodies were labelled with Alexa Fluor 488 or 594 (Molecular Probes, Eugene, OR, U.S.A.). Antigens were retrieved in paraffin sections by incubation with citrate buffer (pH 6·0 for 30 min at 95 °C) prior to immunostaining. Nuclei were counterstained with 4′,6-diamidino-2-phenylindole (Molecular Probes). Sections were also immunohistochemically stained using a 3-amino-9-ethylcarbazole peroxidase substrate kit (Vector Laboratories, Burlingame, CA, U.S.A.). Respective control IgG was stained as a specificity control. Sections were examined and digital images were captured using a confocal laser scanning microscope (LSM510; Carl Zeiss, Jena, Germany). This study was approved by the institutional review boards of the Graduate Schools of Medicine at Ehime and Osaka Universities.

Results

Low-power microscopic evaluation of H&E-stained specimens from the primary lesion in patient 1 revealed adenocarcinoma within the epidermis (Fig. 2b). Higher-power analysis identified dermal invasion by Paget cells (Fig. 2c). Double immunofluorescence for cytokeratin 7 and podoplanin revealed invasion by Paget cells in the direction of tumour-associated lymphatic vessels within the stroma (Fig. 2d, arrowheads). Furthermore, double staining for cytokeratin 7 and the mesenchymal marker N-cadherin showed that invasive Paget cells expressed cell surface N-cadherin (Fig. 2f, arrowheads), whereas Paget cells seen in carcinoma *in situ* did not express N-cadherin (Fig. 2e). This finding suggests that Paget cells adopt an invasive phenotype during epithelial–mesenchymal transition (EMT) seen in tumour progression. Regional lymph nodes obtained by surgical resection indicated the presence of tumour metastasis (Fig. 2g). Differential staining for cytokeratin 7 and podoplanin revealed marked lymphangiogenesis and massive tumour cell invasion towards sinusoidal lymphatic vessels within lymph nodes (Fig. 2h).

Discussion

EMPD should be considered in differential diagnosis of lymphadenopathy of unknown origin. Multiple lymphadenopathy is a major symptom of patients with invasive EMPD. Cutaneous manifestations of EMPD include erythema and/or demarcated plaques resembling dermatitis or psoriasis.[5] Meanwhile, as a common site affected by EMPD is the external genitalia, clinical diagnosis may be delayed due to the unique location of the disease. Indeed, Paget cells can metastasize to regional and/or distant lymph nodes via lymphatic vessels. Nodal lymphangiogenesis has recently been shown to promote enhanced tumour metastasis, leading to progression of nodal metastasis in EMPD.[2] In this report, we observed marked lymphangio-

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genesis in the sinusoidal lymphatic network, which was strikingly enhanced in metastatic lymph nodes. Therefore, multiple lymphadenopathy may be indicative of nodal lymphangiogenesis in patients with EMPD.

Tumour cell invasion of lymphatic vessels is one of the crucial events that predict decreased patient survival in cutaneous malignant neoplasms such as melanoma.[6–8] Clinically, lymphatic invasion of regional lymph nodes is positively correlated with reduced patient survival in EMPD.[2] In the present cases, metastatic Paget cells showed marked lymphatic invasion within regional lymph nodes. These observations corroborate our recent findings indicating the significance of this activity in relation to development of distant lymph node metastases and reduced patient survival.[2] We found that invasive Paget cells in patient 1 express N-cadherin, a mesenchymal marker indicative of EMT, suggesting a molecular mechanism underlying tumour invasion. EMT is reportedly required for invasive phenotypes of tumour cells of epithelial origin, including EMPD.[2,9] Therefore, EMT is likely to promote aggressive phenotypes characteristic of highly metastatic EMPD.

EMPD is classified as either a primary or secondary disorder. Secondary Paget disease represents a cutaneous extension of an underlying adnexal adenocarcinoma.[1,5] In the present cases, no suspicious internal malignancies were detected by general examination. Therefore, both cases are likely to represent primary EMPD. In fact, EMPD is predominantly of cutaneous primary origin, as compared with a secondary disorder, in Asian countries.

What's already known about this topic?

- Tumour metastasis may occur via lymphatic vessels in patients with extramammary Paget disease (EMPD). Nodal lymphangiogenesis has recently been shown to play a key role in mediating distant lymph node and organ metastases in this disease.

What does this study add?

- EMPD should be considered as a differential diagnosis in patients with multiple lymphadenopathies that precede cutaneous manifestations. Lymphadenopathies may represent nodal lymphangiogenesis and lymph node metastases associated with EMPD.

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CASE REPORT

A case of disseminated DLE complicated by atopic dermatitis and Sjögren's syndrome: link between hypohidrosis and skin manifestations

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Abstract We report an unusual case of disseminated discoid lupus erythematosus (DLE) complicated by pre-existing atopic dermatitis (AD) and late-onset Sjögren's syndrome (SS). Disseminated DLE lesions were sparse on the expected sites for AD, such as the medial region of the extremities or v-neck area. The patient fulfilled the diagnostic criteria for AD and SS but not for systemic lupus erythematosus. Histopathological analysis of the crusted erythematous lesions revealed typical DLE with few $FoxP3^+$ cells and a moderate number of $IL\text{-}17^+$ cells. A quantitative sweating test showed impaired sweating of both lesional and non-lesional skin due to underlying hypohidrosis that was related to AD and SS. This finding suggests that dissemination of DLE was triggered by scratching and a Köbner phenomenon-like effect related to hypohidrotic and xerotic skin. To the best of our knowledge, this is the first reported case of disseminated DLE complicated by AD and SS.

Keywords Discoid lupus erythematosus · Atopic dermatitis · Sjögren's syndrome · Hypohidrosis

Introduction

We present the first report of an unusual case of disseminated (widespread) discoid lupus erythematosus (DLE) complicated by pre-existing atopic dermatitis (AD) and late-onset Sjögren's syndrome (SS). Overlapping cases of collagen diseases are not uncommon in clinical practice; however, AD is very rarely complicated by collagen diseases, possibly due to the predominance of a Th2 response in AD. Dissemination of DLE also is thought to be affected by SS via a Th1-dominant immune-privileged state. Furthermore, dissemination may be due to a Kobner phenomenon-like effect related to xerosis of the skin that is induced by impaired sweating characteristic of AD and SS.

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Case report

A 31-year-old female with disseminated itchy, scaly erythematosus lesions presented to our outpatient clinic. She had been treated with topical glucocorticoids and antihistamines following the diagnosis of refractory AD 10 years earlier. The lesions were restricted to the face, neck, inter-scapular region, and interstitial aspect of extremities. She had no previous history of allergic asthma or pollenosis. Three years before the first consultation, she developed itchy, scaly lesions on the extensor sites of the trunk and extremities. She had not noticed photosensitivity, alopecia, or systemic symptoms such as fever or joint pain except xerostomia with decreased salivary flow. Family and past histories did not reveal any genetic association.

Upon physical examination, pea-sized brownish-violaceous erythematous lesions with a white lamellar scale/crust were observed to be distributed symmetrically over the whole body with extensor predominance (Fig. 1a). Reticular slate-colored mottled pigmentation and slight lichenified eczematous lesions were present on her neck and interstitial aspects of extremities (Fig. 1b, c) where violaceous erythemas were nearly absent. Diffuse erythematous scaly patches with crusted and papular eruptions

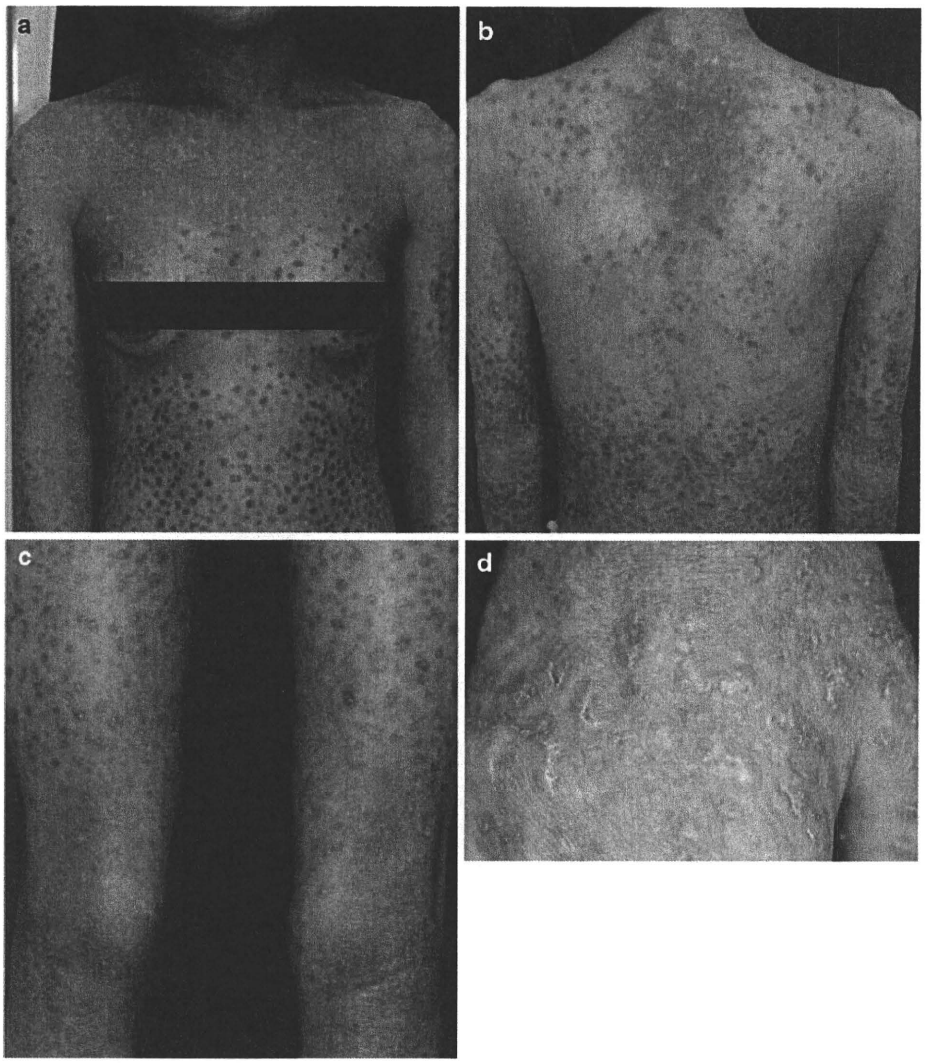

Fig. 1 Clinical appearance of the patient. **a** Pea-sized brownish-violaceous erythema with white lamellar scale/crust distributed symmetrically over the whole body with extensor predominance. **b**, **c** Reticular slate-colored mottled pigmentation and slight lichenified eczematous lesions were present on her neck and interstitial aspects of the extremities where violaceous erythemas were nearly absent. **d** Diffuse erythematous scaly patches with crusted and papular eruptions were observed on her face, scalp, and dorsal aspect of the hands

were observed on the face, scalp, and dorsal aspect of the hands (Fig. 1d). Oral mucosa and the genital regions were devoid of any lesions.

Laboratory findings were as follows: white blood cells: 4.91 × 10^3/μL; neutrophils: 54.5%; lymphocytes: 38.0%; monocytes: 1.1%; eosinophils: 6.1%; basophils: 0.3%; red blood cells: 4.09 × 10^6/μL; platelets: 22.2 × 10^5/μL; hemoglobin: 12.1 g/dL; hematocrit: 35.9%; blood urea nitrogen: 11 mg/dL; creatinine: 0.42 mg/dL; aspartate transaminase: 29 U/L; alanine transaminase: 27 U/L; γ-glutamyl transpeptidase: 16 U/L; LDH: 248 U/L; C-reactive protein: 0.05 mg/dL; immunoglobulin (Ig) G: 3795 mg/dL; IgA: 446 mg/dL; IgM: 169 mg/dL; serum interleukin-2R: 2103 U/ml; IgE: 24,800 IU/mL; Dog-dander-IgE-RAST: >100 IU/mL; dermatophagoides-IgE-RAST: >100 IU/mL; TARC: 1,532 pg/mL; ANAx80 anti-SSA (Ro): 62; anti-SS-B: 11.2; anti-Sm(−), anti-Gal(−)-IgG: 304: logC/mL; anti-CCP: >100 U/mL; anti-dsDNA: 3.9 IU/mL; CH50 (total hemolytic complement): 40.1 U/mL; MMP3: 45.7 ng/mL; rheumatoid factor: 628 IU/ml; urine protein (−).

Histopathological findings of biopsied specimens from the scaled erythematous lesions on the extensor aspect of the forearm revealed hyperkeratotic erythematous change with liquefactive degeneration, follicular plugging, pigment incontinence, and peri-appendageal lymphocytic infiltration compatible with DLE (Fig. 2a). Lymphocytic infiltration around the salivary gland (grade 2) (Fig. 2b) was found in the specimen obtained from a minor salivary gland, with positive salivary scintigraphy findings consistent with SS. Salivary (gum test; 9 ml/10 min) and lacrymal flow [Schirmer test; 5 mm (right)/5 mm (left)] were decreased; however, keratoconjunctivitis was not demonstrated. According to the proposed diagnostic criteria of SS in Japan [1], our patient satisfied two criteria: positive anti-SSA antibody and impaired salivary functions. Immunohistochemical analysis revealed marked infiltration of

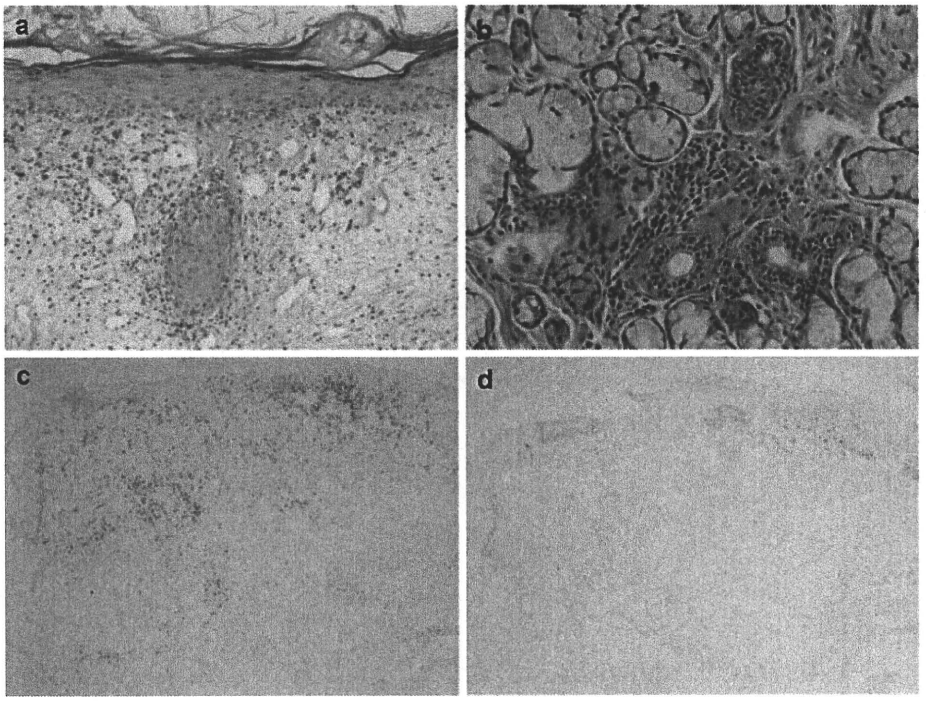

Fig. 2 Histopathological findings. **a** Histopathological findings revealed hyperkeratotic erythematous change with liquefactive degeneration, follicular plugging, pigment incontinence, and peri-appendageal lymphocytic infiltration, all compatible with DLE. **b** Lymphocytic infiltration around the salivary gland was demonstrated in the specimen (obtained from a minor salivary gland), with positive salivary scintigraphy and decreased lacrymal flow, consistent with SS. **c** Immunohistochemical analysis for $CD8^+$ cells, **d** $CD4^+$ cells

$CD8^+$ cells compared to $CD4^+$ cells (Fig. 2c, d), and the number of infiltrating $FoxP3^+$ T-regulatory cells was low (Fig. 3a), which is consistent with chronic LE lesions reported by Kuhn et al. [2, 3]. In contrast, the number of Th17 cells (Fig. 3b) was increased among the infiltrating cells, which is consistent with recent reports of SS [4, 5]. Unfortunately, we could not obtain biopsies of the eczematous lesion of AD. Thus, the patient fulfilled the diagnostic criteria for AD and SS but not for systemic lupus erythematosus (SLE) [1, 6]. From these findings, we made a diagnosis of widespread DLE complicated with AD and SS.

We measured the sweating function in this patient according to a previously described method [7], which revealed that both direct and indirect sweating, induced by iontophoretically applied acetylcholine, were reduced, which is consistent with the pattern seen in SS, as previously reported (Fig. 4) [7].

Discussion

Discoid lupus erythematosus is usually classified as chronic cutaneous lupus erythematosus (CCLE) [8] and rarely complicated by other collagen diseases, with the exception of SLE [9, 10]. The case reported here may be the first documented case of disseminated DLE complicated with SS and AD.

Complications of AD and SLE are relatively, rare and very few case reports have been published [11, 12]

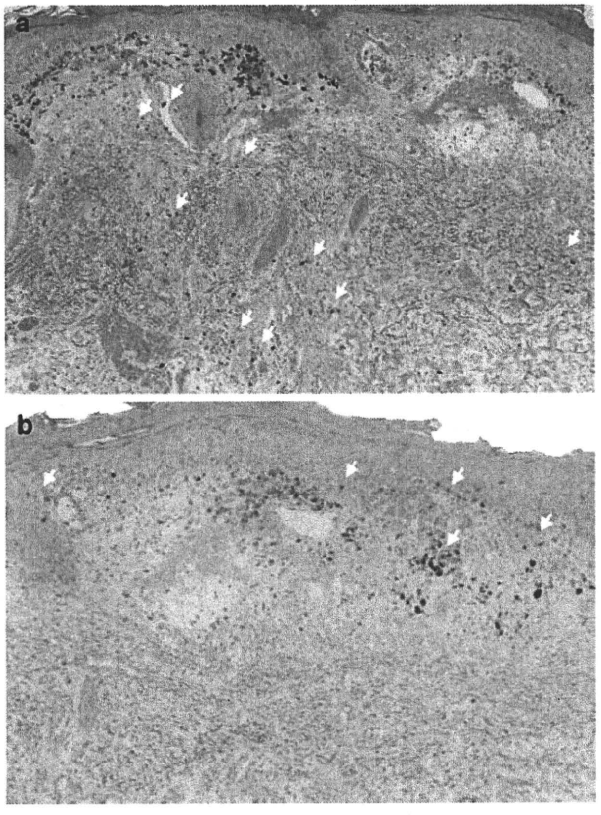

Fig. 3 $FoxP3^+$ cells and interleukin (IL)-17^+ cells in the discoid lupus erythematosus (*DLE*) lesions. **a** Number of Th-17 cells was dominant among the infiltrating cells, **b** number of infiltrating $FoxP3^+$ regulatory cells were decreased

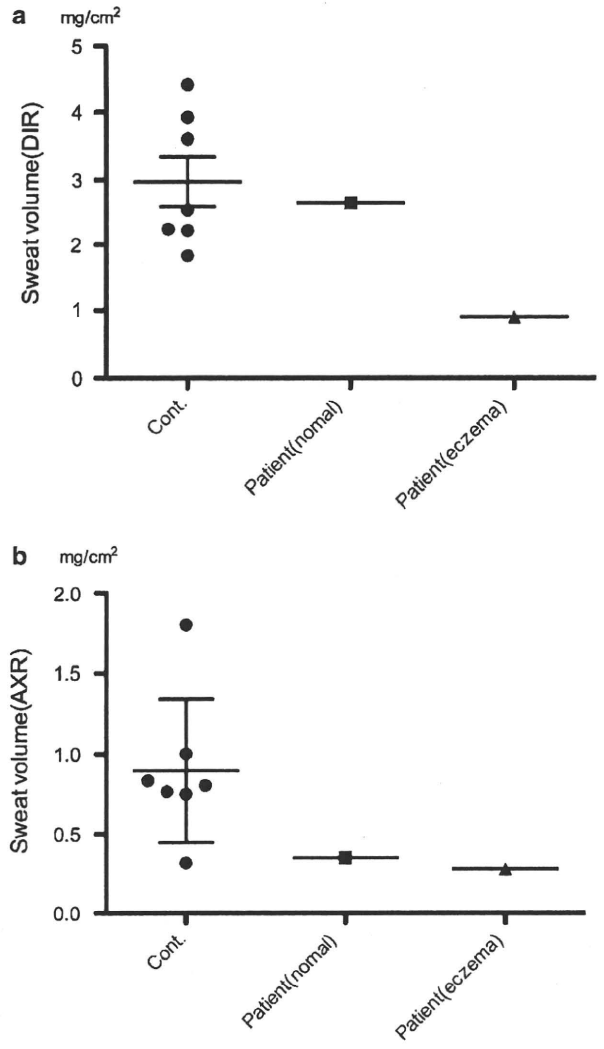

Fig. 4 Quantitative sweating test. The quantitative sweating test was performed according to a previously described method [7]. Both direct and indirect sweating induced by iontophoretically applied acetylcholine were reduced, which is consistent with the pattern seen in Sjögren's syndrome, as previously reported. *DIR* Direct sweat volume, *AXR* axon reflex-mediated indirect sweat volume

although the prevalence of adult AD in Japan is 6.9% [13]. The Th1 and Th2 balance theory or the use of immunosuppressive drugs for SLE has been thought to be responsible for the rare complications of these allergic and systemic autoimmune diseases. Reports of AD and SS as co-morbidities are uncommon in the literature, although SLE is known to occasionally overlap with secondary SS [14].

We previously reported that the sweating function is impaired in patients with AD compared to normal controls as well as in patients with primary SS [7, 15]. In SS, sweating induced by both the direct action of acetylcholine and the axon reflex is impaired, possibly due to eccrine gland dysfunction resulting from autoimmune mechanisms mediated by CD8 T cells [16] or M3 receptor-specific autoantibodies [17], as previously described. In contrast to SS, the reduced sweating function seen in AD is restricted to axon reflex-induced indirect sweating only, which usually is restored to normal levels following improvement of the dermatitis [7]. Therefore, the xerotic skin lesions seen in our patient may have been evoked by AD and SS related-hypohidrosis, which is responsible for the dissemination of DLE.

Interestingly, disseminated DLE lesions were sparse in the areas predisposed to AD, such as antecubital and popliteal fossa or around the neck. The reason for this unique site-specific distribution pattern of DLE is not known at the present time. One possible explanation is the Th1/Th2 balance theory; i.e., AD is known as a typical Th2 cell-mediated allergic skin disease, while SS is considered to be a Th1 or Th17 cell-mediated autoimmune disease [4, 5]. It has been shown that $CD8^+$ T cells are the predominant infiltrating cell type in DLE, as also demonstrated in our case [18]. In support of this explanation is our observation that the number of infiltrating $FoxP3^+$ T cells, which are the counterpart of Th17 cells [2, 4], was reduced in the DLE lesions of our patient (Fig. 3a) compared to AD, respectively (manuscript in preparation).

The patient was treated with topical glucocorticoids and antihistamines after the diagnosis of AD and was subsequently diagnosed with acute dissemination of DLE. Therefore, we may conclude that the patient initially developed AD and underlying SS, which may have aggravated the xerotic eczematous skin lesions due to the sweating dysfunction. Dissemination of DLE is also thought to be affected by SS via a Th1-dominant immunoprivileged state.

Conflict of interest None.

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The skin of patients with systemic sclerosis softened during the treatment with anti-IL-6 receptor antibody tocilizumab

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Abstract

Objective. SSc is an autoimmune disease characterized by fibrosis of the skin and internal organs. Although the aetiology remains uncertain, many reports have suggested that IL-6 is involved in SSc pathogenesis. Tocilizumab, an anti-IL-6 receptor antibody, is an anti-arthritis medicine that works through the blockade of IL-6 functions. To examine the effect of tocilizumab on SSc, we administered tocilizumab to two SSc patients.

Methods. Two dcSSc patients were administered tocilizumab at 8 mg/kg once a month for 6 months. One patient had pulmonary fibrosis assessed by CT and spirometry, and the other had chronic renal failure caused by scleroderma renal crisis. Their skin condition was monitored with a Vesmeter and the modified Rodnan total skin score (mRTSS). Skin biopsies were obtained before and after the tocilizumab treatment to investigate the histological changes.

Results. After tocilizumab treatment, both patients showed softening of the skin with reductions of 50.7 and 55.7% in the total *z*-score of Vesmeter hardness and 51.9 and 23.0% in the mRTSS, respectively. Histological examination showed thinning of the collagen fibre bundles in the dermis. The creatinine clearance in the patient with chronic renal failure improved from 38 to 55 ml/min. However, the fibrotic changes in the lung in the other patient remained unchanged.

Conclusions. In the two cases of SSc that we report here, softening of the skin was observed during the treatment with tocilizumab.

Key words: Systemic sclerosis, Scleroderma, Interleukin-6, Anti-IL-6 receptor antibody, Tocilizumab, Skin score, Vesmeter.

Introduction

SSc is a disease of uncertain aetiology, and is characterized by fibrotic changes in not only the skin but also internal organs. Currently, immunosuppressants, e.g. MTX, are recommended for the treatment of SSc and CYC has also been proved to be effective for interstitial lung diseases and skin thickening [1]. However, the benefits are modest and no highly effective therapy exists. MTX sometimes causes adverse pulmonary reactions. Consequently, it is difficult to use MTX for SSc patients with lung involvement. A new therapeutic method is therefore required to improve the condition of the skin or internal organs that have become harder than normal. To this end, IL-6, one of the pro-inflammatory cytokines, has been implicated in the pathogenesis of SSc. IL-6 expression is reportedly high in both the skin and serum of SSc patients [2], and its elevation depends on the skin score [3]. Tocilizumab, an anti-IL-6 receptor antibody, blocks

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the function of IL-6, and its efficacies for the treatment of RA and Castleman disease have been verified [4]. If IL-6 contributes to the pathological condition of SSc, tocilizumab therapy may be effective for this disease. To clarify the effects of tocilizumab on SSc, we administered tocilizumab to two patients with refractory states of SSc.

Patients and methods

Two patients underwent tocilizumab treatment with the approval of the Ethics Committee of Osaka University Hospital after providing informed consent. The patients met the classification criteria for SSc established by the ARA in 1980 [5]. Tocilizumab was administered at 8 mg/kg every 4 weeks, which is equal to the dosage used for RA. Before this study, the skin condition was evaluated at 17 locations according to the modified Rodnan skin score using a Vesmeter by a single examiner [6]. The modified Rodnan total skin score (mRTSS) was calculated at the same time [7]. Each crude Vesmeter hardness was converted to a *z*-score, which represents the standardized degree of deviation from the normal average, because normal skin hardness varies between body sites. The normal values necessary to calculate *z*-score were referred from our previous study [6]. For identification of histological changes, skin biopsies were obtained from the left forearm before tocilizumab administration. Spirometric evaluation was conducted to assess the restrictive ventilatory impairment as well as lung CT to determine pulmonary fibrosis and an oesophageal radiographic contrast study to evaluate lower oesophageal dilatation. A HAQ for disability index (HAQ-DI) was used to evaluate the activities of daily living [8]. The skin biopsy, spirometry, chest CT and oesophagus radiographic contrast study were performed again after the tocilizumab treatment. Paraffin-embedded biopsy tissues were subjected to haematoxylin and eosin staining as well as immunohistochemical staining using mouse anti-human α-smooth muscle actin (αSMA) antibody 1A4 (DAKO Cytomation, Glostrup, Denmark) to evaluate the number of myofibroblasts using an enzyme-labelled antibody method [9,10]. Briefly, PBS supplemented with 2% BSA was used as a blocking reagent, a 1:50 dilution of the anti-αSMA mAb was used as the primary antibody and a peroxidase-conjugated goat anti-mouse/rabbit immunoglobulin antibody (DAKO Cytomation) was used as the secondary antibody.

Patient 1 was a 42-year-old man who had been suffering from swelling of the fingers and RP since 2005. Treatment with prednisolone at 0.5 mg/kg/day was initiated, but his skin sclerosis continued to spread from his hands to his forearms, upper arms and feet. Serological examinations found neither anti-topo I nor ACAs. ANA was positive with homogeneous and speckled stained patterns. The patient was diagnosed with dcSSc, and because of the severe skin sclerosis (maximum mRTSS: 32), he was treated with ciclosporin at 150 mg/day plus predonisolone at 10 mg/day in 2006. His serum creatinine level suddenly increased from 0.7 to 2.7 mg/dl within 1 month in association with an elevation in blood pressure from 144/84 to 160/104 mmHg in 2007. Laboratory data showed neither fragmented red blood cells nor ANCAs. Daily urine volume was not reduced, but urine analysis showed positive for protein and occult blood. The plasma level of rennin activity in the morning showed 51.9 ng/ml/h (normal: 0.5–2.0 ng/ml/h). Consequently, administration of telmisartan at 80 mg/day was started to suppress his blood pressure in view of a diagnosis of scleroderma renal crisis. Before the first administration of tocilizumab, spirometry and chest CT excluded the presence of pulmonary fibrosis, while a radiographic contrast study of the oesophagus demonstrated lower oesophageal dilatation. The serum creatinine level (1.40 mg/dl) and creatinine clearance (38 ml/min) indicated a decrease in renal function, although proteinuria and urine occult blood were negative when started. The peripheral blood cell count and urinalysis were within normal limits. The CRP level was <0.04 mg/dl (normal: <0.2 mg/dl) and the serum IL-6 concentration was 6.19 pg/ml (normal: <4.0 pg/ml). Administration of prednisolone at 10 mg/day and telmisartan at 80 mg/day was continued. Patient 2 was a 57-year-old woman who had been suffering from RP and swelling of the fingers since 2004. She became aware of dyspnoea on effort and sclerotic changes in the skin of the bilateral hands and forearms as well as the chest. Serological examination was positive for anti-topo I antibodies and ANA with homogeneous and speckled stained patterns. She was diagnosed with SSc and treatment with prednisolone at 10 mg/day was initiated in 2005. However, skin sclerosis spread to her face and upper arms, consistent with diffuse cutaneous disease. It continued to worsen, even though ultraviolet A treatment with 1% psoralen lotion was started in 2007. Owing to the progression of skin sclerosis, the patient was admitted to our hospital. Before initiation of tocilizumab therapy, her peripheral blood cell count, urinalysis and serum creatinine level were confirmed to be within the normal limits. The CRP level was <0.04 mg/dl (normal: <0.2 mg/dl) and the serum IL-6 concentration was 2.77 pg/ml. A CT study revealed patchy infiltrates associated with ground-glass opacities in the bilateral lower lung areas. Spirometry showed that the vital capacity (VC) was 71.6% of the predicted value, forced expiratory volume in 1 s as a percentage of forced VC (FVC) [forced expiratory volume (FEV) 1.0%] was 84.5% and the diffusing capacity for carbon monoxide (DL_{co}) was 35.5%. No lower oesophageal dilatation or retention of contrast agent was observed in a radiographic contrast study. Administration of prednisolone at 10 mg/day was continued.

Results

During the 6-month tocilizumab therapy, both the total *z*-score of Vesmeter hardness and mRTSS decreased (Fig. 1). In Patient 1, the total *z*-score of Vesmeter hardness decreased from 50.1 to 24.7 (50.7% reduction) and mRTSS decreased from 27 to 13 (51.9% reduction). In Patient 2, the total *z*-score of Vesmeter hardness

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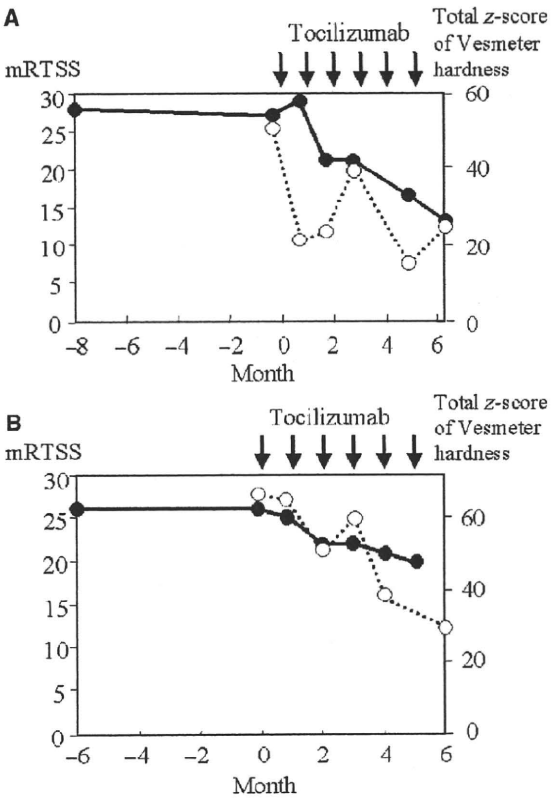
FIG. 1 Clinical courses of Patient 1 (**A**) and Patient 2 (**B**). (○): total *z*-score of Vesmeter hardness; (•): mRTSS.



decreased from 67.5 to 29.9 (55.7% reduction) and mRTSS decreased from 26 to 20 (23% reduction). The decrease in HAQ-DI was observed in both patients as follows: from 0.375 to 0.125 in Patient 1 and from 1.50 to 1.00 in Patient 2. As a result of skin softening, joint mobility also improved. The distance between the palm and third fingertip during forceful gripping was shortened from 17 mm (right) and 13 mm (left) to 0 mm on each side in Patient 1, and from 30 mm (right) and 23 mm (left) to 12 mm and 0 mm, respectively, in Patient 2. The distance between the lips during forceful mouth opening became vertically elongated from 30 mm to 50 mm in Patient 2. Although histological studies did not show any marked changes in the thickness of the dermis, thinning of the collagen fibre bundles in the dermis was observed (Fig. 2A–D). Immunohistochemical staining for αSMA showed positivity in several cells in the dermis and vascular walls, and the number of positive cells in the dermis decreased after the treatment in both patients (Fig. 2E–H). Patient 1 showed kidney involvement, and the serum creatinine level and clearance improved from 1.40 to 1.18 mg/dl and from 38 to 55 ml/min, respectively, during the treatment period, although they had showed only minor changes from 1.31 to 1.40 mg/dl and from 45 to 38 ml/min during the preceding 6 months. Patient 2 did not show kidney involvement and the kidney function did not change during the treatment period. The oesophageal radiographic contrast studies did not show any pronounced changes, although a minor improvement in contraction after the contrast agent had passed was observed in Patient 1. Patient 2 had lung involvement, and re-examination by chest CT showed the same degree of pulmonary fibrosis as in the first examination. Re-examination of the %VC and $\%DL_{CO}$ showed that they remained low at 69.8 and 35.5%, respectively. Adverse reactions were not observed during the periods of this study. Other laboratory data including peripheral blood cell counts, aminotransferases, cholesterols or protein did not show marked change. Patient 1 showed leucocytosis and hypertriglyceridaemia before the study, but they did not show significant change.

Discussion

This is the first report on treating SSc patients with tocilizumab. Although the role of IL-6 in SSc remains unclear, many studies have reported that abnormalities in IL-6 are related to SSc. For example, the culture supernatants of peripheral blood mononuclear cells from patients with SSc were reported to contain higher concentrations of IL-6 than those from normal subjects [11]. Similarly, the culture supernatants of skin tissues from SSc patients were also found to contain elevated IL-6 levels [12]. Furthermore, the serum IL-6 concentrations of SSc patients were reported to be increased and the degree of the increase was dependent on the skin thickness score [2, 3]. Although the patients described here did not show marked elevation of CRP or serum IL-6, overproduction of IL-6 by the affected skin even in the case of SSc patients with normal serum IL-6 levels was reported [12]. It has already been reported that anti-IL-6 antibody suppressed pro-collagen production in fibroblasts from SSc patients [13]. Therefore, tocilizumab, an anti-IL-6 receptor antibody that blocks the function of IL-6, is worth studying for the clinical effect on SSc.

Histological examination revealed changes in the collagen fibre bundles, and immunohistochemical staining showed a decrease of αSMA-positive cells in the dermis. αSMA-positive myofibroblasts have been reported to exhibit abundant production of collagen [9], and their number in the dermis was reported to be correlated with skin hardness [10]. IL-6 may affect the production of extracellular matrix through down-regulation of collagen-producing cells.

The interaction between IL-6 and internal organs has been the subject of discussion [14]. Patient 1 did not show marked change in his oesophageal involvement, and Patient 2 did not show any improvement in her pulmonary fibrosis, although both patients showed an improving tendency for their skin. In this study, the vascular involvement including RP was not evaluated. To clarify these points, the present study needs to be extended to other patients with involvement of internal organs.

The Vesmeter is a new device that can measure the physical properties of skin. We previously reported

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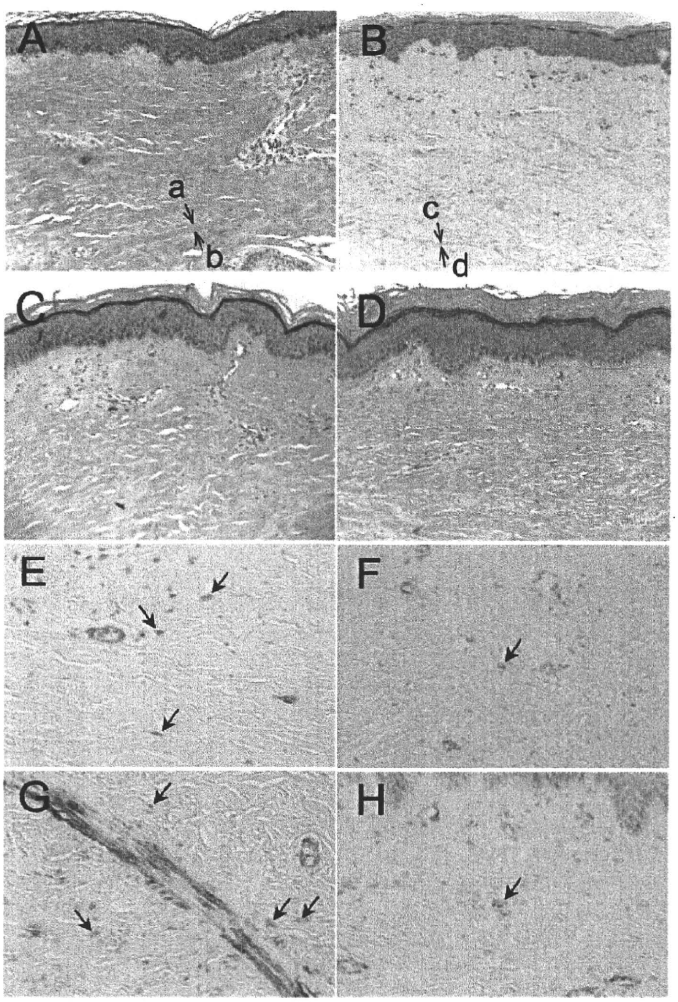
FIG. 2 (**A, B**) Haematoxylin and eosin-stained skin biopsy specimens from the left arm of Patient 1 obtained before tocilizumab therapy (**A**) and after administration of the therapy for 6 months (**B**). Thinning of the collagen fibre bundles in the dermis is observed. A representative example is indicated by the difference between the distances from a to b and c to d. (**C, D**) Haematoxylin and eosin-stained skin biopsy specimens from the left arm of Patient 2 obtained before tocilizumab therapy (**C**) and after administration of the therapy for 6 months (**D**). (Original magnification ×100.) (**E-G**) Immunohistochemical staining with an anti-αSMA antibody of skin biopsy sections obtained from Patient 1 before (**E**) and after (**F**) the tocilizumab therapy, and from Patient 2 before (**G**) and after (**H**) the tocilizumab therapy. αSMA-positive cells outside the vascular wall are indicated by (→). (Original magnification ×200.)



that this device is useful for evaluating the skin condition of patients with SSc [6]. Vesmeter hardness is well correlated with the hardness standards authorized by the American Society for Testing and Materials. We used the Vesmeter for serial evaluations of the skin to assess the drug efficacy, and the results demonstrated that this machine was useful for such studies. As shown in Fig. 1, the total *z*-score of Vesmeter hardness tended to be more highly sensitive than mRTSS. This is the first report of a clinical follow-up study using the Vesmeter.

In this report, we have described two patients with SSc who showed skin softening during the treatment with tocilizumab. Further controlled studies are necessary to properly evaluate its efficacy.

Rheumatology key messages

- The skin of patients with SSc softened during treatment with tocilizumab.
- Vesmeter was useful for assessment of therapy for SSc.

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Disclosure statement: T.K. holds a patent for tocilizumab. A.O. has received an adviser expense as a medical adviser of the subcutaneous injection clinical trial for RA of Chugai Pharmaceutical Co., Ltd from April 2010. All other authors have declared no conflicts of interest.

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CASE REPORT

Pilomatrix carcinoma arising from pilomatricoma after 10-year senescent period: Immunohistochemical analysis

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ABSTRACT

Pilomatrix carcinoma is a rare malignant counterpart of pilomatricoma. To our knowledge, only approximately 90 cases have been published in English literature. Pilomatrix carcinoma is locally aggressive and occasionally shows rapid progression infiltrating to the muscle, bone and vessels. We report a case of pilomatrix carcinoma that developed in a 38-year-old man and started to grow after a long stable period, relapsed for a short time and infiltrated into the muscle underneath. While the initial skin biopsy showed histopathological findings consistent with pilomatricoma, the recurrent tumor contained marked cellular atypia and an aggressive growth pattern. Although it is still controversial whether pilomatrix carcinoma arises *de novo* or through malignant transformation of a pilomatricoma, the present case might be caused by the latter process considering the patient's clinical course. β-catenin is a downstream effecter in the canonical pathway of Wnt, acting as a signal for cell differentiation and proliferation. The characteristic nuclear staining pattern of β-catenin in the basaloid tumor cells, which is usually observed in pilomatrix carcinoma, supported the diagnosis of pilomatrix carcinoma in the present case.

Key words: β-catenin, p53, pilomatrix carcinoma, recurrence, young age.

INTRODUCTION

Pilomatrix carcinoma (PC), also referred to as malignant pilomatrixoma, matrical carcinoma or matrix carcinoma, is an unusual neoplasm, described in 1980 as the malignant variant of pilomatrixoma. To our knowledge, only approximately 90 cases of PC have been reported in English published work and fewer than 10 cases from Japan. PC is locally aggressive and sometimes infiltrates into muscle, bone and vessels. We report here a case of pilomatrix carcinoma in a 38-year-old man which had undergone rapid growth after a long stable period and infiltrated into the muscle underneath.

CASE REPORT

Present history

A 38-year-old Japanese man first visited a dermatological department in November 2007 because of a hard subcutaneous nodule that was 5 cm in diameter on the right posterior neck. An asymptomatic subcutaneous small nodule had appeared on the right posterior aspect of his neck and had not increased in

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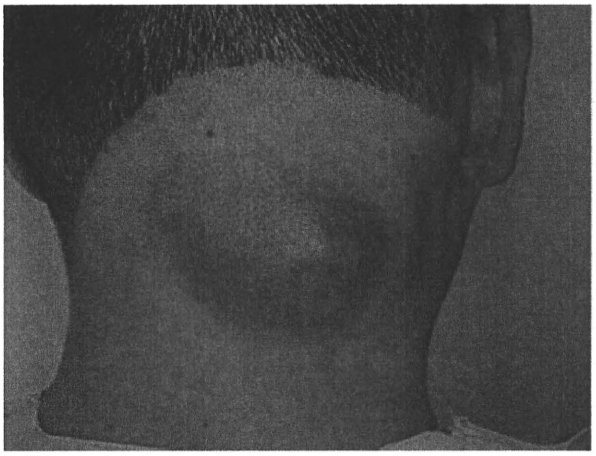

Figure 1. Hard subcutaneous nodule 65 mm × 45 mm in diameter on the right posterior neck.

size for 10 years. It had undergone rapid growth without any trauma 3–4 months before the consultation. Magnetic resonance imaging (MRI) at first visit showed an encapsulated solid mass 32 mm × 59 mm × 44 mm in size and its bottom side appeared to compress the trapezius muscle. Because the patient refused complete resection of the tumor despite our recommendation after an initial biopsy, no additional treatment had been performed. In May 2008, he visited the same hospital again because of local pain and palpable skin elevation over the primary lesion (Fig. 1). We performed a simple section. The histological findings at this time included malignant features of atypical tumor cells, frequent mitosis, infiltrative growth pattern, and necrosis in the peripheral lesions, suggesting PC. The third visit was July 2008. The tumor recurred with muscle invasion visible on MRI examination (Fig. 2). He was introduced to our department for performing a wide resection of the tumor. Computed tomographic imaging revealed no apparent metastatic lesions and all laboratory results were within normal limits at the time of consultation. Therefore, we chose a strategy for whole tumor resection together with the trapezius and sternocleidomastoid muscle. After confirming a complete resection histologically, the skin defect was sequentially reconstructed with meshed split-skin graft. The patient did not have any evidence of local recurrence or metastasis 14 months after the latest surgery without adjuvant chemotherapy or radiation therapy.

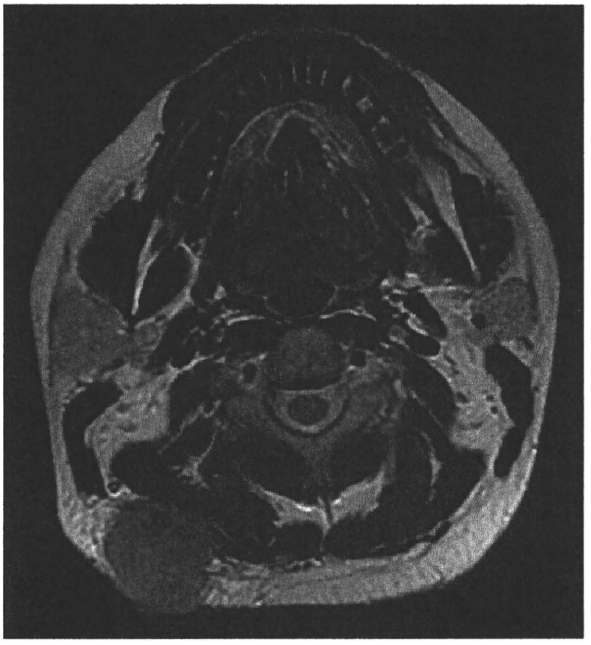

Figure 2. Magnetic resonance imaging with T_2-weighted high-intensity detected a 3.5 cm × 2.8 cm × 3 cm subcutaneous nodule on the right posterior neck. The tumor involved the trapezius muscle underneath.

Histopathology

The first obtained tissue specimen from the primary tumor showed a well-circumscribed nodule in the dermis. The appearance of calcifications and shadow cells suggested a diagnosis of pilomatricoma (Fig. 3a). The second specimen showed a nodular proliferation of basaloid cells from dermis to subcutaneous tissue with poor circumscription. Cystic lesions in the central part of the tumor consisted of transition of metrical cells into shadow cells; on the other hand, peripheral lesions consisted of anaplastic basaloid cells with numerous mitoses. The basophilic cells showed infiltrative growth and transformation into necrotic tissue (Fig. 3b). While the center lesion could be diagnosed as a pilomatricoma, peripheral lesions of the tumor appeared to be a pilomatrix carcinoma. The third specimen obtained from the recurrent tumor 4 months after the second resection intermittently extended and invaded deeply to the muscle layer underneath. Microscopic examination revealed an invasive tumor containing numerous irregular nests composed of basal cell-like atypical cells forming a cobblestone appearance and massive necrosis. Clefts and palisade

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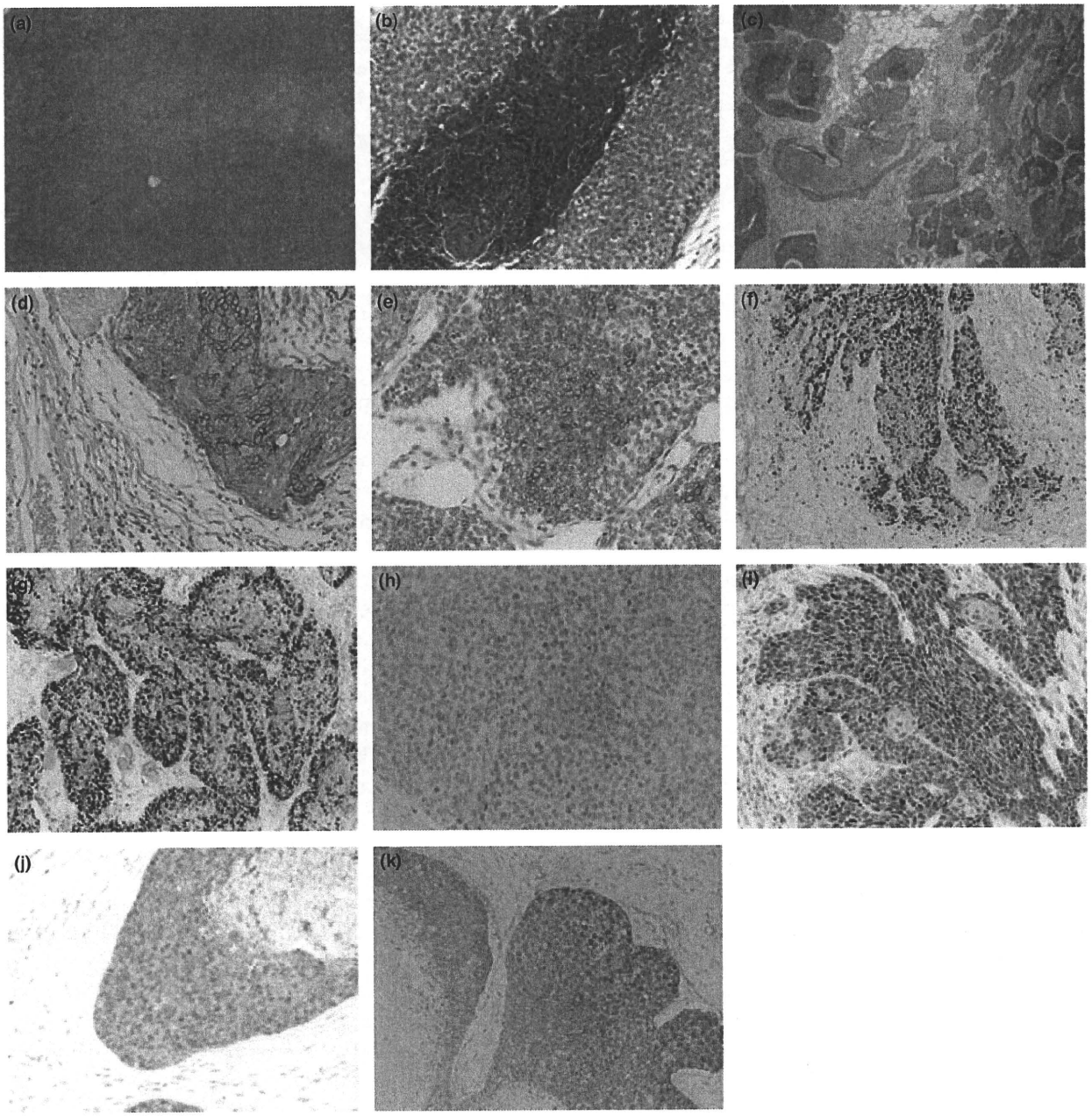

Figure 3. (a) Hematoxylin–eosin (HE) staining of first specimen. Shadow cells were observed. (b) HE staining of second specimen. A necrotic lesion was found in the tumor. (c) HE staining of third specimen. Basal cell-like atypical cells formed islands containing necrosis and infiltrated into the peripheral tissue. Clefts were seen around some of the tumor islands. (d) Hard keratin (AE-13) staining of second specimen. (e) Hard keratin (AE-13) staining of third specimen. Staining for hard keratin was positive in the center of lesions in primary and recurrent tumors. (f) Ki-67 (MIB1) staining of second specimen. (g) Ki-67 (MIB1) staining of third specimen. Stain for Ki-67 indicated basaloid cells to be highly proliferating with >40% of nuclei positive in the peripheral lesion of second and third specimens. (h) p53 protein staining of second specimen. (i) p53 protein staining of third specimen. Staining for p53 was mostly positive in the nuclei of basaloid cells and stronger expression of p53 was observed in the recurrent tumor rather than in the primary one. (j) β-Catenin staining of second specimen. (k) β-Catenin staining of third specimen. Staining for β-catenin was positive in the nuclei and cytoplasm of basaloid cells both in the second and third specimen, reflecting the nuclear translocation of β-catenin.

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Table 1. Notable features of the tumor specimens

	1st	2nd	3rd
Date	November 2007	May 2008	September 2008
Histological diagnosis	Pilomatricoma	Center: pilomatricoma Periphery: pilomatrix carcinoma	Pilomatrix carcinoma
Infiltrative growth	–	+	+
Immunostaining			
Hard keratin	Not decisive	+	
Ki-67	Not decisive	Periphery: >40%	>40%
p53	Not decisive	+	++
β-catenin	Not decisive	NC + CP	NC + CP

NC, nuclear; CP, cytoplasmic.

arrangement were observed around the nests (Fig. 3c). Immunohistochemical staining of second and third resected specimens was performed using antibodies to hard keratin (AE-13), Ki-67 (MIB1), p53 protein and β-catenin (Fig. 3d–k). Staining for hard keratin was positive in the center of lesions in primary and recurrent tumors. Stain for Ki-67 indicated basaloid cells to be highly proliferating with more than 40% of nuclei positive in the peripheral lesion of the second and third specimen. Staining for p53 was mostly positive in the nuclei of basaloid cells and stronger expression of p53 was observed in the recurrent tumor rather than in the primary one. Staining for β-catenin was positive in the nuclei and cytoplasm of basaloid cells both in the second and third specimen, reflecting the nuclear translocation of β-catenin. Notable features of tumor specimens are summarized in Table 1. Immunostaining intensity of p53 is categorized as follows: +, weak staining intensity; and ++, strong staining intensity. The judgment of immunohistochemical classification and categorization, as described above, was evaluated by at least three independent dermatologists.

DISCUSSION

Since pilomatrix carcinoma was initially described by Lopansri and Mihm in 1980,[1] approximately 90 cases of it have been reported. The average age at diagnosis of pilomatrix carcinoma is 60 years and the male : female ratio is 3:1. More than 60% of cases occur on the head and neck region.[2]

Histopathological indicators of malignancy in pilomatrixomas are reported to include asymmetric tumor growth, central necrosis and ulceration on scanning magnification[3] and cytomorphologically atypical and frequent mitoses, nuclear pleomorphism, and infiltration of blood vessels or lymphatics.[4] Because almost all the above histological findings were observed in the second and third specimens of the present case, the diagnosis of pilomatrix carcinoma was considerably made.

It is controversial whether pilomatrix carcinoma arises de novo or through malignant transformation of a pilomatricoma. Not only the present case but also many lesions with diagnosis of pilomatrix carcinoma had been longstanding and suddenly had undergone rapid growth.[5] In addition, some tumors had been histologically diagnosed as benign at first and thereafter underwent carcinomatous alteration at the same site.[6] These previous reports indicate the existence of malignant transformation of pilomatricoma into pilomatrix carcinoma. Thus, we consider that the same transformation might have occurred in the present case.

AE-13 expression in the center may become a rationale to suggest a tumor is matrical. The strong Ki-67 expression in the peripheral portion indicates that the tumor is highly proliferating and tends to invade the surrounding tissues. The fact that there is stronger accumulation of p53 in a recurrent lesion than in a primary lesion is suggestive of turning to higher malignancy in the recurrent tumor.[7] β-Catenin is a downstream effecter in the canonical pathway of Wnt, acting as a signal for cell differentiation and proliferation. Transgenic mice expressing an activated β-catenin localized on the nucleus, developed skin tumors resembling pilomatricoma. Nuclear expression of β-catenin is immunohistochemically observed in pilomatricoma and pilomatrix carcinoma with

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the same intensity.[8–10] It indicates that the staining pattern of the present tumor supports its hair matrix differentiation.

Proliferating pilomatrixoma and basal cell carcinoma with matrical differentiation (basal cell carcinoma (BCC) with matrical differentiation) seem to be histologically important for differential diagnosis. Proliferating pilomatrixoma is usually a symmetrical lesion with an expansive growth pattern, which differs from the asymmetrical infiltrative growth pattern of pilomatrix carcinoma.[11] The invasive growth pattern in the present case differentiates it from proliferating pilomatrixoma. BCC with matrical differentiation is a rare variant of BCC.[12,13] The tumor has typical features of BCC but also has basaloid nests containing shadow cells. Important histological features distinguishing pilomatrix carcinoma from BCC with matrical differentiation are cytological atypia, high mitotic rate and frequent atypical mitoses. Although some clefts and palisade arrangement are present in both, higher degree of nuclear pleomorphism and frequent and atypical mitoses in the basaloid cells preceded diagnosis of pilomatrix carcinoma in the present case. In addition, the predominant nuclear staining of β-catenin observed in basaloid cells of the present case is common in pilomatrix carcinoma, while membranous or cytoplasmatic staining are seen in those in BCC with matrical differentiation.[14,15]

Pilomatrix carcinoma is locally aggressive with a high rate of disease relapse. Also, Autelitano *et al.*[2] reported that pilomatrix carcinoma metastasizes more than described in the previous reports and that the overall probability for metastasis was higher than 10% (10 cases out of 81). Patients need to be observed carefully even after radical resection.

In summary, we report a case of pilomatrix carcinoma which was longstanding for approximately 10 years and had undergone rapid growth. β-Catenin staining may be useful for the precise diagnosis of pilomatrical neoplasms.

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CASE REPORT

Interstitial type granuloma annulare associated with Sjögren's syndrome

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ABSTRACT

We describe a case of granuloma annulare (GA) associated with Sjögren's syndrome (SS) in a 69-year-old woman. She complained of erythematous plaques on the left forearm and neck in addition to dry eyes and mouth. The laboratory and clinical findings also fulfilled the criteria for diagnosis of SS. Histopathological examination revealed the features of interstitial type GA. It is not rare that granulomatous diseases are associated with autoimmune diseases. This case indicated that granulomatous diseases and SS are closely related and that GA should be recognized as a cutaneous manifestation associated with autoimmune diseases, including SS.

Key words: granuloma annulare, histopathology, Sjögren's syndrome.

INTRODUCTION

Sjögren's syndrome (SS) is an autoimmune disease considering its broad organ-specific and systemic manifestations, the most prevalent being diminished lacrimal and salivary gland function, xerostomia, keratoconjunctivitis sicca and parotid gland enlargement. Approximately 60% of SS patients have another autoimmune disorder, such as rheumatoid arthritis, systemic lupus erythematosus (SLE) or systemic sclerosis.[1] While annular erythema – whose clinical features resemble those of granuloma annulare (GA) – is a common cutaneous manifestation of SS, GA is rarely associated with SS.[2] Herein, we report a case of interstitial-type GA associated with SS.

CASE REPORT

A 69-year-old woman presented with a 6-month history of a scaly erythematous plaque on the left forearm and a similar plaque on the neck, which had emerged in the prior 1 month. She also complained of dry eyes and mouth, which had developed during the last year. Physical examination revealed annular erythematous to violaceous plaques on the left forearm and neck (Fig. 1), which were neither painful nor pruriginous. The patient had dry skin but did not have a skin rash, Raynaud's phenomenon or livedo vasculitis. She had hypercholesterolemia, which was being treated with an anti-hyperlipidemic drug for 10 years. Routine laboratory parameters were within the normal ranges, and other laboratory parameters, including C3, C4, CH50, angiotensin 1-converting enzyme and serum lysozyme, were also within normal limits. Abnormal laboratory values were noted for antinuclear antibodies (X160; speckled pattern), anti-Ro/SS-A antibodies (X32), and rheumatoid factor (37 U/mL; normal <15 U/mL). Other autoantibodies, including anti-La/SS-B, anti-double strand DNA, anti-Sm, anti-RNP, anti-Jo-1 and anti-scl-70, were

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