Acquisition of data. Kudo, Nakayama, Inoue.
Analysis and interpretation of data. Kudo, Jinnin, Honda, Kajihara, Makino.

REFERENCES

1. Uitto J, Kouba D. Cytokine modulation of extracellular matrix gene expression: relevance to fibrotic skin diseases. J Dermatol Sci 2000;24:60–9.
2. Trojanowska M, LeRoy EC, Eckes B, Krieg T. Pathogenesis of fibrosis: type I collagen and the skin. J Mol Med (Berl) 1998;76: 266–74.
3. Varga J, Abraham D. Systemic sclerosis: a prototypic multisystem fibrotic disorder. J Clin Invest 2007;117:557–67.
4. Gabrielli A, Avvedimento EV, Krieg T. Scleroderma. N Engl J Med 2009;360:1989–2003.
5. Ihn H, Yamane K, Kubo M, Tamaki K. Blockade of endogenous transforming growth factor β signaling prevents up-regulated collagen synthesis in scleroderma fibroblasts: association with increased expression of transforming growth factor β receptors. Arthritis Rheum 2001;44:474–80.
6. Asano Y, Ihn H, Yamane K, Kubo M, Tamaki K. Impaired Smad7-Smurf-mediated negative regulation of TGF-β signaling in scleroderma fibroblasts. J Clin Invest 2004;113:253–64.
7. Takehara K. Hypothesis: pathogenesis of systemic sclerosis. J Rheumatol 2003;30:755–9.
8. Overbeek MJ, Boonstra A, Voskuyl AE, Vonk MC, Vonk-Noordegraaf A, van Berkel MP, et al. Platelet-derived growth factor receptor-β and epidermal growth factor receptor in pulmonary vasculature of systemic sclerosis-associated pulmonary arterial hypertension versus idiopathic pulmonary arterial hypertension and pulmonary veno-occlusive disease: a case-control study. Arthritis Res Ther 2011;13:R61.
9. Brissett M, Veraldi KL, Pilewski JM, Medsger TA Jr, Feghali-Bostwick CA. Localized expression of tenascin in systemic sclerosis–associated pulmonary fibrosis and its regulation by insulin-like growth factor binding protein 3. Arthritis Rheum 2012;64: 272–80.
10. Kawaguchi Y. IL-1α gene expression and protein production by fibroblasts from patients with systemic sclerosis. Clin Exp Immunol 1994;97:445–50.
11. Feghali CA, Bost KL, Boulware DW, Levy LS. Mechanisms of pathogenesis in scleroderma. I. Overproduction of interleukin 6 by fibroblasts cultured from affected skin sites of patients with scleroderma. J Rheumatol 1992;19:1207–11.
12. Makino T, Fukushima S, Wakasugi S, Ihn H. Decreased serum IL-7 levels in patients with systemic sclerosis. Clin Exp Rheumatol 2009;27:68–9.
13. Blumberg H, Conklin D, Xu WF, Grossmann A, Brender T, Carollo S, et al. Interleukin 20: discovery, receptor identification, and role in epidermal function. Cell 2001;104:9–19.
14. Wegenka UM. IL-20: biological functions mediated through two types of receptor complexes. Cytokine Growth Factor Rev 2010; 21:353–63.
15. Gallagher G, Dickensheets H, Eskdale J, Izotova LS, Mirochnitchenko OV, Peat JD, et al. Cloning, expression and initial characterisation of interleukin-19 (IL-19), a novel homologue of human interleukin-10 (IL-10). Genes Immun 2000;1:442–50.
16. Liao YC, Liang WG, Chen FW, Hsu JH, Yang JJ, Chang MS. IL-19 induces production of IL-6 and TNF-α and results in cell apoptosis through TNF-α. J Immunol 2002;169:4288–97.
17. Jiang H, Lin JJ, Su ZZ, Goldstein NI, Fisher PB. Subtraction hybridization identifies a novel melanoma differentiation associated gene, mda-7, modulated during human melanoma differentiation, growth and progression. Oncogene 1995;11:2477–86.
18. Hsu YH, Li HH, Hsieh MY, Liu MF, Huang KY, Chin LS, et al. Function of interleukin-20 as a proinflammatory molecule in rheumatoid and experimental arthritis. Arthritis Rheum 2006;54: 2722–33.
19. Li HH, Cheng HH, Sun KH, Wei CC, Li CF, Chen WC, et al. Interleukin-20 targets renal mesangial cells and is associated with lupus nephritis. Clin Immunol 2008;129:277–85.
20. Wei CC, Chang MS. A novel transcript of mouse interleukin-20 receptor acts on glomerular mesangial cells as an aggravating factor in lupus nephritis. Genes Immun 2008;9:668–79.
21. Sa SM, Valdez PA, Wu J, Jung K, Zhong F, Hall L, et al. The effects of IL-20 subfamily cytokines on reconstituted human epidermis suggest potential roles in cutaneous innate defense and pathogenic adaptive immunity in psoriasis. J Immunol 2007;178: 2229–40.
22. Subcommittee for Scleroderma Criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee. Preliminary criteria for the classification of systemic sclerosis (scleroderma). Arthritis Rheum 1980;23:581–90.
23. Maricq HR, McGregor AR, Diat F, Smith EA, Maxwell DB, LeRoy EC, et al. Major clinical diagnoses found among patients with Raynaud phenomenon from the general population. J Rheumatol 1990;17:1171–6.
24. Maricq HR, Weinrich MC, Keil JE, Smith EA, Harper FE, Nussbaum AI, et al. Prevalence of scleroderma spectrum disorders in the general population of South Carolina. Arthritis Rheum 1989;32:998–1006.
25. Ihn H, Sato S, Tamaki T, Soma Y, Tsuchida T, Ishibashi Y, et al. Clinical evaluation of scleroderma spectrum disorders using a points system. Arch Dermatol Res 1992;284:391–5.
26. Honda N, Jinnin M, Kira-Etoh T, Makino K, Kajihara I, Makino T, et al. miR-150 down-regulation contributes to the constitutive type I collagen overexpression in scleroderma dermal fibroblasts via the induction of integrin β3. Am J Pathol 2013;182:206–16.
27. Jinnin M, Makino T, Kajihara I, Honda N, Makino K, Ogata A, et al. Serum levels of soluble vascular endothelial growth factor receptor-2 in patients with systemic sclerosis. Br J Dermatol 2010;162:751–8.
28. Noda S, Asano Y, Akamata K, Aozasa N, Taniguchi T, Takahashi T, et al. Constitutive activation of c-Abl/protein kinase C-δ/Fli1 pathway in dermal fibroblasts derived from patients with localized scleroderma. Br J Dermatol 2012;167:1098–105.
29. Asano Y, Trojanowska M. Phosphorylation of Fli1 at threonine 312 by protein kinase C δ promotes its interaction with p300/CREB-binding protein-associated factor and subsequent acetylation in response to transforming growth factor β. Mol Cell Biol 2009;29:1882–94.
30. Ihn H, Ohnishi K, Tamaki T, LeRoy EC, Trojanowska M. Transcriptional regulation of the human α2(I) collagen gene: combined action of upstream stimulatory and inhibitory cis-acting elements. J Biol Chem 1996;271:26717–23.
31. Filippova M, Song H, Connolly JL, Dermody TS, Duerksen-Hughes PJ. The human papillomavirus 16 E6 protein binds to tumor necrosis factor (TNF) R1 and protects cells from TNF-induced apoptosis. J Biol Chem 2002;277:21730–9.
32. Wolk K, Witte E, Warszawska K, Schulze-Tanzil G, Witte K, Philipp S, et al. The Th17 cytokine IL-22 induces IL-20 production in keratinocytes: a novel immunological cascade with potential relevance in psoriasis. Eur J Immunol 2009;39:3570–81.
33. Yamamoto T, Takagawa S, Katayama I, Yamazaki K, Hamazaki Y, Shinkai H, et al. Animal model of sclerotic skin. I. Local injections of bleomycin induce sclerotic skin mimicking scleroderma. J Invest Dermatol 1999;112:456–62.
34. Tanaka C, Fujimoto M, Hamaguchi Y, Sato S, Takehara K, Hasegawa M. Inducible costimulator ligand regulates bleomycin-induced lung and skin fibrosis in a mouse model independently of the inducible costimulator/inducible costimulator ligand pathway. Arthritis Rheum 2010;62:1723–32.
35. Marotta M, Martino G. Sensitive spectrophotometric method for the quantitative estimation of collagen. Anal Biochem 1985;150: 86–90.

36. Czuwara-Ladykowska J, Shirasaki F, Jackers P, Watson DK, Trojanowska M. Fli-1 inhibits collagen type I production in dermal fibroblasts via an Sp1-dependent pathway. J Biol Chem 2001;276: 20839–48.
37. Czuwara-Ladykowska J, Sementchenko VI, Watson DK, Trojanowska M. Ets1 is an effector of the transforming growth factor β (TGF-β) signaling pathway and an antagonist of the profibrotic effects of TGF-β. J Biol Chem 2002;277:20399–408.
38. Jinnin M, Ihn H, Yamane K, Mimura Y, Asano Y, Tamaki K. α2(I) collagen gene regulation by protein kinase C signaling in human dermal fibroblasts. Nucleic Acids Res 2005;33:1337–51.
39. Asano Y, Ihn H, Yamane K, Jinnin M, Mimura Y, Tamaki K. Phosphatidylinositol 3-kinase is involved in α2(I) collagen gene expression in normal and scleroderma fibroblasts. J Immunol 2004;172:7123–35.
40. Morris E, Chrobak I, Bujor A, Hant F, Mummery C, Ten Dijke P, et al. Endoglin promotes TGF-β/Smad1 signaling in scleroderma fibroblasts. J Cell Physiol 2011;226:3340–8.
41. Clements P, Lachenbruch P, Seibold J, White B, Weiner S, Martin R, et al. Inter and intraobserver variability of total skin thickness score (modified Rodnan TSS) in systemic sclerosis. J Rheumatol 1995;22:1281–5.
42. LeRoy EC, Medsger TA Jr. Criteria for the classification of early systemic sclerosis. J Rheumatol 2001;28:1573–6.
43. Hsieh MY, Chen WY, Jiang MJ, Cheng BC, Huang TY, Chang MS. Interleukin-20 promotes angiogenesis in a direct and indirect manner. Genes Immun 2006;7:234–42.
44. Heuze-Vourc'h N, Liu M, Dalwadi H, Baratelli FE, Zhu L, Goodglick L, et al. IL-20, an anti-angiogenic cytokine that inhibits COX-2 expression. Biochem Biophys Res Commun 2005;333: 470–5.
45. Asano Y, Markiewicz M, Kubo M, Szalai G, Watson DK, Trojanowska M. Transcription factor Fli1 regulates collagen fibrillogenesis in mouse skin. Mol Cell Biol 2009;29:425–34.
46. Apras S, Ertenli I, Ozbalkan Z, Kiraz S, Ozturk MA, Haznedaroglu IC, et al. Effects of oral cyclophosphamide and prednisolone therapy on the endothelial functions and clinical findings in patients with early diffuse systemic sclerosis. Arthritis Rheum 2003;48:2256–61.
47. Pope JE, Bellamy N, Seibold JR, Baron M, Ellman M, Carette S, et al. A randomized, controlled trial of methotrexate versus placebo in early diffuse scleroderma. Arthritis Rheum 2001;44: 1351–8.
48. Haustein UF. Systemic sclerosis-scleroderma. Dermatol Online J 2002;8:3.

JDA Japanese Dermatological Association
THE JOURNAL OF DERMATOLOGY
doi: 10.1111/1346-8138.12508
Journal of Dermatology 2014; **41**: 539–541

CONCISE COMMUNICATION

Evaluation of sentinel node biopsy for cutaneous squamous cell carcinoma

Satoshi FUKUSHIMA,[1] Shinichi MASUGUCHI,[1] Toshikatsu IGATA,[1] Miho HARADA,[1] Jun AOI,[1] Azusa MIYASHITA,[1] Satoshi NAKAHARA,[1] Yuji INOUE,[1] Masatoshi JINNIN,[1] Shinya SHIRAISHI,[2] Yasuyuki YAMASHITA,[2] Tsuyoshi ISHIHARA,[1] Hironobu IHN[1]

[1]*Department of Dermatology and Plastic Surgery, Faculty of Life Sciences, and* [2]*Department of Diagnostic Radiology, Kumamoto University, Kumamoto, Japan*

ABSTRACT

Sentinel lymph node biopsy (SLNB) is a standard care for cutaneous melanoma but its role in cutaneous squamous cell carcinoma (SCC) has not been established. Clinical data was obtained from 54 patients with SCC who received SLNB with the usage of blue dye and radioisotope colloid methods. The positive rate of SLNB in SCC was 7.4%. If the cases were limited to more than T2, the positive rate was 12.9%. Three of 41 patients who was estimated negative LN metastasis by the preoperative tests had micrometastasis (7.3%). Among 13 patients who were suggested to have metastasis in the preoperative tests, only one patient had histological metastasis. One patient with SCC located in the lower lip showed negative SLNB and subsequently developed node recurrence. In conclusion, the efficacy of SLNB in SCC is comparable to that of melanoma in the positive rate. There are two kinds of benefit, avoidance of unnecessary complete lymph node dissection and early detection of metastasis.

Key words: false-negative, false-positive, lymph node metastasis, non-melanoma skin cancer, skin surgery.

INTRODUCTION

Sentinel lymph node biopsy (SLNB) is a standard care for melanoma.[1] However, the data on patients with non-melanoma skin cancers (NMSC) are still limited.[2,3] It is no doubt that the patients with NMSC, such as squamous cell carcinoma (SCC) or extramammary Paget's disease (EMP), sometimes show lymph node metastasis. Several cases of SLNB for NMSC have been reported.[4–6] In addition, recently, the effectiveness of SLNB for EMP has been reported.[7–9] However, there has been no report of a large number of case series or a prospective study. It is important to collect the data of SLNB in NMSC and examine its efficacy. To our knowledge, this is the largest number of case series of SLNB for SCC at one institution.

METHODS

Clinical data was obtained from 54 patients with SCC who visited Kumamoto University Hospital between 2006 and 2012 (Table 1). Clinical staging was estimated according to the 7th edition of the American Joint Committee on Cancer Staging Manual. We performed SLNB using the standard technique with blue dye and radioisotope colloid as described previously.[10] Briefly, SLNB was performed with a Techne Phytate kit (FUJIFILM, RI Pharma, Tokyo, Japan) and 2% patent blue violet. Radioisotopes were detected using a Gamma Finder II probe (World of Medicine, Berlin, Germany). We clinicians often experience "false-positive" cases of preoperative tests for SCC. Because SCC is often associated with bacterial infection, and the sentinel lymph node (SLN) is shown as lymphadenopathy. Therefore, we also performed SLNB on the 13 patients in whom the existence of metastasis was "suggested" or "not denied" by the radiologists in the preoperative tests. Institutional review board approval and written informed consent were obtained before the patients were entered into this study, according to the Declaration of Helsinki. Sections of tissue were stained with hematoxylin–eosin and examined by immunohistochemical analysis with the use of antibodies to AE1/AE3. If the nodes were found to contain metastases, complete lymph node dissection (CLND) was performed shortly thereafter. The mean postoperative follow-up period was 33.2 months, with the range 3.2–97.1 months.

RESULTS

Fifty-four patients with SCC received SLNB (Table 1). Four of 54 patients who received SLNB had histological metastasis (7.4%) (Table 2). Three of 41 patients who were estimated as having no lymph node metastasis by the preoperative tests using ultrasonography (US) or positron emission tomography/computed tomography (PET/CT) were found to have the histological metastasis by SLNB (Table 3). All false-negative cases

Correspondence: Satoshi Fukushima, M.D., Ph.D., Department of Dermatology and Plastic Surgery, Faculty of Life Sciences, Kumamoto University, 1-1-1 Honjo, Kumamoto 860-8556, Japan. Email: satoshi.fukushima.tb@gmail.com
Received 31 January 2014; accepted 2 April 2014.

Table 1. Clinical details of patients with squamous cell carcinoma

Age (years)*	69 (20–87)
Sex	
Male	37
Female	17
Primary tumor (T category)	
T1	23
T2	23
T3	3
T4	5
Primary region	
Head and Neck	25
Upper limb	13
Lower limb	12
Trunk	2
Genital area	2

*Median (range).

Table 2. Summary of micrometastasis detected by SLNB and node recurrence after SLNB

Summary of micrometastasis detected by SLNB	
All cases	4/54 (7.4%)
Cases ≥T2	4/31 (12.9%)
Cases ≥T3	1/8 (12.5%)
Summary of node recurrence after negative SLNB	
All cases	1/54 (1.9%)
SCC (head and neck)	1/25 (4.0%)
SCC (head and neck, ≥T2)	1/13 (7.7%)

SCC, squamous cell carcinoma; SLNB, sentinel lymph node biopsy.

were T2. On the other hand, only one of the 13 patients in whom the existence of metastasis was "suggested" or "not denied" had histological metastasis (Table 3). The patient was a 79-year-old man with SCC on the scrotum. PET/CT was not performed. The primary tumor was located slightly left from the midline. Two apparent lymph node metastases of his left inguinal lesion were suggested by US. Therefore, we conducted CLND on the left inguinal lymph nodes. At the same time, we performed SLNB on the right inguinal lesion because radioimmunoassay lymphoscintigraphy indicated only one lymph node located in the right inguinal lesion. We considered that the occlusion of the lymphatic vessels around the metastatic nodes of the left inguinal lesion might have changed the normal lymphatic drainage. Actually, the patient had micrometastasis in his right SLN. Furthermore, one patient showed negative SLNB but subsequently developed node recurrence 3 months after the first surgery (1.9%) (Table 2). The primary tumor was T2 and located in the lower lip. Pathological total resection was achieved at the first operation and no local recurrence was observed in the follow-up period. If the cases were limited to those who have SCC (≥T2) located in the head and neck, the percentage of cases who showed negative SLNB but subsequently developed lymph node recurrence increased up to 7.7% (Table 2).

Table 3. Summary of preoperative tests of 54 cases

Preoperative tests	Metastasis positive	Metastasis negative
Positive	1	12
Negative	3	38

DISCUSSION

In total, the positive rate of SLN metastasis in SCC at our institution was 7.4%. In addition, if the cases were limited to those whose tumor stage was more than T2, the positive rate became 12.9% (Table 2). In our case series, there was no patient with T1 tumor who showed positive SLNB. SLNB is recommended for the patients with intermediate-thickness melanomas (Breslow thickness, 1–4 mm) of any anatomical site; use of SLNB in this population provides accurate staging.[11] Han *et al.* reviewed that SLN metastases were detected in 56 (6.3%) of 891 melanomas of 0.75 mm or more but in only nine (2.5%) of 359 melanomas of less than 0.75 mm. No SLN metastases were detected in melanomas of less than 0.5 mm.[12] In 2011, Kwon *et al.* reported a review of SLNB for high-risk cutaneous SCC. They identified 130 reported cases of SLNB for SCC. The SLNB positivity rate was found to be 14.1%, 10.1% and 18.6%; false-negative rate was 15.4%, 0%, and 22.2%; and the negative predictive value was 97.8%, 100% and 95.2% for all sites, head/neck and truncal/extremity sites, respectively.[2] Recently, a systematic review of SLNB for head and neck cutaneous SCC was reported.[3] Ten patients among 73 had a positive SLN (13.7%). Tumor diameter was not associated with SLN status. We think that the efficacy of SLNB in SCC is comparable to that of melanoma in the positive rate.

In our cases, one patient with SCC showed negative SLNB but subsequently developed node recurrence (Table 2) in the lower lip. Recently, similar cases have been reported.[6] The standard technique using blue dye and radioisotope has established a reliable method for SLNB for melanoma. However, the detection rate of cervical SLN is still lower than that of inguinal or axillary SLN, because of the complexity of lymphatic drainage in the head and neck region and the "shine-through" phenomenon.[13] Nakamura *et al.* recently reported that indocyanine green fluorescence imaging (ICG-FI) could improve detection of cervical SLN.[13] ICG-FI detected SLN more efficiently than the conventional methods, and these "occult" SLN may offer an explanation for some false-negative cases.[5] It is possible that the occlusion of the lymphatic vessels by metastatic tumor cells may change the normal lymphatic drainage. Such a case has been reported.[14] The potential remodeling and functionality of tumor-draining lymphatic vessels has remained unclear. Recently, Proulx *et al.* reported that lymphatic flow could be obstructed by the growth of SLN metastasis using mice models.[15] They revealed that this resulted in rerouting of both lymphatic flow and the spread of tumor cells to alternative lymph nodes.[15] Therefore, development of a non-invasive and more sensitive imaging method for lymphatic drainage is needed to reduce the false-negative rates.

Currently, in the standard care for SCC, CLND is performed when patients show the positive preoperative tests. However, only one patient had histological metastasis among the 13 patients in whom the existence of metastasis was "suggested" or "not denied" by the radiologists in the preoperative tests in our experience (Table 3). This means that these 12 patients were undamaged from the unnecessary CLND. On the other hand, three of 41 patients who were estimated to have negative lymph node metastasis by the preoperative tests were found to have micrometastasis by SLNB. It can be suggested that we could have detected and excised the early metastasis in these three patients.

Whether CLND after positive SLNB improves overall survival is the subject of the ongoing Multicenter Selective Lymphadenectomy Trial II.[11] In addition, it remains unclear whether SLNB has a survival benefit in patients with SCC. A large-scale, multicenter, prospective study on NMSC should be conducted in the future to confirm our results.

ACKNOWLEDGMENTS: This work was supported by JSPS Grant-in-Aid for Young Scientists (B) 25870545 and National Cancer Center Research and Development Fund (23-A-22). We thank Yukie Hirayama for data collection.

CONFLICT OF INTEREST: None declared.

REFERENCES

1 Morton DL. Overview and update of the phase III Multicenter Selective Lymphadenectomy Trials (MSLT-I and MSLT-II) in melanoma. *Clin Exp Metastasis* 2012; **29**: 699–706.
2 Kwon S, Dong ZM, Wu PC. Sentinel lymph node biopsy for high-risk cutaneous squamous cell carcinoma: clinical experience and review of literature. *World J Surg Oncol* 2011; **9**: 80.
3 Ahmed MM, Moore BA, Schmalbach CE. Utility of head and neck cutaneous squamous cell carcinoma sentinel node biopsy: a systematic review. *Otolaryngol Head Neck Surg* 2014; **150**: 180–187.
4 Tsujino Y, Mizumoto K, Matsuzaka Y *et al.* Fluorescence navigation with indocyanine green for detecting sentinel nodes in extramammary Paget's disease and squamous cell carcinoma. *J Dermatol* 2009; **36**: 90–94.
5 Fujisawa Y, Nakamura Y, Kawachi Y *et al.* Indocyanine green fluorescence-navigated sentinel node biopsy showed higher sensitivity than the radioisotope or blue dye method, which may help to reduce false-negative cases in skin cancer. *J Surg Oncol* 2012; **106**: 41–45.
6 Takeda A, Akimoto M, Nemoto M *et al.* Preoperative risk factors of lymph node metastasis in cutaneous squamous cell carcinoma. *J Plast Surg Hand Surg* 2013; **47**: 204–208.
7 Hatta N, Morita R, Yamada M *et al.* Sentinel lymph node biopsy in patients with extramammary Paget's disease. *Dermatol Surg* 2004; **30**: 1329–1334.
8 Hatta N, Yamada M, Hirano T *et al.* Extramammary Paget's disease: treatment, prognostic factors and outcome in 76 patients. *Br J Dermatol* 2008; **158**: 313–318.
9 Nakamura Y, Fujisawa Y, Ishikawa M *et al.* Usefulness of sentinel lymph node biopsy for extramammary Paget disease. *Br J Dermatol* 2012; **167**: 954–956.
10 Ishihara T, Kaguchi A, Matsushita S *et al.* Management of sentinel lymph nodes in malignant skin tumors using dynamic lymphoscintigraphy and the single-photon-emission computed tomography/computed tomography combined system. *Int J Clin Oncol* 2006; **11**: 214–220.
11 Wong SL, Balch CM, Hurley P *et al.* Sentinel lymph node biopsy for melanoma: American Society of Clinical Oncology and Society of Surgical Oncology joint clinical practice guideline. *J Clin Oncol* 2012; **30**: 2912–2918.
12 Han D, Zager JS, Shyr Y *et al.* Clinicopathologic predictors of sentinel lymph node metastasis in thin melanoma. *J Clin Oncol* 2013; **31**: 4387–4393.
13 Nakamura Y, Fujisawa Y, Maruyama H *et al.* Improvement of the sentinel lymph node detection rate of cervical sentinel lymph node biopsy using real-time fluorescence navigation with indocyanine green in head and neck skin cancer. *J Dermatol* 2013; **40**: 453–457.
14 Iwata H, Kamiya H, Kitajima Y. False-negative sentinel lymph node biopsy resulting from obstruction of lymphatic basin by nodal metastasis: a case report of malignant melanoma. *J Dermatol* 2011; **38**: 1121–1124.
15 Proulx ST, Luciani P, Christiansen A *et al.* Use of a PEG-conjugated bright near-infrared dye for functional imaging of rerouting of tumor lymphatic drainage after sentinel lymph node metastasis. *Biomaterials* 2013; **34**: 5128–5137.

REFERENCES

1 Draelos ZD. Skin lightening preparations and the hydroquinone controversy. *Dermatol Ther* 2007; **20**: 308–313.
2 To-o K, Nomura K, Sugimoto K, Nishimura T, Kuriki T, Itoh M. Experimental study on cross-reactivity of α-arbutin toward *p*-phenylenediamine and hydroquinone in guinea pigs. *J Dermatol* 2010; **37**: 455–462.
3 Mishra SN, Dhurat RS, Deshpande DJ, Nayak CS. Diagnostic utility of dermatoscopy in hydroquinone-induced exogenous ochronosis. *Int J Dermatol* 2013; **52**: 413–417.
4 Kligman AM, Willis I. A new formula for the depigmenting human skin. *Arch Dermatol* 1975; **111**: 40–48.
5 Nath AK, Thappa DM. Kumkum-induced dermatitis: an analysis of 46 cases. *Clin Exp Dermatol* 2007; **32**: 385–387.

Immunoglobulin G4-related disease in a psoriasis vulgaris patient treated with ustekinumab

Dear Editor,

Immunoglobulin (Ig)G4-related disease (IgG4-RD) is a new proposed disease characterized by elevated serum IgG4 levels and the infiltration of IgG4-positive cells.[1] To our knowledge, the current case is the first one of IgG4-RD in a patient of psoriasis vulgaris treated with ustekinumab.

A 71-year-old man had a 20-year history of severe psoriasis vulgaris. Although he had been treated using a topical steroid and phototherapy, the therapeutic efficacy was limited. He was admitted to our hospital in January 2011. Although infliximab therapy was effective, it was stopped for 1 month because of the incidence of bacterial pneumonia. In March 2012, his skin condition became worse (Psoriasis Area and Severity Index [PASI], 37.9). At that time, blood examination showed the following results: white cell count, 8100/μL; C-reactive protein, 0.06 mg/dL; urea nitrogen, 16.9 mg/dL; and creatinine, 0.94 mg/dL. Whole-body computed tomography (CT) showed aorta dissection and coronary artery calcification. Thus, ustekinumab was administrated at weeks 0 and 4 and every 12 weeks (45 mg per s.c. injection).

At 14 months after initiation of ustekinumab therapy, renal function became slowly worse (serum urea nitrogen, 20.4 mg/dL; serum creatinine, 1.73 mg/dL) although his psoriatic lesion improved (PASI, 3.6). Whole-body plain CT revealed mediastinal lymphoadenopathy, retroperitoneal fibrosis and bilateral hydronephrosis (Fig. 1). Additional laboratory studies revealed the following values: white cell count, 6800/μL; C-reactive protein, 2.03 mg/dL; serum IgG, 3714 mg/dL (normal range, 800–1600); and serum IgG4, 311 mg/dL (normal, <105). These results indicated that he had IgG4-related retroperitoneal fibrosis. Although contrast enhanced CT-guided biopsy was essential for definite diagnosis, it could not be conducted because of his kidney dysfunction. Thus, he was clinically diagnosed as having IgG4-RD. We discontinued ustekinumab and started oral prednisolone therapy (1.0 mg/kg per day; 60 mg/day). Three months later, serum IgG4 levels had increased, and renal dysfunction and retroperitoneal fibrosis had largely improved. Currently, the patient takes oral prednisolone (2.5 mg/day) and cyclosporin (100 mg/day) because of recurrence of psoriasis without the exacerbation of IgG4-RD.

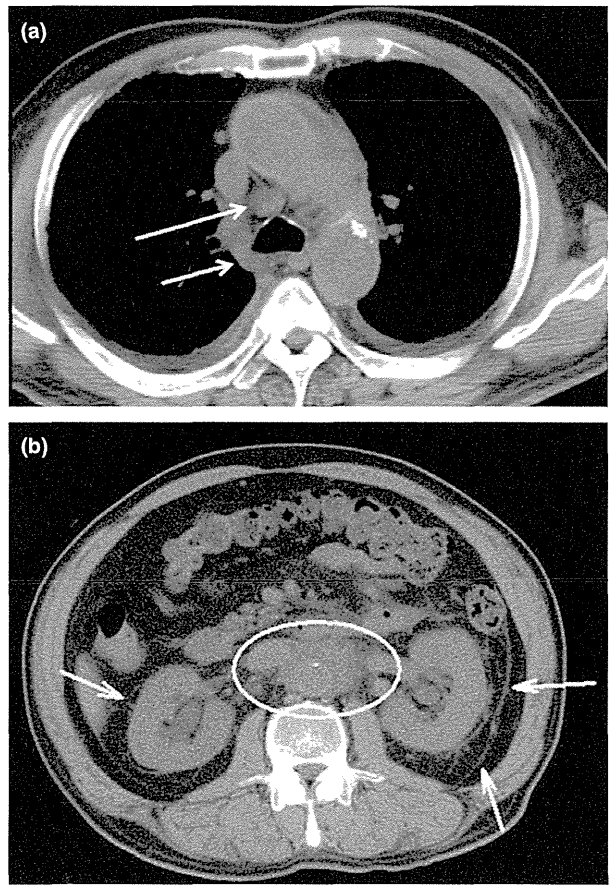

Figure 1. Computed tomography showed (a) mediastinal lymphoadenopathy (arrows) and (b) retroperitoneal fibrosis (circle) and its secondary bilateral hydronephrosis (arrows).

Correspondence: Ikko Kajihara, M.D., Department of Dermatology and Plastic Surgery, Faculty of Life Sciences, Kumamoto University, 1-1-1 Honjo, Kumamoto 860-8556, Japan. Email: kajiderma@gmail.com

Biologics occasionally induce severe adverse events. Regarding rare autoimmune adverse events, some reports indicated that the occurrence of eosinophilic pneumonia[2] and alopecia areata[3] may be elicited by ustekinumab. To our knowledge, this patient is the first case with IgG4-RD related to ustekinumab although our case may be occasional. IgG4-RD has the following features: (i) IgG4-RD can occur in various organs; (ii) IgG4-RD mainly affects middle-aged to elderly men; (iii) many patients with IgG4-RD can be treated effectively by steroid therapy; and (iv) the severity of fibrosis is dependent on the individual organ involvement.[1] Although we could not perform biopsy from the retroperitoneum, IgG4-RD was clinically diagnosed on the basis of elevated serum IgG4 levels and effectiveness of oral steroid therapy. In fact, it is difficult to perform a biopsy in patients with IgG4-related retroperitoneal fibrosis as pointed out in a previous report.[1]

In conclusion, IgG4-RD may be recognized as a severe complication of biologics by many stocks in future.

CONFLICT OF INTEREST: None.

Ikko KAJIHARA,[1] Katsunari MAKINO,[1] Asako ICHIHARA,[1] Satoshi FUKUSHIMA,[1] Masatoshi JINNIN,[1] Sayuri HIROOKA,[2] Keisuke KOJIMA,[2] Hirotsugu KOUROGI,[2] Hironobu IHN[1]

[1]*Departments of Dermatology and Plastic Surgery and* [2]*Respiratory Medicine, Faculty of Life Sciences, Kumamoto University, Kumamoto, Japan*

doi: 10.1111/1346-8138.12528

REFERENCES

1 Umehara H, Okazaki K, Masaki Y *et al.* Comprehensive diagnostic criteria for IgG4-related disease (IgG4-RD), 2011. *Mod Rheumatol* 2012; **22**: 21–30.

2 Yashiro M, Maejima H, Taniguchi T *et al.* Psoriasis vulgaris complicated by eosinophilic pneumonia during ustekinumab treatment. *Eur J Dermatol* 2013; **23**: 396–397.

3 Tauber M, Beneton N, Reygagne P *et al.* Alopecia areata developing during ustekinumab therapy: report of two cases. *Eur J Dermatol* 2013; **23**: 912–913.

Brief Report

DOI: 10.5582/bst.2014.01020

Serum levels of soluble carbonic anhydrase IX are decreased in patients with diffuse cutaneous systemic sclerosis compared to those with limited cutaneous systemic sclerosis

Katsunari Makino, Masatoshi Jinnin*, Takamitsu Makino, Ikko Kajihara, Satoshi Fukushima, Yuji Inoue, Hironobu Ihn

Department of Dermatology and Plastic Surgery, Faculty of Life Sciences, Kumamoto University, Kumamoto, Japan.

Summary **Hypoxia may play an important role in the pathogenesis of systemic sclerosis (SSc). Carbonic anhydrase IX (CA IX) is one of the hypoxia markers and its extracellular domain can be released into the serum. However, the clinical significance of serum CA IX levels in SSc is still unknown. The aim of this study is to evaluate the possibility that serum CA IX levels can be a specific disease marker of SSc. Serum samples were obtained from SSc patients and healthy controls. Patients diagnosed as scleroderma spectrum disorder (SSD), who did not fulfill the ACR criteria of SSc but were thought that they might develop SSc in the future, were also included in this study. Serum CA IX levels were measured with specific enzyme-linked immunosorbent assays. SSD patients had significantly lower CA IX levels than diffuse cutaneous SSc (dcSSc), limited cuntaneous SSc (lcSSc) and healthy control groups. Also, we found a significant decrease in the values in dcSSc patients compared to those of lcSSc patients. Serum levels of CA IX may be useful for the differentiation of lcSSc from SSD. Decreased serum CA IX levels in spite of the presence of hypoxia in SSc may indicate an impaired response to hypoxia, which leads to the persistent hypoxic condition. Our results suggest that the abnormal response to hypoxia may already exist in SSD patients, and may be involved in its pathogenesis.**

Keywords: Carbonic anhydrase IX, collagen disease, hypoxia, systemic sclerosis

1. Introduction

Systemic sclerosis (SSc) or scleroderma is an acquired disorder which typically results in fibrosis of the skin and internal organs. Although the pathogenesis of this disease is still unclear, it includes inflammation, autoimmune attack, and vascular damage, leading to activation of fibroblasts and abnormal accumulation of extracellular matrix, mainly collagen (*1,2*).

Microangiopathy is one of the primary pathologic components of SSc (*3*). Raynaud's phenomenon or aberrant nailfold bleeding is known as an early vascular event of this disease. Telangiectasia, pitting scars, skin ulcers, impaired wound healing or pulmonary hypertension are frequently observed in the disease process, and they can severely affect the quality of life in these patients.

The microangiopathy causes a reduction of blood flow, which results in tissue hypoxia. The tissue ischemia leads usually to the expression of angiogenic growth factors, which act against the ischemic conditions. Hypoxia induced factor (HIF)-1α, one of the hypoxic markers, is a transcription factor which regulates cellular adaptation to low oxygen tension (*4*). Under normoxic conditions, the expression of HIF-1α is maintained at a low level by ubiquitination and degradation (*5*). In hypoxic conditions, HIF-1α is up-regulated and translocated to the nucleus where it induces transcription of target genes essential for survival and adaptation to hypoxic environments, such as vascular endothelial growth factor (VEGF), glucose transporter 1 (GLUT-1), and erythropoietin (*6*).

**Address correspondence to:*
Dr. Masatoshi Jinnin, Department of Dermatology and Plastic Surgery, Faculty of Life Sciences, Kumamoto University, 1-1-1 Honjo, Kumamoto, Japan.
E-mail: mjin@kumamoto-u.ac.jp

Cutaneous hypoxia in patients with SSc was reported (*7*). Distler O *et al.* describe that despite severely reduced oxygen levels, protein levels of HIF-1α in the skin of SSc patients were even below the levels seen in healthy control skin (*8*). Therefore, the impaired response to tissue hypoxia in SSc patients may lead to persistence of hypoxic conditions. Hypoxia contributes directly to progression of fibrosis by activation of fibroblasts in SSc. On the other hand, excess extracellular matrix deposition increases diffusion distances from blood vessels to cells, and induces further hypoxic conditions (*9*). This vicious circle is thought to be associated with the pathogenesis of SSc.

In this study, we focused on carbonic anhydrase IX (CA IX), another hypoxia marker. Carbonic anhydrases (CAs) are a family of zinc-containing enzymes, that catalyze a reversible conversion of carbon dioxide to bicarbonate and a proton in the reaction: $CO_2+H_2O \leftrightarrow HCO_3^-+H^+$ (*10*). These enzymes participate in a variety of biological processes, including respiration, ion transport, pH balance, and bone resorption (*11*). Human CAs exist in at least 15 isoforms (*12*). Among them, CA IX is a membrane-associated protein and is known as a biomarker of hypoxia or certain malignant tumors. The expression of CA IX can only be detected in a few normal tissues, whereas it is abnormally induced in hypoxic conditions or malignant tumors (*13*). The relationship of CA IX with hypoxia has been explained by the notion that the CA IX promoter contains a hypoxia response element (HRE) to which HIF-1 can bind (*14*). Because the extracellular domain of CA IX can be released into cell culture medium or into the body fluids (*15*), unlike HIF-1α, CA IX can be detected in serum. As described above, hypoxia plays an important role in the pathogenesis of SSc. Although the clinical significance of serum CA IX levels in SSc is still unknown, they can be correlated with disease activity. Thus, in this study, we try to evaluate the possibility that serum levels of CA IX can be a useful marker of SSc.

2. Materials and Methods

2.1. *Clinical assessment and patient material*

Serum samples were obtained from 43 patients with SSc (7 men and 36 women; age range, 7-85 years; mean, 57.4 years). All patients fulfilled the criteria proposed by the American College of Rheumatology, and were grouped according to the classification system proposed by LeRoy *et al.* (*16*): 20 patients had diffuse cutaneous SSc (dcSSc) and 23 patients had limited cutaneous SSc (lcSSc), as described previously (*17*). Clinical and laboratory data reported in this study were obtained at the time of serum sampling. Patients were evaluated for the presence of gastrointestinal, pulmonary, cardiac, renal or muscle involvement as described previously (*17*). Control serum samples were also collected from healthy age- and sex-matched volunteers. Five patients diagnosed as scleroderma spectrum disorder (SSD), who did not fulfill the ACR criteria of SSc but were thought that they might develop SSc in the future based on the criteria proposed by Ihn *et al.*, were also included in this study (*18-20*). Institutional review board approval and written informed consent were obtained before patients and healthy volunteers were entered into this study according to the Declaration of Helsinki. All serum samples were stored at –80°C prior to use.

2.2. *Measurement of serum CA IX concentrations*

Levels of serum CA IX were measured with a specific ELISA kit (R&D Systems, Minneapolis, MN, USA). Briefly, anti-CA IX monoclonal antibodies were precoated onto microtiter wells. Aliquots of serum were added to each well, followed by peroxidase-conjugated antibodies to CA IX. Color was developed with hydrogen peroxide and tetramethylbenzidine peroxidase and the absorbance at 450 nm was measured. Wavelength correction was performed by absorbance at 570 nm. The concentration of CA IX in each sample was determined by interpolation from a standard curve.

2.3. *Statistical analysis*

Statistical analysis was carried out with a Welch two sample *t*-test for the comparison of means, and Fisher's exact probability test for the analysis of frequency. *p* values less than 0.05 were considered significant.

3. Results and Discussion

The serum CA IX levels in patients with SSc and in healthy control subjects are shown in Figure 1. Serum samples were obtained from 43 patients with SSc. Twelve healthy control subjects and 5 SSD patients, who did not fulfill the criteria of SSc but were thought that they might develop SSc in the future, were also included in this study. The SSD patients had 4-8 points using a point system proposed by Ihn *et al.* (*20*).

Although mean serum CA IX levels were higher in SSc patients (146 ± 198 pg/mL) than in healthy control subjects (118 ± 115 pg/mL), there was no statistically significant difference between the two groups. However, when SSc patients were classified into lcSSc and dcSSc as described in 'Patients and Methods', we found a significant decrease in the values of dcSSc patients than in those of lcSSc patients (82 ± 68 *vs.* 201 ± 254 pg/mL, $p < 0.05$). The mean serum levels were higher in lcSSc patients and lower in dcSSc patients than those in healthy controls, but there were no significant difference.

On the other hand, CA IX levels in all 5 SSD patients were decreased as compared to other groups;

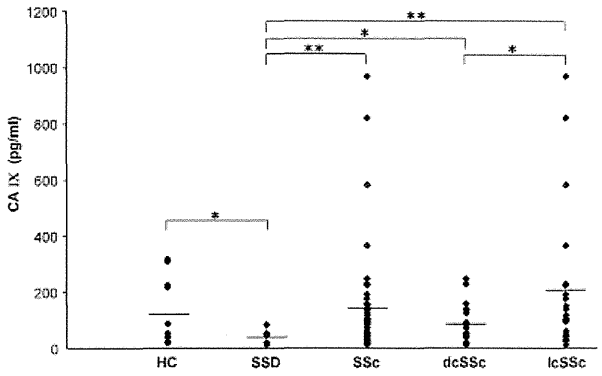

Figure 1. Serum concentrations of soluble CA IX in patients with systemic sclerosis (SSc), scleroderma spectrum disorders (SSD), and in healthy control subjects (HC). Serum CA IX levels were measured with ELISA kits as described in 'Materials and Methods'. Serum CA IX concentrations are shown on the ordinate. Bars show means. p values less than 0.05 are interpreted as significant. dcSSc, diffuse cutaneous SSc; lcSSc, limited cutaneous SSc. $^{*}p < 0.05$, $^{**}p < 0.01$.

SSD patients had significantly lower CA IX levels than healthy controls (42 ± 28 *vs.* 118 ± 114 pg/mL, $p < 0.05$) and SSc patients (42 ± 28 *vs.* 146 ± 198 pg/mL, $p < 0.01$). In addition, the difference between SSD patients and lcSSc patients (42 ± 28 *vs.* 201 ± 254 pg/mL, $p < 0.01$) was more significant than that between SSD patients and dcSSc patients (42 ± 28 *vs.* 82 ± 68 pg/mL, $p < 0.05$). Taken together, the serum CA IX levels were decreased in patients with SSD and dcSSc in that order, with statistical significance.

Table 1 shows the association of serum CA IX levels with the clinical features in SSc patients. Considering that HIF-1α expression in the skin of SSc patients was previously reported to be below the levels seen in healthy control skin (*8*), and that the serum CA IX levels in SSD and dcSSc patients tended to be decreased in our study, we regarded reduction of CA IX levels as the meaningful change in SSc patients. As shown in Table 1, in SSc patients with reduced CA IX levels, the percentage of dcSSc was significantly increased compared to those with normal CA IX levels (dcSSc:lcSSc = 14:8 *vs.* 6:15, $p = 0.022$). There was no statistically significant difference between these groups in terms of sex, mean age at onset, duration of disease, and other clinical or laboratory features including therapy, smoking history, respiratory dysfunction or anemia.

In this study, although we expected that hypoxia marker CA IX was up-regulated in SSc sera due to its hypoxic conditions, there was no significant difference in serum CA IX between healthy controls and SSc patients. However, we found that SSD patients had significantly lower CA IX levels than control subjects, dcSSc or lcSSc patients. In addition, serum CA IX levels were significantly lower in dcSSc patients compared to lcSSc patients.

The concept of SSD was originally proposed by

Table 1. Correlation of serum CA IX levels with clinical features in patients with systemic sclerosis (SSc)

Items	Patients with normal CA IX levels (n =21)	Patients with reduced CA IX levels (n =22)
Sex (female : male)	18:3	18:4
Type (diffuse : limited)	6:15*	14:8*
Clinical Features		
Raynaud's phenomenon	76.2	90.9
Pitting scar	47.6	36.4
Ulcer	33.3	27.3
Nailfold bleeding	33.3	50.0
Cobblestone on hands	4.8	13.6
Hyperkeratotic plaque	0	9.1
Telangiectagia	23.8	18.2
Contracture of phalanges	66.7	72.7
Calcinosis	4.8	0
Inflammatory erythema	95.2	45.5
Diffuse pigmentation	19.0	18.2
Short subungal flenurum	52.4	45.5
Sicca symptoms	47.6	18.2
Organ involvement		
Oesophagus	19.0	18.2
Ileus	0	0
Pulmonary fibrosis	23.8	50.0
Heart	52.4	13.6
Liver	19.0	13.6
Kidney	0	0
Joint	23.8	34.8
Thrombosis	0	0
Thyroiditis	28.6	27.3
Others		
Complication of malignancy	0	0

Unless noted otherwise, values are percentages. Fisher's exact probability test was performed to compare the frequency of clinical findings of patients. P values less than 0.05 were considered significant. $^{*}p < 0.05$.

Maricq *et al.* to unify typical SSc, early forms of SSc and closely related disorders including mixed connective tissue disease (MCTD) (*18*). Thereafter, Ihn *et al.* defined SSD as patients who did not fulfill the criteria of SSc but were thought that they might develop SSc in the future, and established a new diagnostic method using a points system to distinguish patients with SSD from those with early SSc. A total score was obtained as the sum of the following five factors: (1) extent of skin sclerosis (maximum, 10 points); (2) pulmonary changes (maximum, 4 points); (3) antinuclear antibodies (maximum, 5 points); (4) pattern of Raynaud's phenomenon (maximum, 3 points); and (5) nailfold bleeding (maximum, 2 points). The authors suggest the conditions with 9 or more points are consistent with SSc and those with 5 to 8 points are consistent with SSD (*20*). Because progressive fibrosis of SSc is often irreversible, at least clinically, there is an urgent need to develop new strategies to diagnose patients as early as possible and follow them carefully. For that purpose, the concept of SSD should be further understood and characterized. Our study is the first to perform ELISA experiments using SSD sera.

To note, there were significant difference between

SSD patients and lcSSc patients. The diagnosis of SSc presents little problem when the clinical features have fully developed. However, it may be difficult to distinguish lcSSc from SSD, because skin sclerosis is sometimes not apparent in lcSSc, especially in a very early stage. Serum levels of CA IX may be useful for the differentiation of lcSSc from SSD. Moreover, we frequently encounter SSD patients with an increased risk of future development of SSc. Serial time-course measurement of serum CA IX concentration in SSD patients may lead to early detection of developing SSc.

As described above, CA IX is thought to be a down-stream target of HIF-1α (*14*). Thus, reduced CA IX levels seen in dcSSc patients in our study is consistent with down-regulation of HIF-1α in SSc skin in spite of the presence of hypoxia (*8*), which may result in the persistent hypoxic condition of the disease. Our results suggest that such dysregulation of HIF-1α as well as CA IX and subsequent persistent hypoxia may already occur in SSD patients. Considering that microangiopathy is one of the primary symptoms of SSc and tissue fibrosis is observed in the disease process, the impaired response to microangiopathy-induced hypoxic conditions may be involved in the pathogenesis of SSD. Furthermore, a sustained abnormal response may lead to the development of severe skin sclerosis in dcSSc patients, whereas the reduction of CA IX may be transient in lcSSc. Otherwise, the discrepancy between dcSSc and lcSSc in CA IX levels may indicate heterogeneity between the two clinical subtypes.

There are some limitations to our results. First, we could not collect large number of SSD patients because of the rarity of this condition. However, our approach may be effective to clarify the properties of SSD. Larger studies are needed in the future. In addition, recent studies show transforming growth factor (TGF)-β, one of the key cytokines in the pathogenesis of SSc, can also regulate CA IX gene expression (*21*). Thus, there may be another pathway other than HIF-1α in the regulation of CA IX. The regulatory mechanisms of CA IX in this disease should be clarified.

Acknowledgements

This study was supported in part by a grant for scientific research from the Japanese Ministry of Education, Science, Sports and Culture, by project research for progressive systemic sclerosis from the Japanese Ministry of Health and Welfare, and by Lydia O'leary Memorial Foundation.

References

1. Korn J. Immunologic aspects of scleroderma. Curr Opin Rheumatol. 1989; 1:479-484.
2. Mauch C, Kreig T. Fibroblast-matrix interactions and their role in the pathogenesis of fibrosis. Rheum Dis Clin North Am. 1990; 16:93-107.
3. Müller-Ladner U, Distler O, Ibba-Manneschi L, Neumann E, Gay S. Mechanisms of vascular damage in systemic sclerosis. Autoimmunity. 2009; 42:587-595.
4. Wang G, Jiang B, Rue E, Semenza G. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension. Proc Natl Acad Sci U S A. 1995; 92:5510-5514.
5. Lee J, Bae S, Jeong J, Kim S, Kim K. Hypoxia-inducible factor (HIF-1)α: Its protein stability and biological functions. Exp Mol Med. 2004; 36:1-12.
6. Gleadle J, Ratcliffe P. Induction of hypoxia-inducible factor-1, erythropoietin, vascular endothelial growth factor, and glucose transporter-1 by hypoxia: evidence against a regulatory role for Src kinase. Blood. 1997; 89:503-509.
7. Silverstein J, Steen V, Medsger TJ, Falanga V. Cutaneous hypoxia in patients with systemic sclerosis (scleroderma). Arch Dermatol. 1988; 124:1379-1382.
8. Distler O, Distler J, Scheid A, Acker T, Hirth A, Rethage J, Michel B, Gay R, Müller-Ladner U, Matucci-Cerinic M, Plate K, Gassmann M, Gay S. Uncontrolled expression of vascular endothelial growth factor and its receptors leads to insufficient skin angiogenesis in patients with systemic sclerosis. Circ Res. 2004; 95:109-116.
9. Beyer C, Schett G, Gay S, Distler O, Distler J. Hypoxia. Hypoxia in the pathogenesis of systemic sclerosis. Arthritis Res Ther. 2009; 11:220.
10. Swietach P, Patiar S, Supuran C, Harris A, Vaughan-Jones R. The role of carbonic anhydrase 9 in regulating extracellular and intracellular ph in three-dimensional tumor cell growths. J Biol Chem. 2009; 284:20299-20310.
11. Sly W, Hu P. Human carbonic anhydrases and carbonic anhydrase deficiencies. Annu Rev Biochem. 1995; 64:375-401.
12. Zatovicova M, Sedlakova O, Svastova E, Ohradanova A, Ciampor F, Arribas J, Pastorek J, Pastorekova S. Ectodomain shedding of the hypoxia-induced carbonic anhydrase IX is a metalloprotease-dependent process regulated by TACE/ADAM17. Br J Cancer. 2005; 93:1267-1276.
13. Ivanov S, Liao S, Ivanova A, Danilkovitch-Miagkova A, Tarasova N, Weirich G, Merrill M, Proescholdt M, Oldfield E, Lee J, Zavada J, Waheed A, Sly W, Lerman M, Stanbridge E. Expression of hypoxia-inducible cell-surface transmembrane carbonic anhydrases in human cancer. Am J Pathol. 2001; 158:905-919.
14. Wykoff C, Beasley N, Watson P, Turner K, Pastorek J, Sibtain A, Wilson G, Turley H, Talks K, Maxwell P, Pugh C, Ratcliffe P, Harris A. Hypoxia-inducible expression of tumor-associated carbonic anhydrases. Cancer Res. 2000; 60:7075-7083.
15. Závada J, Závadová Z, Zat'ovicová M, Hyrsl L, Kawaciuk I. Soluble form of carbonic anhydrase IX (CA IX) in the serum and urine of renal carcinoma patients. Br J Cancer. 2003; 89:1067-1071.
16. LeRoy E, Black C, Fleischmajer R, Jablonska S, Krieg T, Medsger TJ, Rowell N, Wollheim F. Scleroderma (systemic sclerosis): Classification, subsets and pathogenesis. J Rheumatol. 1988; 15:202-205.
17. Ihn H, Sato S, Fujimoto M, Kikuchi K, Igarashi A, Soma Y, Tamaki K, Takehara K. Measurement of anticardiolipin antibodies by ELISA using β2-glycoprotein I (β2-GPI) in systemic sclerosis. Clin Exp

Immunol 1996; 105:475-479.
18. Maricq H, Weinrich M, Keil J, Smith E, Harper F, Nussbaum A, LeRoy E, McGregor A, Diat F, Rosal E. Prevalence of scleroderma spectrum disorders in the general population of South Carolina. Arthritis Rheum. 1989; 32:998-1006.
19. Maricq H, McGregor A, Diat F, Smith E, Maxwell D, LeRoy E, Weinrich M. Major clinical diagnoses found among patients with Raynaud phenomenon from the general population. J Rheumatol. 1990; 17:1171-1176.
20. Ihn H, Sato S, Tamaki T, Soma Y, Tsuchida T, Ishibashi Y, Takehara K. Clinical evaluation of scleroderma spectrum disorders using a points system. Arch Dermatol Res. 1992; 284:391-395.
21. Yildirim H, Köçkar F. TGF-β upregulates tumor-associated carbonic anhydrase IX gene expression in Hep3B cells. Cell Biol Int. 2009; 33:1002-1007.

(Received February 13, 2014; Revised June 10, 2014; Accepted June 13, 2014)

http://dx.doi.org/10.5021/ad.2014.26.4.536

Clinical Significance of Serum Vascular Endothelial-Cadherin Levels in Inflammatory Skin Diseases

Haruka Kuriyama, Masatoshi Jinnin, Hisashi Kanemaru, Asako Ichihara, Akihiko Fujisawa, Chikako Moriya, Satoshi Fukushima, Hironobu Ihn

Department of Dermatology and Plastic Surgery, Faculty of Life Sciences, Kumamoto University, Kumamoto, Japan

Dear Editor:

Inflammatory skin diseases are sometimes accompanied by vascular abnormalities; e.g. Auspitz phenomenon in psoriasis or white dermographism in atopic dermatitis (AD)[1,2]. However, the detailed mechanism(s) and role of such vasculopathy in the pathogenesis of each disease are still unclear.

Vascular endothelial (VE)-cadherin is one of the major components of adherens junctions between endothelial cells. The critical role of VE-cadherin is vascular morphogenesis during embryogenesis. Such function of VE-cadherin is regulated by vascular endothelial cell growth factor (VEGF) signaling through the VEGF receptor leads to the increased detachment of endothelial cells and transendothelial permeability by promoting VE-cadherin internalization[3]. On the other hand, VE-cadherin limits the

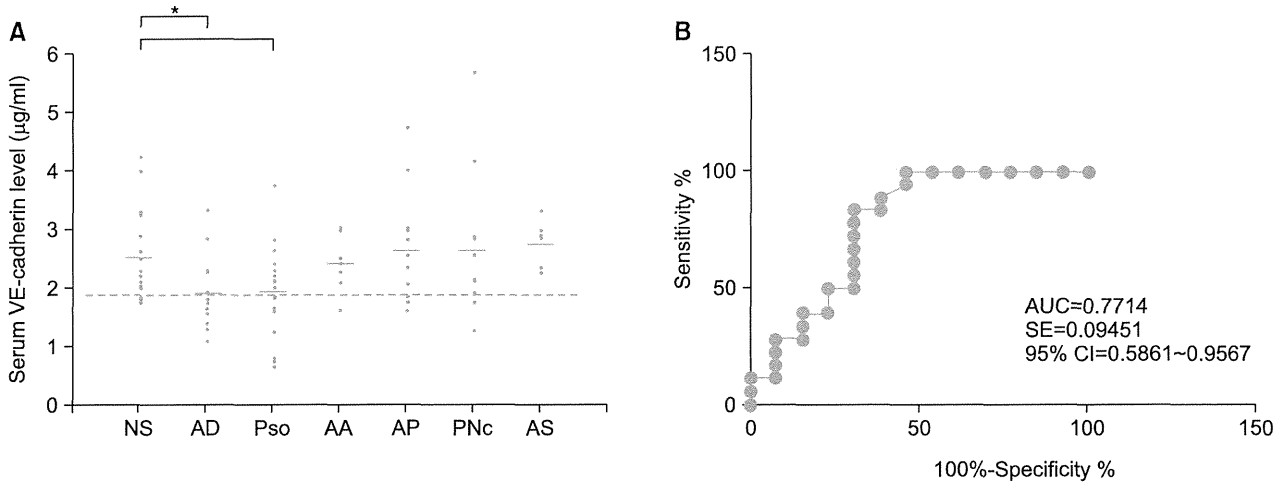

Fig. 1. (A) Serum soluble VE-cadherin levels in patients with various skin diseases. The serum levels of VE-cadherin determined by using ELISA are shown on the ordinate; the horizontal bars show the mean value in each group. The dotted line indicates the cutoff. VE: vascular endothelial, NS: normal subjects. AD: atopic dermatitis, Pso: psoriasis, AA: alopecia areata, AP: anaphylactoid purpura, PNc: cutaneous polyarteritis nodosa, AS: angiosarcoma. *$p<0.05$ by using the Mann-Whitney U-test. (B) Receiver operating characteristic curve for serum VE-cadherin levels in patients with AD. AUC: areas under curves, SE: standard error, CI: confidence interval.

Received July 24, 2013, Accepted for publication August 6, 2013

Corresponding author: Masatoshi Jinnin, Department of Dermatology and Plastic Surgery, Faculty of Life Sciences, Kumamoto University, 1-1-1 Honjo, Kumamoto 860-8556, Japan. Tel: 81-96-373-5233, Fax: 81-96-373-5235, E-mail: mjin@kumamoto-u.ac.jp

proliferation of endothelial cells by preventing the internalization of the VEGF receptor[4]. However, the role of VE-cadherin in the vascular abnormalities of inflammatory skin diseases has not been investigated. Full-length VE-cadherin is an insoluble transmembrane protein, whereas the extracellular domain of VE-cadherin is secreted as a soluble protein through a metalloproteinase-dependent mechanism[5]. Soluble VE-cadherin may function as an antagonist of full-length VE- cadherin, which is suggested by its inhibitory effect on tumor angiogenesis and tumor growth *in vivo*[6]. To date, although soluble VE-cadherin has been detected in serum *in vivo*, the clinical significance of serum soluble VE- cadherin levels is still unknown. Therefore, in this study, we attempted to evaluate the possibility that the serum VE-cadherin level can be a useful marker for inflammatory skin diseases. Serum samples were obtained from 13 patients with AD, 33 patients with psoriasis, and 7 patients with alopecia areata. Control serum samples were also collected from 18 healthy volunteers. Sera of 12 patients with anaphylactoid purpura, 11 patients with cutaneous polyarteritis nodosa, and 6 patients with angiosarcoma were also included as the disease controls. This research was approved by the Ethics Review Committee in Kumamoto University (No. 177). Written informed consents were obtained before patients and healthy volunteers were enrolled into this study according to the Declaration of Helsinki.

Table 1. Association of serum VE-cadherin levels with clinical and serological features

	VE-cadherin	
	Normal	Reduced
Patients with atopic dermatitis		
Mean duration of disease (yr)	15.3	20.3
Mean SCORAD (score)	56.1	47.4
Mean eosinophil (%)	11.9	9.3
Mean serum LDH (U/L)	321.0	237.4
Mean serum TARC (pg/ml)	9,428.0	4,386.0
Mean serum IgE (ng/ml)	539.3	881.8
Total	5	8
Patients with psoriasis		
Mean duration of disease (yr)	5.9	3.9
Mean PASI (score)	16.6	15.7
Mean BSA (%)	40.3	31.0
Arthritis (%)	25.0	28.6
Nail change (%)	53.8	21.4*
Total	17	16

SCORAD: scoring atopic dermatitis, LDH: lactate dehydrogenase, TARC: thymus and activation-regulated chemokine, IgE: immunoglobulin E, PASI: psoriasis area and severity index, BSA: body surface area of the involved skin.
*$p<0.05$ vs. patients with normal VE-cadherin levels by using the Fisher exact probability test.

The serum soluble VE-cadherin levels were measured with a specific enzyme-linked immunosorbent assay kit (R&D Systems, Minneapolis, MN, USA)[7] (Fig. 1A). The mean serum VE-cadherin level tended to be lower in patients with AD (1.903 μg/ml) and those with psoriasis (1.915 μg/ml) in comparison to normal subjects (2.511 μg/ml). We found a statistical significance in these decreases ($p=0.01$ in patients with AD, and $p=0.04$ in patients with psoriasis). The serum VE-cadherin levels in patients with alopecia ($p=0.81$), anaphylactoid purpura ($p=0.90$), cutaneous polyarteritis nodosa ($p=0.96$), or angiosarcoma ($p=0.13$) were similar to those in normal subjects. When the cutoff value was set at 1.880 μg/ml on the basis of the normal range provided by the manufacturer, reduced serum VE-cadherin levels were found in 3 of the 18 healthy volunteers (16.7%), 8 of the 13 patients with AD (61.5%), 16 of the 33 patients with psoriasis (48.5%), 1 of the 7 patients with alopecia (14.3%), 4 of the 12 patients with anaphylactoid purpura (33.3%), 2 of the 11 patients with cutaneous polyarteritis nodosa (11.2%), and 0 of the 6 patients with angiosarcoma. Thus, the low serum VE-cadherin levels may be more specific to AD or psoriasis than cutaneous vasculitis or vascular tumor.

In the receiver operating characteristic curve analysis of patients with AD (Fig. 1B), the area under curve (AUC) was 0.77 (95% confidence interval [95% CI], 0.59~0.96). An AUC of >0.7 indicates that the serum VE-cadherin levels can effectively distinguish patients with AD from normal subjects. On the other hand, the AUC was 0.67 (95% CI, 0.53~0.82) in patients with psoriasis, indicating that serum VE-cadherin is less useful in diagnosing psoriasis. Accordingly, serum VE-cadherin may be more effective for diagnosing AD than psoriasis.

Next, we determined the association of serum VE-cadherin levels with the clinical and serological features of patients with AD (Table 1); 5 disease activity markers (SCORAD, percentage of eosinophil counts, serum lactate dehydrogenase levels, serum thymus and activation-regulated chemokine levels, and serum immunoglobulin E level) and the duration of the disease (between symptom onset and the first visit to the hospital) were evaluated. However, we could not find a significant difference in these factors between patients with reduced VE-cadherin levels and those with normal levels. On the other hand, in patients with psoriasis, when 4 activity indicators (psoriasis area and severity index score, body surface area of the involved skin, arthritis, and nail change) and disease duration were evaluated (Table 1), patients with reduced VE-cadherin levels showed a significantly lower prevalence of nail change ($p=0.03$). Thus, serum VE-cadherin

levels are correlated with clinical symptom in patients with psoriasis but not in patients with AD. Taken together, our results suggest that the serum VE-cadherin level in patients with AD can be the diagnostic marker rather than the disease activity marker, whereas that in patients with psoriasis may be more useful as the marker for disease activity.

This is the first report measuring serum VE-cadherin levels in patients with various skin diseases. Furthermore, as far as we searched, decreased serum VE-cadherin levels have not been reported in human diseases. Although the role of VE-cadherin in skin diseases is unknown, skin erythema, for example, is one of the common features of AD and psoriasis and is caused by dilated vessels. As described above, soluble VE-cadherin may function as an antagonist of full-length VE-cadherin. Thus, lower serum soluble VE-cadherin levels may activate transmembrane full-length VE-cadherin, which may contribute to the pathogenesis of AD and psoriasis through erythema formation. Clarifying the mechanism by which VE-cadherin-mediated vascular abnormality contributes to the pathogenesis may lead to the understanding of these diseases and to novel therapeutic strategies. However, this study has a limitation; although the VE-cadherin levels were lower in patients with psoriasis than in healthy volunteers, the nail change is less frequent in patients with psoriasis with decreased VE-cadherin levels. This paradoxical finding may be due to the small number of patients. A larger study with an increased number of patients is needed in the future.

REFERENCES

1. Genovese A, Detoraki A, Granata F, Galdiero MR, Spadaro G, Marone G. Angiogenesis, lymphangiogenesis and atopic dermatitis. Chem Immunol Allergy 2012;96:50-60.
2. Steinhoff M, Steinhoff A, Homey B, Luger TA, Schneider SW. Role of vasculature in atopic dermatitis. J Allergy Clin Immunol 2006;118:190-197.
3. Gavard J, Gutkind JS. VEGF controls endothelial-cell permeability by promoting the beta-arrestin-dependent endocytosis of VE-cadherin. Nat Cell Biol 2006;8:1223-1234.
4. Lampugnani MG, Orsenigo F, Gagliani MC, Tacchetti C, Dejana E. Vascular endothelial cadherin controls VEGFR-2 internalization and signaling from intracellular compartments. J Cell Biol 2006;174:593-604.
5. Herren B, Levkau B, Raines EW, Ross R. Cleavage of beta-catenin and plakoglobin and shedding of VE-cadherin during endothelial apoptosis: evidence for a role for caspases and metalloproteinases. Mol Biol Cell 1998;9:1589-1601.
6. Li H, Shi X, Liu J, Hu C, Zhang X, Liu H, et al. The soluble fragment of VE-cadherin inhibits angiogenesis by reducing endothelial cell proliferation and tube capillary formation. Cancer Gene Ther 2010;17:700-707.
7. Soeki T, Tamura Y, Shinohara H, Sakabe K, Onose Y, Fukuda N. Elevated concentration of soluble vascular endothelial cadherin is associated with coronary atherosclerosis. Circ J 2004;68:1-5.

JAPANESE DERMATOLOGICAL ASSOCIATION
THE JOURNAL OF DERMATOLOGY
doi: 10.1111/1346-8138.12563
Journal of Dermatology 2014; **41**: 746–748

CONCISE COMMUNICATION

Serum levels of matrix metalloproteinase-13 in patients with eosinophilic fasciitis

Yoshihide ASANO,[1] Hironobu IHN,[2] Masatoshi JINNIN,[2] Zenshiro TAMAKI,[1] Kunihiko TAMAKI,[1] Shinichi SATO[1]

[1]*Department of Dermatology, University of Tokyo Graduate School of Medicine, Tokyo, and* [2]*Department of Dermatology & Plastic and Reconstructive Surgery, Faculty of Medical and Pharmaceutical Sciences, Kumamoto University, Kumamoto, Japan*

ABSTRACT

Matrix metalloproteinase-13 (MMP-13), a member of the collagenase family, has been implicated in the pathogenesis of connective tissue diseases characterized by extracellular matrix remodeling. Since serum MMP-13 levels reflect disease severity of systemic sclerosis and localized scleroderma, we evaluated the clinical significance of serum MMP-13 levels in eosinophilic fasciitis (EF). All the EF patients had serum MMP-13 levels lower than the mean – 2SD of healthy controls. Serum MMP-13 levels were also significantly decreased in EF patients compared with diffuse cutaneous systemic sclerosis, limited cutaneous systemic sclerosis, and generalized morphea patients. Although serum MMP-13 levels did not reflect any clinical and serological features of EF, these results indicate that MMP-13 may be involved in the development of this disease.

Key words: **eosinophilic fasciitis, extracellular matrix, fibrosis, matrix metalloproteinase-13, scleroderma.**

INTRODUCTION

Eosinophilic fasciitis (EF), also known as Shulman syndrome, is clinically characterized by symmetrical scleroderma-like induration of skin over distal extremities, the absence of Raynaud's phenomenon and visceral involvement, peripheral eosinophilia, hypergammaglobulinemia, elevated erythrocyte sedimentation rate, and favorable response to systemically administered corticosteroids. Pathologically, EF features thickening of the fascia with intense inflammatory cell infiltrates composed of lymphocytes, macrophages, and eosinophils.[1,2] Although its pathogenesis still remains elusive, the imbalance between extracellular matrix (ECM) production and degradation is the central event of EF.

Matrix metalloproteinases (MMPs) are important breakdown enzymes of various ECM components. MMPs can be divided into subgroups; collagenases, stromelysins, stromelysins-like MMPs, gelatinases, membrane-type MMPs, and others. MMP-13 belongs to the collagenase family, which degrades various ECM proteins including fibrillar collagens and plays a key role in the MMP activation cascade.[3] MMP-13 expression has been well studied in cancer and rheumatoid arthritis. Elevated MMP-13 expression is observed in highly invasive tumors and correlates with tumor behavior and patient prognosis.[4] In rheumatoid arthritis, MMP-13 is of special interest for the pathogenesis because it cleaves type II collagen of hyaline cartilage more efficiently than the other human collagenases, MMP-1 and MMP-8.[5] Indeed, MMP-13 is highly expressed in the synovial fibroblast-like cells of this disease.[5,6] On the other hand, in systemic sclerosis (SSc) and localized scleroderma, which share the pathological fibrotic process with EF, serum MMP-13 levels correlate with disease severity.[7,8] Thus, MMP-13 may contribute to the development of connective tissue diseases characterized by ECM remodeling.

Based on these backgrounds, to assess if MMP-13 is involved in the pathogenesis of EF, we investigated serum MMP-13 levels and their clinical correlation in EF patients.

METHODS

Patients

Serum samples, frozen at −80°C until assayed, were obtained from 10 EF patients, 40 SSc patients, 10 generalized morphea (GM) patients, and 10 healthy individuals after getting written informed consent. No patients treated with corticosteroids or immunosuppressives were included. All EF patients had the typical clinical and pathological features as described in the Introduction.[1,2] Clinical manifestations and laboratory findings were obtained at the time of serum sampling. Forty SSc patients, including 20 diffuse cutaneous SSc (dcSSc) and 20 limited cutaneous SSc (lcSSc) patients, and 10 GM patients were identical to those enrolled in our previous studies.[7,8] The whole study was performed according to the Declaration of Helsinki and approved by an ethical committee (University of Tokyo Graduate School of Medicine).

Correspondence: Yoshihide Asano, M.D., Ph.D., Department of Dermatology, University of Tokyo Graduate School of Medicine, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan. Email: yasano-tky@umin.ac.jp
Received 16 March 2014; accepted 29 May 2014.

The measurement of serum MMP-13 levels

According to the manufacturer's instruction, specific kits were used for the measurement of serum MMP-13 levels (Amersham Pharmacia Biotech, Piscataway, NY, USA).[7,8] Briefly, polystyrene cups coated with F(ab')$_2$ goat anti-mouse were incubated with mouse anti-MMP-13 antibodies at 37°C for 2 h and subsequently incubated with 100 μL of 10-fold diluted serum at 4°C for 24 h. Then, the cups were washed and incubated at 37°C for 1 h with 50 μL of 0.5 mmol/L p-aminophenylmercuric acetate solution, which changes pro MMP-13 into its active form. Next, the detection reagent, which includes a pro detection enzyme and a specific chromogenic peptide substrate, was added and the absorbance at 405 nm was immediately measured. Then, the cups were incubated at 37°C for 2 h and the absorbance at 405 nm was measured again. A standard curve was generated by plotting $\delta Absorbance_{405}$ (*y* axis) against ng/mL standard (*x* axis) and serum MMP-13 levels were calculated from $\delta Absorbance_{405}$ of each samples using this standard curve. Serum MMP-13 levels of dcSSc, lcSSc, and GM patients in this study were identical to the data reported in our previous studies.[7,8]

Statistical analysis

Statistical analysis was carried out with one-way ANOVA followed by Tukey *post hoc* test for multiple comparison, with the Mann–Whitney *U*-test for two-group comparison, Spearman's rank correlation coefficient to evaluate the correlation with clinical data, and the Shapiro-Wilk normality test to confirm a normal distribution. Statistical significance was defined as a *P* value of <0.05.

RESULTS

Serum MMP-13 levels in EF patients

Serum MMP-13 levels were normally distributed in EF patients and healthy controls. EF patients exhibited significantly lower serum MMP-13 levels than normal controls (33.8 ± 9.8 vs 73.2 ± 11.5 ng/mL, $P < 0.0001$; Fig. 1). Importantly, all the EF patients had serum MMP-13 levels lower than the mean – 2SD of normal controls.

Since we measured serum MMP-13 levels of EF, dcSSc, lcSSc, and GM patients simultaneously on the same ELISA plates (the data of dcSSc, lcSSc, and GM patients were previously reported[7,8]), we next compared serum MMP-13 levels among EF patients, dcSSc patients (53.4 ± 14.1 ng/mL), lcSSc patients (59.4 ± 14.8 ng/mL), GM patients (54.0 ± 18.7 ng/mL), and healthy controls. Notably, one-way ANOVA showed statistically significant differences among five groups ($P < 0.0001$). According to Tukey's multiple comparison test, serum MMP-13 levels were significantly decreased in EF patients compared with all the other four groups ($P < 0.05$ for all). There was also a significant decrease in serum MMP-13 levels of dcSSc patients and GM patients compared with those of healthy controls ($P < 0.05$ for both). These results indicate that MMP-13 may be involved in the pathological fibrotic process of EF as well as SSc and GM.

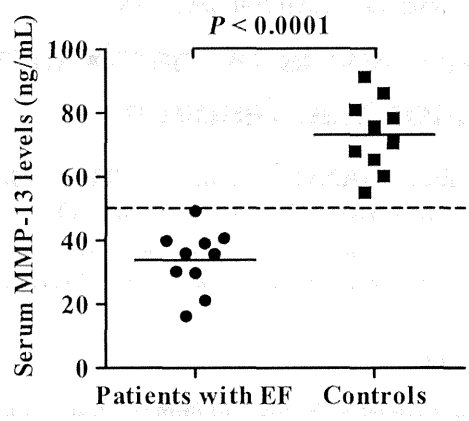

Figure 1. Comparison of serum matrix metalloproteinase-13 (MMP-13) levels between patients with eosinophilic fasciitis and healthy controls. Serum levels of MMP-13 were normally distributed in both patients with eosinophilic fasciitis (EF) and healthy controls. The dotted line indicates the mean – 2SD of serum MMP-13 levels in healthy controls. The horizontal bars indicate the mean value in each group. Statistical analysis was carried out with the Mann–Whitney *U*-test.

Correlation of serum MMP-13 levels with clinical and serological features

To clarify the clinical significance of serum MMP-13 levels in EF patients, we examined a possible correlation of serum MMP-13 levels with various laboratory data, including serum IgG, peripheral cell count of eosinophils, erythrocyte sedimentation rate, and aldolase, but failed to detect any significant correlation (data not shown). Furthermore, there was no significant difference in serum MMP-13 levels between EF patients with morphea-like plaque and those without (33.6 ± 8.1 [$n = 4$] vs 35.2 ± 13.8 [$n = 6$] ng/mL). We also evaluated the correlation of serum MMP-13 levels with serum TIMP-1 levels, which are significantly increased and serve as a useful marker of disease activity in EF patients,[9] but no correlation was found.

DISCUSSION

This study was undertaken to clarify the clinical significance of serum MMP-13 levels in EF patients. Serum MMP-13 levels were significantly decreased in EF patients compared with normal controls. More importantly, all the EF patients had serum MMP-13 levels lower than the mean – 2SD of healthy controls. Although serum MMP-13 levels did not reflect any clinical and serological features of EF, these results indicate that MMP-13 may be implicated in the pathogenesis of this disease.

The function of MMP-13 has been well studied in human fetal skin fibroblasts and human gingival fibroblasts. In these cells, the expression of MMP-13 is increased by TGF-β, a growth factor implicated in ECM accumulation in wound repair and fibrosis.[10–12] It is therefore possible that MMP-13 may play an important role in rapid turnover of collagenous ECM in granulation tissue during normal repair of fetal and gingival wounds, resulting in minimal scar formation. By contrast, TGF-β shows

no significant effect on the expression of MMP-13 in human neonatal skin fibroblasts.[11] However, the expression of MMP-13 is induced by three-dimension culture in human neonatal skin fibroblasts[13,14] and the elevated expression of MMP-13 is observed by *in situ* hybridization in fibroblast- and macrophage-like cells in fibrotic areas of chronic dermal wound.[13] Considering that MMP-13 is a collagenolytic enzyme with a wide substrate specificity, these previous data suggest that the decreased expression of MMP-13 contributes to the establishment of fibrosis through reducing the degradation of ECM proteins. Consistently, serum MMP-13 levels were significantly decreased in EF as well as dcSSc, lcSSc, and GM, which are characterized by the altered ECM remodeling leading to fibrosis. It is noteworthy that serum MMP-13 levels were significantly decreased in patients with EF as compared to those with dcSSc, lcSSc, and GM. These results indicate that the mechanism of fibrosis is different between these diseases and MMP-13 may play a central role in the fibrotic process in EF.

We previously demonstrated that the expression of TIMP-1, an inhibitor of MMP-1, is elevated in the fascia of EF and serum TIMP-1 levels correlate with its disease activity,[9] indicating that the decreased ECM degradation plays a central role in the pathogenesis of EF as well as the excessive ECM production. This notion is strongly supported by the present observation that serum MMP-13 levels are significantly decreased in patients with EF. Further *in vivo* and *in vitro* studies are required to clarify the significance of MMP-13 in the pathogenesis of EF.

CONFLICT OF INTEREST: None.

REFERENCES

1 Shulman LE. Diffuse fasciitis with eosinophilia: a new syndrome? *Trans Assoc Am Physicians* 1975; **88**: 70–86.
2 Shewmake SW, Lopez DA, McGlamory JC. The Shulman syndrome. *Arch Dermatol* 1978; **114**: 556–559.
3 Leeman MF, Curran S, Murray GI. The structure, regulation, and function of human matrix metalloproteinase-13. *Crit Rev Biochem Mol Biol* 2002; **37**: 149–166.
4 Ala-aho R, Kähäri VM. Collagenases in cancer. *Biochimie* 2005; **87**: 273–286.
5 Konttinen YT, Salo T, Hanemaaijer R *et al.* Collagenase-3 (MMP-13) and its activators in rheumatoid arthritis: localization in the pannus-hard tissue junction and inhibition by alendronate. *Matrix Biol* 1999; **18**: 401–412.
6 Petrow PK, Wernicke D, Schulze Westhoff C *et al.* Characterisation of the cell type-specificity of collagenase 3 mRNA expression in comparison with membrane type 1 matrix metalloproteinase and gelatinase A in the synovial membrane in rheumatoid arthritis. *Ann Rheum Dis* 2002; **61**: 391–397.
7 Asano Y, Ihn H, Kubo M *et al.* Clinical significance of serum levels of matrix metalloproteinase-13 in patients with systemic sclerosis. *Rheumatology (Oxford)* 2006; **45**: 303–307.
8 Asano Y, Ihn H, Kubo M *et al.* Clinical significance of serum matrix metalloproteinase-13 levels in patients with localized scleroderma. *Clin Exp Rheumatol* 2006; **24**: 394–399.
9 Jinnin M, Ihn H, Yamane K, Asano Y, Yazawa N, Tamaki K. Serum levels of tissue inhibitor of metalloproteinase-1 and 2 in patients with eosinophilic fasciitis. *Br J Dermatol* 2004; **151**: 407–412.
10 Ravanti L, Häkkinen L, Larjava H *et al.* Transforming growth factor-b induces collagenase-3 expression by human gingival fibroblasts via p38 mitogen-activated protein kinase. *J Biol Chem* 1999; **274**: 37292–37300.
11 Ravanti L, Toriseva M, Penttinen R *et al.* Expression of human collagenase-3 (MMP-13) by fetal skin fibroblasts is induced by transforming growth factor b via p38 mitogen-activated protein kinase. *FASEB J* 2001; **15**: 1098–1100.
12 Uría JA, Jiménez MG, Balbín M, Freije JM, López-Otín C. Differential effects of transforming growth factor-b on the expression of collagenase-1 and collagenase-3 in human fibroblasts. *J Biol Chem* 1998; **273**: 9769–9777.
13 Vaalamo M, Mattila L, Johansson N *et al.* Distinct populations of stromal cells express collagenase-3 (MMP-13) and collagenase-1 (MMP-1) in chronic ulcers but not in normally healing wounds. *J Invest Dermatol* 1997; **109**: 96–101.
14 Ravanti L, Heino J, López-Otín C, Kähäri VM. Induction of collagenase-3 (MMP-13) expression in human skin fibroblasts by three-dimensional collagen is mediated by p38 mitogen-activated protein kinase. *J Biol Chem* 1999; **274**: 2446–2455.

Acta Derm Venereol 2014; 94: 563–567

CLINICAL REPORT

The Proportion of Lymphocytic Inflammation with CD123-positive Cells in Lupus Erythematous Profundus Predict a Clinical Response to Treatment

Azusa MIYASHITA, Satoshi FUKUSHIMA, Takamitsu MAKINO, Yuichiro YOSHINO, Junji YAMASHITA, Noritoshi HONDA, Jun AOI, Asako ICHIHARA, Masatoshi JINNIN, Yuji INOUE and Hironobu IHN

Department of Dermatology and Plastic Surgery, Faculty of Life Sciences, Kumamoto University, 1-1-1 Honjo, Chuo-ku, Kumamoto, Japan

Lupus erythematosus profundus is a rare inflammatory disorder of subcutaneous fat in patients with lupus erythematosus. Previous reports suggested that plasmacytoid dendritic cells, which expressed CD123 and CD303 antigens, play a central proinflammatory role in the pathogenesis of lupus erythematosus. To find the factors that determine the response to treatment, we analysed 23 skin specimens from the patients with lupus erythematosus profundus. The patients with considerable lymphocytic inflammation with high percentages of CD123$^+$ cells in dermis and subcutaneous fat significantly responded to the systemic corticosteroid therapies. On the other hand, the patients with minor lymphocytic inflammation with low percentages of CD123$^+$ cells showed poor response to treatments. The mean percentage of CD123$^+$ cells in patients who showed good response to therapy was significantly higher than those that showed poor response ($p=0.027$). These results suggest that the clinical response to treatment of lupus erythematosus profundus could be predicted from the histological features. *Key words: lupus erythematosus profundus; plasmacytoid dendritic cells; CD123; CD303.*

Accepted Oct 2, 2013; Epub ahead of print Dec 17, 2013

Acta Dermato Venereol 2014; 94: 563–567.

Satoshi Fukushima, MD, PhD, Department of Dermatology and Plastic Surgery, Faculty of Life Sciences, Kumamoto University, 1-1-1 Honjo, Kumamoto, 860-8556, Japan. E-mail: satoshi.fukushima.tb@gmail.com

Lupus erythematosus profundus (LEP) is an inflammatory disorder of the subcutaneous fat in patients with lupus erythematosus (LE). LEP accounts for approximately 1–3% of patients with cutaneous LE (1, 2). The typical clinical presentations are deep subcutaneous nodules or plaques. It may be observed in patients with discoid lupus erythematosus (DLE), systemic lupus erythematosus (SLE) or as an isolated phenomenon without systemic or other cutaneous findings (3, 4). Most commonly the proximal limbs, the trunk and head and neck are involved. Most patients are adults between 20 and 60 years old, with a female to male ratio of approximately 2:1.

Previous reports suggested that plasmacytoid dendritic cells (pDCs) and type I interferon (IFN) play a central proinflammatory role in the pathogenesis of LE (5–7). Plasmacytoid DC have been identified as the main IFN-producing cells in LE and expressed high level of CD123 antigen, which is also known as IL-3 receptor α chain. Alternatively, CD303 (BDCA-2), a type II transmembrane C-type lectin, was reported to be strictly expressed on pDCs. Elevated serum levels of type I IFN in patients with SLE and increased levels of type I IFN in skin lesions of DLE were also reported previously (8–10).

In this study, we investigated clinical, histopathological and immunophenotypical features of LEP, and assessed the association between the histological features and clinical manifestations. Furthermore, we examined the proportion of CD123$^+$ cells in LEP and association between these and the clinical response to treatment.

PATIENTS AND METHODS

Clinical assessment and patient material

Twenty-three patients with LEP and 17 patients with SLE who visited Kumamoto University Hospital between 2004 and 2013 were investigated. The diagnosis of LEP was confirmed by both clinical and histological findings. Diagnosis of SLE was defined as the presence of at least 4 items of American College of Rheumatology (ACR) criteria (4). We analysed their age, sex, duration of disease, clinical symptoms, lesions, histopathologic sections, therapy and clinical response to the treatment. Clinical and laboratory data reported in this study were obtained at the time of sampling of tissue or serum. LE is classified into 5 types, as listed below (5). cutaneous LE (CLE): cutaneous manifestation only, intermediate LE (ILE) I: basically CLE, associated with slight systemic and/or laboratory findings, ILE II: basically SLE, not satisfying the criteria of SLE, SLE I: lacking systemic and/or laboratory findings listed below, SLE II: associated with renal involvement, central nervous system (CNS) involvement, thrombocytopenia, haemolytic anaemia and/or serositis. Institutional review board approval and written informed consent were obtained before patients were entered into this study according to the Declaration of Helsinki.

Antinuclear antibodies

Antinuclear antibodies (ANA) were detected by indirect immunofluorescence using HEp-2 cells as the substrate and double immunodiffusion, as described previously (6).

 doi: 10.2340/00015555-1777
 ISSN 0001-5555

Histology

Twenty-three skin samples were obtained from the lesions clinically suggesting panniculitis. Seventeen skin samples were obtained from patients of SLE. Haematoxylin-eosin stained sections were examined and Peters and Su histologic criteria were used for the diagnosis of LEP (3): lymphocytic aggregates, hyaline degeneration of the fat, lobular or septal panniculitis, lymphocytic vasculitis, and calcification criteria. Lesions of DLE were seen in about half of cases. In this study, we examined the presence of the following morphologic criteria: (*i*) Epidermal involvement: atrophy and/or flattening of the epidermis, vacuolar degeneration of the basal layer, necrotic keratinocytes, and hyperkeratosis. (*ii*) Dermal involvement: pattern of infiltration, involvement of the hair follicles, mucin deposition, and presence of fibrosis and/or sclerosis. (*iii*) Subcutaneous involvement: pattern of infiltration (lobular or septal) and presence or absence of the features: fibrosis of the septae, hyaline fat necrosis, calcium deposits, vasculitis, granulomatous reaction and lymphocytic nuclear dust.

To investigate an association between the histopathological features and the clinical manifestations, we evaluated the patterns of infiltrated lymphocytes and classified the histopathological patterns of the cases into 3 types listed below. Type 1: involves all layers of the skin (associated with DLE), type 2: involves dermis and fat only, type 3: involves only fat tissue. These samples were estimated independently by the 2 observers in a blinded manner (A.M. and S.F.). Some researchers have used the term LEP as a synonym for lupus panniculitis, and some have used the term LEP to designate the patients who have both lupus panniculitis and DLE lesions, and the term lupus panniculitis to refer to those having only subcutaneous lesions (11, 12). In this study, we used the term LEP for lupus panniculitis including the cases that have only subcutaneous lesions.

Direct immunofluorescence study

For the direct immunofluorescence staining, 4 μm sections were cut in a cryostat at –20°C and then placed on special slides. Direct immunofluorescence studies were performed using fluorescein isothiocyanate labelled anti-human IgG, IgM, IgA, complement C3 and fibrin (Dako, Tokyo, Japan) according to the instructions.

Immunohistochemistry

Skin samples were fixed in 10% neutral-buffered formalin and embedded in paraffin. Staining with mouse anti-human CD123 antibody (clone 7G3; BD Biosciences, CA, USA) was performed as a surface marker of pDCs. Deparaffinised sections were retrieved by incubation with citrate buffer, pH9, for 5 min in an autoclave. Endogenous peroxidase activity was inhibited after which sections were incubated with 5% normal goat serum for 20 min at room temperature. Anti-human CD123 antibody was used at a concentration of 1:50 and incubated with the specimens overnight in a humidifying chamber. After excess antibody was washed off with PBS, samples were incubated with horseradish peroxidase (HRP)-labelled goat anti-mouse antibody (Nichirei, Tokyo, Japan) for 60 min. The reaction was visualised by the DAB substrate system (Dojindo, Kumamoto, Japan). Slides were counterstained with Mayer's haematoxylin, and mounted using aqueous medium. Primary isotype monoclonal mouse antibodies (clone MG2a-53; abcam, Tokyo, Japan) was used as negative control. Stained sections were evaluated in the percentages of positive cells for CD123 staining among the infiltrated mononuclear cells. The cells were countered in 5 different 400-fold magnification fields under a light microscope.

Double-colour immunofluorescence staining

Double-colour immunofluorescent staining of cryostat sections of skin samples was performed using anti-CD303 mAb (clone AC144; Miltenyi Biotec, CA, USA) and anti-CD123-PerCP-Cy 5.5 mAb (clone 7G3; BD Biosciences, CA, USA). Staining with non-conjugated primary mAb was revealed by secondary staining with Alexa Fluor 488-conjugated anti-mouse IgG Ab (Invitrogen, Oregon, USA). The sections were finally mounted with a medium containing 4,6-diamidino-2-phenylindole (DAPI) (VECTER; CA, USA) for nuclear fluorescent staining, and viewed under fluorescence microscope. Primary isotype monoclonal mouse antibodies (clone MOPC-31C; BD Pharmingen, Tokyo, Japan and clone MG2a-53; abcam, Tokyo, Japan) were used as negative controls.

Statistical analysis

Statistical analysis was carried out with Mann-Whitney's U test and Kruskal-Wallis test using the statistical software Excel (Microsoft Corp., Redmond, WA, USA) and Statcel (OMS Publishing Inc, Tokyo, Japan). Correlation analysis was done by Spearman's rho test. p-value <0.05 was considered statistically significant.

RESULTS

Clinical findings

The profiles of 23 patients were shown in Table SI[1]. The majority of the patients (74%) were over 30 years of age, mean age at diagnosis was 36.8 years (range 12–59 years). A female to male ratio was 20:3. Proximal limbs were the most commonly involved sites (61%) followed by head and/or neck (35%) and trunk (8%). The majority of clinical symptoms was indurated erythema or indurated plaques. Duration of symptoms varied from 1 month to 14 years (up to 6 months: 61%, more than 6 months: 39%). Five patients (21%) fulfilled the ACR criteria for SLE.

Laboratory findings

Antinuclear antibodies were positive in 13 of 19 cases (68%). Anti-double-stranded DNA antibodies were positive in 3 of 23 cases (13%). Peripheral blood counts abnormalities including anaemia, thrombocytopenia, and leucopenia were observed in 3 of 23 patients (13%).

Histopathologic findings

Skin biopsies were performed in all patients. The histopathologic findings in the lesions clinically suggesting panniculitis are characterised by lymphocytic panniculitis, hyaline degeneration of the fat, perivascular lymphocytic infiltration in dermis and lymphoid nodular structures in the lower dermis and subcutaneous tissue. Lesions of DLE were observed in 12 of 23 cases (52%). Mucin depositions were seen in 8 of 23 cases

[1]http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1777