FIGURE LEGENDS

Figure 1

(a)

Clinical appearance of the nodule and papule on the left upper eyelid.

(b-e)

Skin biopsy findings: Hematoxylin and eosin staining reveals abundant infiltrate of lymphocytes, plasma cells, histiocytes, and giant cells. Low-power magnification (b), and high-power magnification (c). The periodic acid-Schiff stain (d) and Grocott methenamine silver stain (e) show the presence of round and budding yeast-like cells in the dermis.

(f, g)

Cultures of the biopsy specimen on Sabouraud's dextrose agar and potato dextrose agar: dark brown velvety colonies form (f). From slide cultures, branching hyphae with slender, tapering conidiophores rising at right angles are seen. The apex of the conidiophore bears pear-shaped or almost round conidia, giving it a flower-like appearance. Dark brown conidia are produced singly along the hyphae and conidiophores (g).

Figure 2

(a)

Clinical appearance of the nodule on the left mandible.

(b-d)

Skin biopsy findings: Hematoxylin and eosin staining reveals chronic inflammatory granuloma with infiltration of neutrophils, histiocytes, and giant cells. Low-power magnification (b), and high-power magnification (c). The Grocott methenamine silver stain (d) shows a few round yeast-like cells in the dermis.

(e, f)

Cultures of the biopsy specimen using Sabouraud's dextrose agar: a dark brown velvety colonies yield grows (e). Slide cultures from colonies show fine branching, septate hyphae with spherical conidia at the tips of the conidiophores, and thick-walled dematiaceous conidia along the conidiophores and hyphae (f).

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Case Letter for *The Journal of the American Academy of Dermatology*

Aleukemic leukemia cutis with extensive bone involvement

Maria Maroto Iitani, Riichiro Abe, Teruki Yanagi, Asuka Hamasaka, Yasuki Tateishi, Yukiko Abe, Miki Ito*,Takeshi Kondo**, Kanako Kubota***, Hiroshi Shimizu

Department of Dermatology, Hokkaido University Graduate School of Medicine, Sapporo, Japan

* Department of Dermatology, National Hospital Organization Hokkaido Cancer Center, Sapporo, Japan

** Department of Gastroenterology and Hematology, Hokkaido University Graduate School of Medicine, Sapporo, Japan

***Department of Surgical Pathology, Hokkaido University Hospital, Sapporo, Japan

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Correspondence and reprint requests to:

Maria Maroto Iitani, M.D.

Department of Dermatology,

Hokkaido University Graduate School of Medicine.

North 15, West 7, Kita-ku, Sapporo 060-8638, Japan,

Phone: +81-11-716-1161 ext. 5962

Fax: +81-11-706-7820

e-mail: miitani@med.hokudai.ac.jp

To the editor: Aleukemic leukemia cutis (ALC) is a rare condition that is characterized by the invasion of leukemic cells into the skin prior to such cells being observed in the peripheral blood.[1] Here, we present a case of ALC with multiple bone metastases.

An 81-year-old male presented with a two-month history of an asymptomatic nodule on his left thigh. A careful physical examination revealed five subcutaneous nodules measuring up to 10mm in size on his trunk and legs together with a firm, slightly violaceous nodule measuring 20 mm in diameter on his left thigh (Fig. 1). A complete blood cell count and chemical analysis showed no pathologic changes. Histopathologic examination revealed dense, nodular, diffuse infiltrate of monotonous uniform cells with round nuclei, prominent single or multiple nucleoli, and abundant pale, slightly eosinophilic cytoplasmic cells throughout the dermis and subcutaneous fat (Fig. 2a). Atypical mitotic figures were scant (Fig. 2b). Histological diagnosis of myeloid leukemia cutis with possible monocytic lineage was made. However, bone marrow aspiration showed neither an increase in blasts nor abnormal cell infiltration, and repeated peripheral blood counts were normal, with no atypical cells. A diagnosis of ALC was established. Positron emission tomography revealed extensive high-density areas on the bone and the subcutaneous tissue, suggesting multiple metastases (Fig. 3). At seven weeks after the first visit, peripheral blood examination disclosed 8% of atypical monocytic cells indicating a diagnosis of acute myeloid leukemia (AML). The patient died about one week later.

ALC is a rare form of leukemia with poor prognosis. It was first reported by Yoder in 1976.[2] The term aleukemic has been used to designate a form of leukemia in which there are no leukemic cells in the blood.[2] ALC precedes to the peripheral blood or bone marrow abnormalities at least one month before the systemic findings. Once leukemic cells appear in the peripheral blood or bone marrow, the mean survival time ranges from 3 to 30 months.[3,4] The clinical features of ALC include multiple papules, nodules or infiltrated plaques with a red-brown or plum coloured surface. Histological findings show infiltration of leukemic cells in the dermal or subcutaneous tissues. The cytologic features of the tumour cells include the large size, vesicular nuclei, and multiple prominent nucleoli.[3] There is no consensus on the treatment of choice for ALC, due to rarity of the disease; radiotherapy, chemotherapy and total body electron therapy have been reported to achieve varying results.[1,3,4,5,6,7,8] A study of the largest group of ALC patients, by Chang et al., showed the commonest extramedullary site in ALC after the skin to be the lymph node (8 patients out of 31), followed by the spleen (2 patients out of 31).[4] Although no reports of clinical presentation of ALC with multiple bone infiltration were found in a thorough search of the English-language literature, extramedullary leukemia is known to occur in the bone.[5] It is to be emphasized that routine assessment of a patient with ALC should include systemic investigations such as PET scan in our case, taking into consideration the possibility of bone involvement..

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Fig. 1. A slightly violaceous nodule on the left thigh.

Fig. 2. Skin biopsy findings: (a) Dense, nodular, diffuse infiltrate of monotonous uniform cells involving the dermis and subcutaneous fat. (b) A nodule of cells with round nuclei, prominent single or multiple nucleoli and abundant pale, slightly eosinophilic cytoplasm and scant atypical mitotic figures.

Fig. 3. PET reveals extensive high-density areas on the bone and in the subcutaneous tissue on the trunk and the extremities, suggesting multiple metastases.

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HOW I DO IT

Controlling the Histological Margin for Non-Melanoma Skin Cancer Conveniently Using a Double-Bladed Scalpel

SATORU AOYAGI, MD, PhD,* HIROO HATA, MD, ERINA HOMMA, MD, AND HIROSHI SHIMIZU, MD, PhD
Department of Dermatology, Hokkaido University Graduate School of Medicine, Sapporo, Japan

Background: In some countries, intraoperative histological evaluation to control the surgical margin for non-melanoma skin cancer is widely used instead of Mohs micrographic surgery. Nevertheless, this evaluation by frozen section analysis is usually limited to suspicious areas.
Objectives: To evaluate the efficacy of double-bladed scalpel for intraoperative histological margin control for non-melanoma skin cancers.
Methods: Between 2005 and 2009, 10 basal cell carcinomas and 5 squamous cell carcinomas were underwent complete histological margin control in which a double-bladed scalpel was used during the surgery at the Hokkaido University Hospital in Japan.
Results: The mean number of re-excisions required for complete tumor resection was 1.4 times. Nine (60%) of the 15 patients obtained histological clearance of all surgical margins at the first re-excision. The mean size of total surgical margin was 6.1 mm (range: 2–12 mm). The median time from the first tumor excision to reconstruction was 124 min. No local recurrences have been reported.
Conclusions: This method may be used as an alternative for complete histological margin control at many hospitals where it is difficult to perform Mohs micrographic surgery.
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KEY WORDS: **basal cell carcinoma; squamous cell carcinoma; Mohs micrographic surgery; intraoperative histological evaluation; frozen section**

INTRODUCTION

Non-melanoma skin cancer (NMSC) is the most common malignancy in the world, and its incidence continues to rise [1]. The majority of these are basal cell carcinomas (BCCs) and squamous cell carcinomas (SCCs). Standard excision with adequate surgical margin is the most common surgical treatment for these, providing a high cure rate for small, low-risk skin cancers [2]. In conventional surgery, where the samples are sent to pathology for margin assessment, the tissues are sliced vertically at 2- to 4-mm intervals to make microscopic sections.

Some of these cancers that grow asymmetrically and with a much wider subclinical extension tend to show high recurrence rates [3,4]. For these high-risk continuous skin cancers, Mohs micrographic surgery (MMS), which is based on the concept of excising skin cancer layer by layer and examining horizontally cut specimen sections, affords a high cure rate by achieving precise removal [5]. MMS is a well-established technique; it is the gold standard of care for selected cases of skin cancers in Western countries [5].

However, due to differences among countries in insurance systems and surgical approaches, MMS is still uncommon in Asian countries [6]. In some communities, intraoperative histological evaluation to control the surgical margin is widely used instead of MMS [7]. Nevertheless, intraoperative histological evaluation by frozen section analysis is usually limited to suspicious areas. The accuracy of such analysis for detecting hisitological surgical margin of skin cancer is highly dependent on the methods used to obtain and analyze the margins. For institutions at which it is difficult to perform MMS, we introduce double-bladed scalpel (DBS) as a novel simple method for complete histological margin control (Fig. 1). This improves the pathodiagnostic reliability of conventional intraoperative histological evaluation.

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MATERIALS AND METHODS

Between September 2005 and August 2009, 15 patients with biopsy-proven BCC or SCC were referred to our institution for treatment of their lesion and underwent complete histological margin control in which a DBS was used during the surgery at the Department of Dermatology, Hokkaido University Hospital in Japan. The indications for this procedure were histologically aggressive subtype (BCC: morpheaform, infiltrative, micronodular; SCC: poorly differentiated), high risk of recurrence (BCC on the H-zone of the face; SCC on the lip and ear) and tumor with ill-defined clinical margins. Information recorded included the patient age and gender, the lesion site and clinical features, the tumor size, the histological diagnosis and subtype, the type of anesthesia, the total time required for the procedure, the type of reconstruction, the number of re-excisions required to achieve tumor-free margins, the initial and final surgical margin, and the outcome.

Prior to surgery, the gross macroscopic extent of the tumor was measured as the long axis and recorded as the tumor size. Each margin after re-excision was added to the initial surgical margin. After surgery had achieved a histologically negative margin, the measurement of this margin was recorded as the final surgical margin. The clinical

*Correspondence to: Satoru Aoyagi, MD, PhD, Department of Dermatology, Hokkaido University Graduate School of Medicine, N15 W7, Kita-ku, Sapporo 060-8638, Japan. Fax: +81-11-706-7820.
E-mail: saoyagi@med.hokudai.ac.jp

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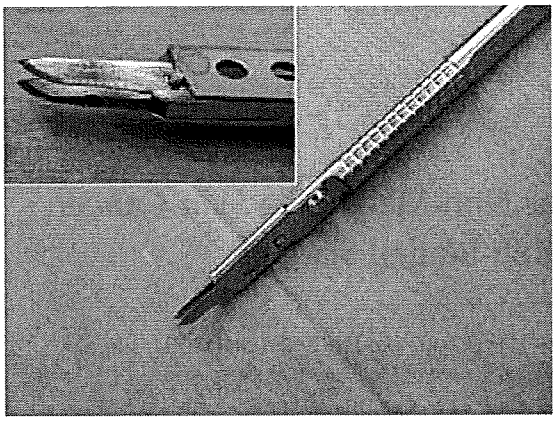

Fig. 1. A newly devised DBS set with a 2-mm interblade gap with a lightweight cylindrical holder.

and histological features of the patients are shown in Table I. This procedure is detailed in Figure 2.

Surgical Technique

Before injection of local anesthesia, the gross tumor and surgical margins were marked with blue ink under meticulous dermoscopic observation. Initial surgical margins of 2–5 mm were proposed, with the size depending on the condition of the patient. The adequate margin will vary with tumor size, location, clinical definition of border and surgeon's preference. Next, using a DBS, the inside blade was set at the surgical margin line and an excision was made at least to the depth of the subcutaneous tissue (Fig. 2A). Then two parallel lines were made to excise a 2-mm width around the tumor.

The gross tumor with initial surgical margin was then excised in a bowl shape (Fig. 2B) and sent to the pathology lab for conventional paraffin-embedded vertical sections, for assessment a few days after the surgery.

After excision of the tumor, the defect was sub-sectioned into two to four regions (Fig. 2C). The sub-sectioned defect was excised along with the outer 2-mm strips of skin made by DBS. It was excised at a uniform width by scissors with great care to avoid tearing tissue (Fig. 2D), as in re-excision of an additional layer for histological analysis in conventional MMS.

The re-excised specimens were flattened and face down from the tumor side for the preparation of a frozen tissue block of specimen. Horizontal sections were sliced starting from the true margin side. Sections were stained in the conventional manner with hematoxylin and eosin. The horizontal sectioning of the re-excised tissue layer allows for the identification of both deep and peripheral margins on the same section. This is an important essential technique of MMS in our method, as the complete surgical margin can be evaluated histologically.

All slides were examined by a dermatologic surgeon and pathologist together during the surgery. If a tumor-positive margin in a re-excision specimen was obtained, additional layers of tissue were excised from the tumor-positive area. This second excision specimen was sent for permanent-section analysis a few days after the surgery. Defect repair was performed by a dermatologic surgeon on the same day, except when a tumor-positive margin was obtained in re-excision. When any of the additional layers of the margins ware positive, the reconstruction was scheduled for the postoperative day after tumor-free margins were confirmed by conventional margin assessment in permanent slides.

RESULTS

All 15 of the primary tumors (10 BCCs and 5 SCCs) from 15 patients were completely resected with complete histological margin control using DBS during the surgery. These tumors were also histologically confirmed by permanent section later. The 15 patients (8 male (53.3%), 7 female (46.7%)) met the criteria of histologically aggressive subtype, high risk of recurrence and tumor with ill-defined clinical margins. The median age was 72.6 years (range: 48–90). All patients were ethnic Japanese. All tumors were located on the head and neck, with five (33.3%) on the nose, five (33.3%) on the lip, two (13.3%) on the cheek, one (6.7%) on the cantus, one on the neck and one on the ear. The median size of tumor was 18.5 mm (Table 1).

Four (26.7%) of the 15 patients had the surgery under general anesthesia. The mean number of re-excisions required for complete tumor resection was 1.4 times. Nine (60%) of the 15 patients obtained histological clearance of all surgical margins at the first re-excision. Six patients (40%) required an additional re-excisional layer to achieve clear margins. The mean size of initial surgical margin was 4.6 mm (range: 2–10 mm) and the total surgical margin was 6.1 mm (range: 2–12 mm). Surgical defects were reconstructed with primary closure in 2 patients (13.3%), local flap in 9 patients (60%), and combination of local flap and tissue grafting in 4 patients (26.7%). The median time

TABLE I. Summary of Clinical Histological Features of NMSC Cases in the Present Study

Case	Age/gender	Diagnosis	Tumor site	Tumor size (mm)	Clinical feature of the lesion	Histologic subtype
1	81/F	BCC	Cantus	8	Ill-demarcated, pink colored plaque	Micronodular
2	87/M	BCC	Lip	14	Ill-demarcated, pink colored nodule	Micronodular
3	75/M	BCC	Neck	32	Well-demarcated, slightly pigmented, ulcerated nodule	Micronodular
4	64/F	BCC	Cheek	10	Ill-demarcated, pink colored nodule	Micronodular
5	77/F	BCC	Cheek	11	Ill-Demarcated, pink colored nodule	Micronodular
6	77/M	BCC	Ear	8	Ill-demarcated, slightly pigmented, ulcerated plaque	Morpheic
7	69/F	BCC	Nose	7	Ill-demarcated, red colored plaque	Morpheic
8	68/M	BCC	Nose	11	Well-demarcated, slightly pigmented nodule	Micronodular
9	71/F	BCC	Nose	25	Ill-demarcated, pink colored, ulcerated nodule	Morpheic
10	56/M	BCC	Nose	30	Ill-demarcated, slightly pigmented, ulcerated plaque	Morpheic
11	82/F	SCC	Nose	20	Well-demarcated, red colored, ulcerated nodule	Poorly differentiated
12	90/M	SCC	Lip	20	Well-demarcated, pink colored, keratotic nodule	Moderately differentiated
13	48/M	SCC	Lip	32	Ill-demarcated, red colored, keratotic nodule	Poorly differentiated
14	65/M	SCC	Lip	10	Well-demarcated, pink colored, ulcerated nodule	Moderately differentiated
15	79/F	SCC	Lip	40	Ill-demarcated, red colored, ulcerated nodule	Moderately differentiated

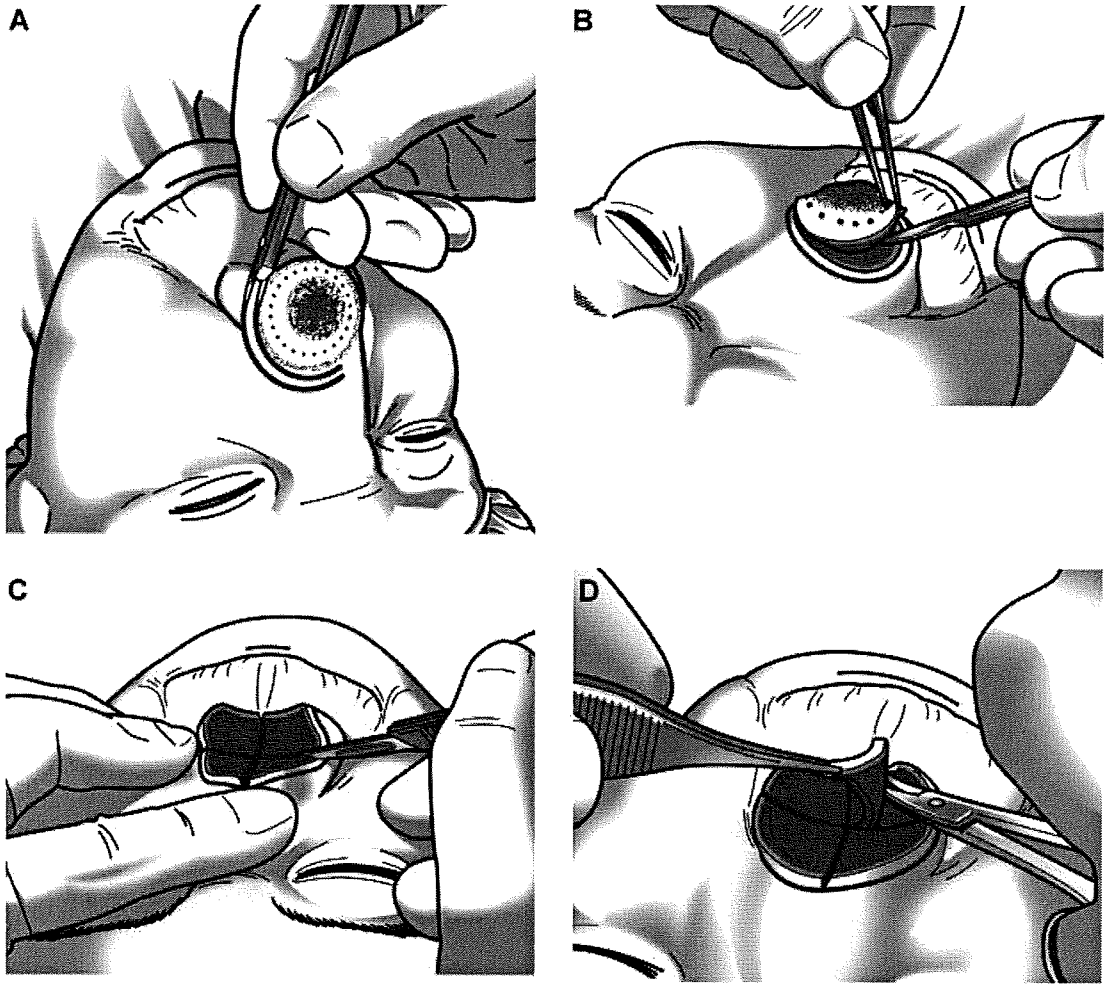

Fig. 2. Schematic diagrams of our technique. **A**: Using a DBS, parallel excisions are made around the tumor. **B**: The gross tumor with initial surgical margin is excised in a bowl shape along the DBS inner excision. **C**: The defect is sub-sectioned into two to four regions, varying with tumor size. **D**: An additional layer of tissue along with 2-mm outer strips of the skin made by DBS is excised at a uniform width by scissors as in conventional MMS.

TABLE II. Summary of Results of NMSC Cases Treated by Micrographic Surgery in Our Simple Method for Complete Histological Margin Control

Case	Anesthesia	Total procedure time (min)	Type of reconstruction	Number of re-excision	Initial surgical margin (mm)	Final margin (mm)	Recurrence
1	Local	104	Flap	2	5	7	None
2	Local	89	Flap	1	2	2	None
3	Local	116	Flap	1	3	3	None
4	Local	80	Flap	1	3	3	None
5	Local	132	Flap	1	5	5	None
6	Local	64	Closure	1	10	10	None
7	Local	131	Flap	2	3	7	None
8	Local	124	Flap	2	3	5	None
9	General	133	Flap, skin graft	2	6	12	None
10	General	232	Combination flap, cartilage graft	2	6	10	None
11	General	167	Combination flap, cartilage graft	1	6	6	None
12	Local	58	Closure	1	2	2	None
13	General	151	Flap	1	7	7	None
14	Local	107	Flap	1	4	4	None
15	Local	170	Combination flap	2	4	9	None

from the first tumor excision to reconstruction, including the assessment of the frozen section, was 124 min. No local recurrences have been reported to date, after a median follow-up of 14.9 months (range: 1–48 months) (Table II). As shown in Figure 3, SCC on the lower lip who underwent complete histological margin control was reconstructed with combination of local flap.

DISCUSSION

Although MMS is the most useful surgical method for high-risk skin cancer in USA and European countries, it is generally performed only under local anesthesia by experienced Mohs surgeons and technicians [5]. However, some cases of large and deep defect require reconstruction under general anesthesia. In these cases, reconstructive surgery should be planned not on the same day of the resection. In contrast, intraoperative histological evaluation is frequently performed in many institutions at which it is difficult to perform a complete MMS procedure due to health insurance and other reasons [7]. Intraoperative histological evaluation is especially common for surgery under general anesthesia in which resection and reconstruction are completed together [8]. To save time and increase the accuracy of histological analysis for surgical margins, it is easier and more convenient to add an essential technique of MMS to conventional intraoperative histological evaluation than to introduce a new MMS system itself from the beginning. The technical simplicity of this procedure allows it be used by non-MMS surgeons in the treatment of non-melanoma patient at most institutions.

Micrographic surgery in which complete histological visualization and evaluation of surgical margins, is used to detect unseen tumor outgrowth [9]. In addition to the traditional Mohs micrographic surgery, many variations of this histopathological approach for surgery have been developed and described in the literature [10]. Although the approach differs for each variation, methods are categorized as those that use a frozen section versus using a permanent section [11,12], and those that evaluate the peripheral margin only versus including the deep margin [13].

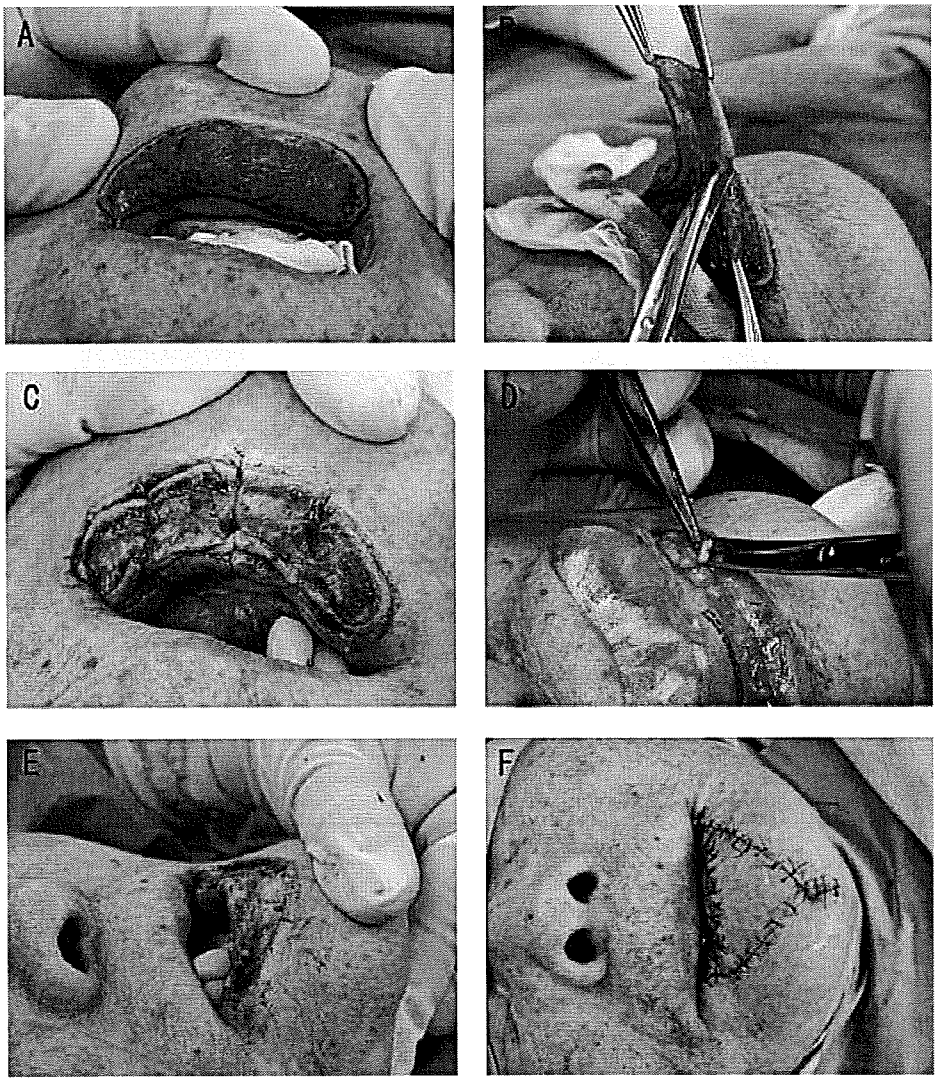

Fig. 3. A 79-year-old woman with SCC on the lower lip who underwent complete histological margin control by frozen section (case 15 in Table I and II). **A**: The initial surgical margin is 2 mm (solid line) from the clinical border (dotted line). **B**: After parallel excision by DBS, the central tumor can be easily resected along the inner line. **C**: After excision of the tumor, the defect is sub-sectioned into four regions. **D**: Each piece is excised easily in uniform width of skin. **E**: The final defect after complete excision of tissue under histological evaluation by frozen section. **F**: Double V-Y advancement flap and mucous transposition flap from the right cheek are used to reconstruct the lower lip.

A basic element of our procedure is resection of the whole tumor followed by concurrent excision of an additional outer layer for complete histological evaluation of the excision margins in three dimensions. That element involves completing the first two steps of MMS together by using permanent sections for histological evaluation of the tumor and using the frozen section only in re-excision of specimens of an additional outer layer from the tumor defect. Our system requires fewer frozen tissue sections to be processed than for MMS. There is a definite difference in total procedure time between our procedure and MMS. In our procedure, a number of each process for making frozen section has to limit in once or twice. Therefore, one distinguishing feature our procedure is the detection and removal of subclinical irregular invasions of tumor cells rather than aesthetic preservation through maximum tissue conservation.

To improve the efficiency of our procedure, an additional layer should excised correctly after gross tumor resection, and this layer should include skin with uniform width for ease in preparing frozen sections. Thus, the distance between the double blades is set at 2 mm. Minor successive improvements to the scalpel holder allow a parallel excision line to be made in a single motion. The greatest advantage of the DBS over the single blade is that it makes it easier for the surgeon to harvest tissue strips of uniform width. The uniform strips are more easily processed for frozen section evaluation, which increases the efficiency of the total intraoperative procedure time and the accuracy of histological analysis for surgical margins.

The DBS consists of a handle with two parallel scalpel blades that may be set at variable widths by adjusting metal spacers. The concept of this device was described in 1977 by Coiffman [14] and the device was originally created to harvest donor strips for hair transplantation. The DBS has been described with respect to the removal of surgical scars [15], BCCs [16], non-melanoma skin cancers [17] and dermatosarcoma protuberans [18]. A few articles detail the potential advantages of the DBS in micrographic surgery [18]. However previous reports describe methods using DBS only for peripheral margin evaluation or for staged margin evaluation, and not for true MMS procedures [18].

There is the concern that the procedure will increase the time required to complete tumor removal, compared to conventional intraoperative histological evaluation. However, 60% of our procedures required only one re-excison for complete histologic margin control. Complete surgical margin negative required only two re-excisions in total. The median procedure time was 107 min to clear the surgical margin with one re-excision and 149 min to clear it with two re-excisions. These procedure times would not differ significantly from those for patients undergoing conventional surgical excision with intraoperative histological evaluation. Furthermore, no local recurrences have been reported in on our short-term follow-up (14.9 months). We believe that the reliability of our method is high, although long-term follow-up data are required to substantiate the effectiveness of this surgical approach.

CONCLUSION

In conclusion, DBS may be used as an alternative for complete histological margin control at many hospitals where complete MMS is not available, especially in Asia. This method appears to be time-saving and easy to apply with existing systems. It is convenient and may require fewer excisions than with MMS.

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Correspondence

Desmoglein1 and BP 180 ELISA indexes correlating with disease activity in a patient with coexisting pemphigus foliaceus and bullous pemphigoid

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An 88-year-old Japanese woman presented with erosions in the oral cavity. On physical examination, oral erosions, tense vesicles and erythema with partially crusted erosions were seen on the trunk. Acantholysis (Fig. 1a) and subepidermal blisters (Fig. 1b) were seen in skin biopsy specimens taken from the trunk. Direct immunofluorescence of the skin revealed *in vivo* deposition of IgG in both the cell surface and the basement membrane zone (BMZ) of the epidermis (Fig. 1c). Using indirect immunofluorescence using human skin section as substrate, IgG autoantibodies against cell surface and BMZ were detected at a titre of > 1 : 160 (Fig. 1d). Immunoblotting assays using normal human epidermal extracts and BP180 NC16a domain

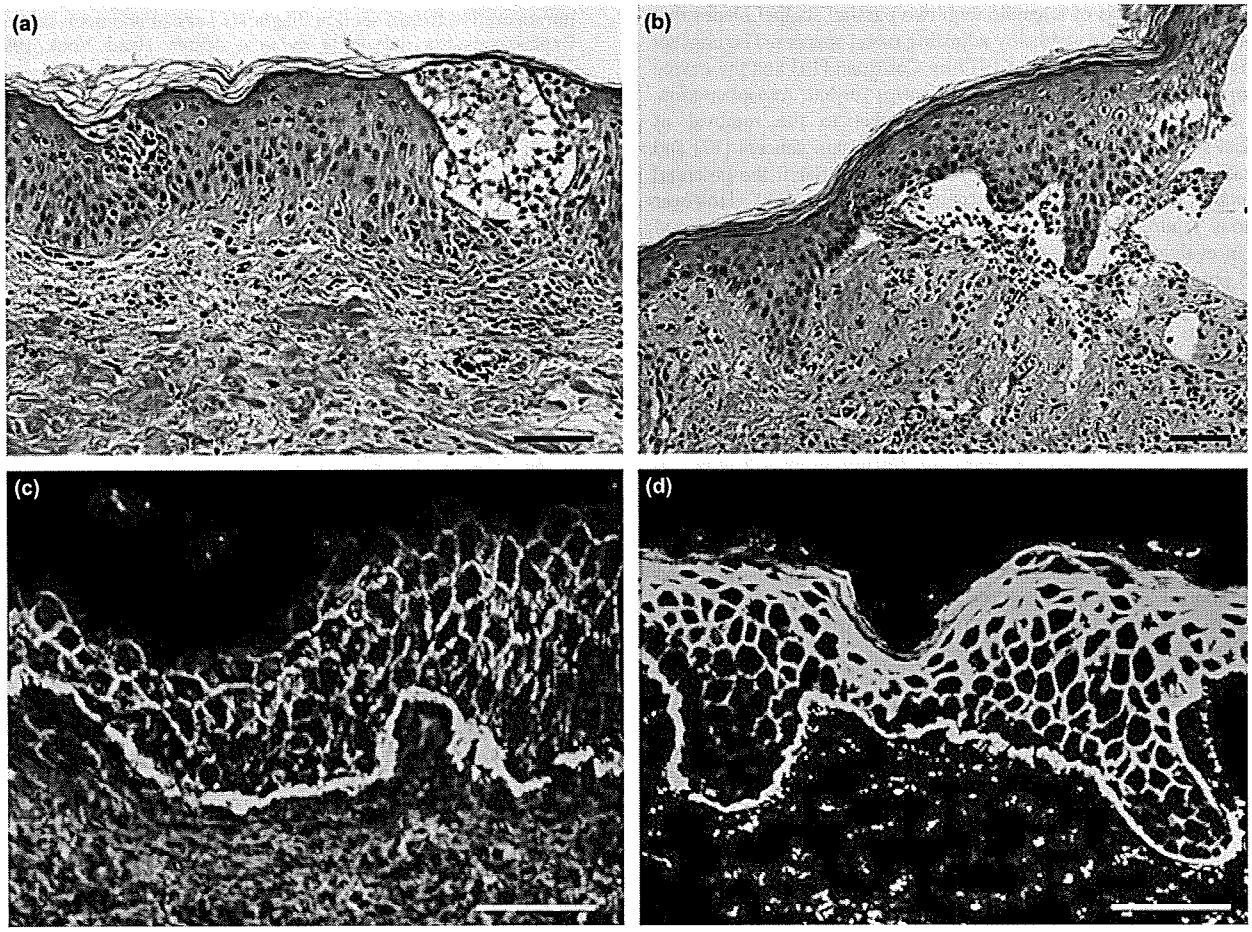

Figure 1 (a) Acantholysis and (b) subepidermal blisters (haematoxylin and eosin). Both (c) direct immunofluorescence of the skin and (d) indirect immunofluorescence using human skin sections confirmed IgG antibodies to the cell surface and basement membrane zone of the epidermis. Scale bar, 100 μm.

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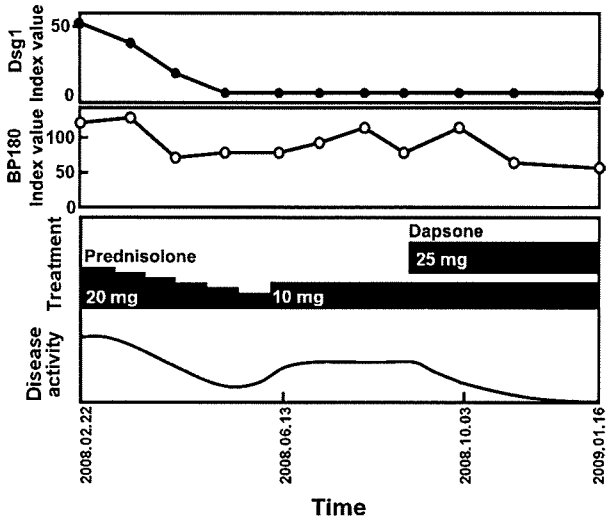

Figure 2 Desmoglein (Dsg)1 and BP180 ELISA indexes correlated with disease activity. With oral prednisolone 20 mg/day, the erosions and blisters disappeared, and the Dsg1 ELISA index became negative. Additional treatment with dapsone 25 mg/day, resulted in resolution of the bullous lesions and the BP180 ELISA index gradually decreased.

recombinant protein confirmed the presence of serum IgG antibodies against BP180 and the BP180 NC16a domain, respectively. The desmogein (Dsg)1 and BP180 ELISA indexes were 54 (normal range < 20) and 125 (normal range < 8), respectively. Oral prednisolone 20 mg/day and topical steroid application were effective, and the Dsg1 ELISA index decreased to 10. However, the BP180 ELISA index did not decrease, and it was still 88 when the patient had a flare while on oral prednisolone 5 mg/day. Oral dapsone 25 mg/day plus prednisolone 10 mg/day cleared the bullous skin lesions, and the BP180 ELISA index gradually decreased (Fig. 2).

In this case, the clinical features, histological findings, immunofluorescence and ELISA indexes confirmed the simultaneous coexistence of PF and BP. Previous reports[1–3] reported the simultaneous coexistence of PF and BP based on histological or immunofluorescence findings. None of the three cases had coexistence of anti-Dsg1 antibodies and anti-BP180 antibodies by ELISA. To our knowledge, our patient is the first case of coexistence of PF and BP confirmed by positive ELISA results for both anti-Dsg1 and anti-BP180 antibodies.

It was previously reported that ELISAs for Dsg1, Dsg3 and BP180 are more sensitive and specific than indirect immunofluorescence and that ELISA indexes tend to correlate with the disease activity.[4,5] In our case, Dsg1 and BP180 ELISA indexes correlated with disease activity along the time course. ELISA is a valuable tool for monitoring disease activity and provides the important information for determining treatments for various immunobullous diseases.

S. Ando, K. C. Sato-Matsumura, M. Kasai, I. Nemoto-Hasebe,* D. Hoshina,* B. Ohyama,† T. Hashimoto† and H. Shimuzu*

*Department of Dermatology, Sapporo Social Insurance General Hospital, Sapporo, Hokkaido, Japan; *Department of Dermatology, Hokkaido University School of Medicine, Sapporo, Hokkaido, Japan; and †Department of Dermatology, Kurume University School of Medicine, Kurume, Fukuoka, Japan*

E-mail: satoan@sapporo-shaho.jp

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Comparison of skin barrier function and sensory nerve electric current perception threshold between IgE-high extrinsic and IgE-normal intrinsic types of atopic dermatitis

T. Mori, K. Ishida,* S. Mukumoto, Y. Yamada, G. Imokawa,† K. Kabashima, M. Kobayashi, T. Bito, M. Nakamura, K. Ogasawara‡ and Y. Tokura

Department of Dermatology, University of Occupational and Environmental Health, 1-1 Iseigaoka, Yahatanishi-ku, Kitakyushu 807-8555, Japan
*Personal Health Care Laboratories, Kao Corporation, Tokyo, Japan
†School of Bioscience and Biotechnology, Tokyo University of Technology, Tokyo, Japan
‡Department of Immunobiology, Institute of Development, Aging and Cancer, Tohoku University, Sendai, Japan

UNCORRECTED PROOF

Correspondence
Tomoko Mori.
E-mail: tomoko-n@med.uoeh-u.ac.jp

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Summary

Background Two types of atopic dermatitis (AD) have been proposed with different pathophysiological mechanisms underlying this seemingly heterogeneous disorder. The extrinsic type shows high IgE levels presumably as a consequence of skin barrier damage and feasible allergen permeation, whereas the intrinsic type exhibits normal IgE levels and is not mediated by allergen-specific IgE.

Objectives To investigate the relationship between pruritus perception threshold and skin barrier function of patients with AD in a comparison between the extrinsic and intrinsic types.

Methods Enrolled in this study were 32 patients with extrinsic AD, 17 with intrinsic AD and 24 healthy individuals. The barrier function of the stratum corneum was assessed by skin surface hydration and transepidermal water loss (TEWL), and pruritus perception was evaluated by the electric current perception threshold (CPT) of sensory nerves upon neuroselective transcutaneous electric stimulation.

Results Skin surface hydration was significantly lower and TEWL was significantly higher in extrinsic AD than intrinsic AD or normal controls. Although there was no statistically significant difference in CPT among extrinsic AD, intrinsic AD and normal controls, CPT was significantly correlated with skin surface hydration and inversely with TEWL in intrinsic AD and normal controls, but not extrinsic AD. Finally, CPT was correlated with the visual analogue scale of itch in the non-lesional skin of patients with extrinsic but not intrinsic AD.

Conclusions Patients with extrinsic AD have an impaired barrier, which increases the pre-existing pruritus but rather decreases sensitivity to external stimuli. In contrast, patients with intrinsic AD retain a normal barrier function and sensory reactivity to external pruritic stimuli.

Atopic dermatitis (AD) is a chronic inflammatory skin disease with complicated pathophysiological mechanisms and causative agents. Two subtypes of AD have been proposed: extrinsic AD and intrinsic AD. The extrinsic type is the IgE-mediated common form of AD and is associated with respiratory allergies, such as rhinitis and asthma, and high serum levels of IgE.[1–3] In contrast, intrinsic AD is characterized by the absence of allergen-specific IgE and thus shows normal total IgE levels, although this newly introduced concept is still controversial among academic dermatologists.[1–3] Approximately 20%[4] or fewer[5] patients are estimated as having intrinsic AD. Its characteristics include female predominance, absence of atopic diseases, later onset of disease, and milder disease severity.[3–6] A history of atopy, recurrent conjunctivitis, palmar hyperlinearity, keratosis pilaris, pityriasis alba, and hand and/or foot eczema are significantly less present in the intrinsic type, but Dennie-Morgan fold is positively associated with intrinsic AD.[3]

Several studies have suggested differences in various aspects of pathophysiology between extrinsic and intrinsic AD.

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Increased transepidermal water loss (TEWL) and reduced skin surface hydration are hallmarks of atopic skin, and there are some differences in these values between the two types of AD.[4] Immunologically, surface expression of the high- and low-affinity receptor for IgE and of the interleukin (IL)-4Rα chain is elevated in monocytes from patients with extrinsic AD, but serum levels of IL-13 are significantly increased in patients with intrinsic AD.[7] Skin lesions of extrinsic AD show high levels of chemokines such as CCL18.[8] Expression of neurotrophins is increased comparably in both types.[6]

The stratum corneum of the epidermis, consisting of more than 10 layers of corneocytes and intercellular lipids, serves as the skin barrier.[9] In extrinsic AD, impairment of the skin barrier may be the primary condition which facilitates permeation of environmental allergens and leads to immunological responses such as elevation of allergen-specific IgE.[1] A recent finding of filaggrin gene mutations in a high percentage of patients with AD,[10,11] together with an older finding of ceramide reduction in the stratum corneum,[12,13] have further suggested the presence of skin barrier damage in extrinsic AD. On the other hand, intrinsic AD shows normal or mildly elevated serum IgE, in striking contrast to extrinsic AD.[14] The mechanisms underlying intrinsic AD remain unclear and more speculative than those underlying extrinsic AD.[2,6,8,15]

Patients with AD are well known to be sensitive to irritation from the environment due to the impaired skin barrier function. Given that the extrinsic and intrinsic types are different from each other in the skin barrier condition, each type might respond to external stimuli in a different manner. However, little is known regarding the difference in sensitivity to irritants and in elicitability of pruritus between the two types. It appears that most previous studies on sensitivity were performed in patients with extrinsic AD because of its higher incidence.

There are several reported methods to assess the threshold for the itch sensation to various environmental stimuli.[16–18] Local administration of histamine, either by needle injection or by iontophoresis, is one of the most common procedures for this purpose.[19] Electrically evoked itch is another useful method with the use of a neuroselective transcutaneous electrical stimulator, Neurometer™ CPT/C (Neurotron Inc., Baltimore, MD, U.S.A.), in a noninvasive fashion. Evaluation of electric current perception threshold (CPT) quantifies the sensory threshold to electric stimulation of the sensory nerves.[20,21] This device has been used mainly by neurologists to demonstrate abnormalities in a variety of neuropathic conditions. It does not measure the sensation only to histamine, but the device directly excites large- and small-diameter sensory nerve fibres in a differentiating fashion, independent of local factors such as skin thickness, temperature and substances involved in the induction of pruritus.[20,22] The CPT for 250- and 5-Hz frequency current emitted by the Neurometer CPT/C has been reported to enable quantification of the sensory threshold of Aδ- and C-fibres, respectively, that are thought to transmit the itch sensation from the skin. Therefore, this instrument allows us to investigate the elicitability of pruritus in patients with AD. Kobayashi *et al.*[23] have reported that patients with AD showed lower CPT than healthy controls, and CPT was inversely correlated with TEWL after tape stripping in normal subjects.

To address the differences in the mechanisms between extrinsic and intrinsic AD, we measured CPT in patients with AD, together with measurements of stratum corneum function. Our results show that there are prominent differences in the relationship of CPT with the skin barrier function between the two types.

Materials and methods

Participants

Patients over 18 years of age from our department were included in this study. Forty-nine patients with AD (25 men and 24 women), diagnosed in accordance with the Hanifin and Rajka classification,[24] and 24 healthy controls were enrolled in this study. The distribution of skin symptoms in all patients was characterized for adult AD. The hands, shoulders, neck, flexures and face were the predilection sites, while the extremities were less involved. Patients who had total serum IgE levels < 220 U mL^{-1} (normal range for Japanese subjects) were classified as having intrinsic AD, and those with levels > 400 U mL^{-1} were classified as having extrinsic AD. There was no patient with IgE levels between 220 and 400 U mL^{-1}. IgE RAST for *Dermatophagoides pteronyssinus* was measured in 20 patients. The disease activity was assessed by SCORAD (severity scoring of AD). Patient details are listed in (Table 1). All participants provided written informed consent, and the institutional review board approved this study.

Electric current perception threshold and stratum corneum function

C-fibres are sensory nerves conducting itch and pain. Transcutaneous electric current with 5-Hz sine wave stimulates C-fibres, as assessed by the active action potentials of rat dorsal root ganglia.[21,25] Depending on the body surface site, 5-Hz electric current induces itch and/or pain. In addition, transcutaneous 250-Hz current has also been known to stimulate Aδ-fibres, thus inducing itch.[25] In this direct stimulation of nerve fibres with transcutaneous electric current, the condition of the stratum corneum possibly modifies the perception by affecting the current or other factors.

We measured CPT, skin surface hydration and TEWL at the nonlesional flexor forearm, the nonlesional lower leg, and at lesional skin on the trunk or extremities. When the patients had skin lesions on the flexor forearms or lower legs we avoided these regions and chose clinically normal areas on the volar skin as the sites to perform measurements on nonlesional skin. The patients did not apply any ointment or cream to the examined sites for at least 2 days before the measurements. Concerning the inflammatory state at the clinically normal sites tested, we have previously demonstrated

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Table 1 Patients and healthy individuals enrolled in this study

	Extrinsic AD	Intrinsic AD	Healthy controls
Number of subjects	32 (21 men and 11 women)	17 (four men and 13 women)	24 (nine men and 15 women)
Age (years), mean ± SD (range)	30·0 ± 8·1 (19–51)	33·0 ± 10·4 (18–57)	28·9 ± 3·8 (23–37)
IgE (U mL^{-1}), mean ± SD (range)	5034·8 ± 7538·0 (436–30 000)	110·5 ± 66·8 (11–219)	–
SCORAD, mean ± SD (range)	41·8 ± 19·0 (4·6–84·5)	27·1 ± 20·6 (3·5–73)	–
VAS (nonlesional forearm), mean ± SD	30·9 ± 20·6	15·8 ± 22·1	–
VAS (nonlesional lower leg), mean ± SD	36·0 ± 24·9	20·4 ± 28·5	–
VAS (lesional skin), mean ± SD	55·3 ± 28·3	47·3 ± 36·0	–

AD, atopic dermatitis; VAS, visual analogue scale.

that clinically normal-appearing skin of patients with AD has no histological evidence of inflammation.[26] As control, we measured CPT, skin surface hydration and TEWL on the mid-flexor forearm and lower leg in healthy individuals. CPT was measured by using the Neurometer CPT/C as described previously.[23] Skin surface hydration was evaluated by capacitance using the Corneometer CM825 (Courage & Khazaka Electronic GmbH, Cologne, Germany) and was expressed as arbitrary units.[27] TEWL was measured by detecting the evaporated water using the VapoMeter SWL-2 (Delfin Technologies Ltd, Kuopio, Finland).

Visual analogue scale

All patients rated current itching on a 100-mm visual analogue scale (VAS)[28] at the following sites: nonlesional forearm, nonlesional lower leg and lesional skin.

Statistical analyses

Data were expressed as mean ± SD and assessed for statistical significance. We used Student's t-test to compare skin surface hydration, TEWL and CPT. A linear regression analysis was performed for correlations between the skin surface hydration or VAS and CPT, using Peason's correlation coefficient. For all tests, $P < 0{\cdot}05$ was considered statistically significant.

Results

Impaired skin barrier function in extrinsic but not intrinsic atopic dermatitis

Patients were classified as having extrinsic or intrinsic AD by means of IgE level (> 400 and < 220 U mL^{-1}, respectively). IgE RAST was scored by index values 0–6 according to the manufacturer's criteria (BML, Tokyo, Japan). An index value > 3 to *D. pteronyssinus* was obtained in 11 of 12 (92%) patients with extrinsic AD, but in only one of eight (12·5%) patients with intrinsic AD. Moreover, 67% of the patients with extrinsic AD showed a RAST score index value of 6, and none of the patients with intrinsic AD showed this highest score. As summarized in (Table 1), more patients had extrinsic AD than intrinsic AD, and women predominated in the intrinsic type, as previously reported.[3–5] No significant difference was noted in age between the two types. There was a tendency that SCORAD and VAS at the three test sites were higher in extrinsic than intrinsic AD, as reported previously.[3–5]

As extrinsic AD is caused by external allergens invading through the damaged skin barrier, we initially examined the skin surface hydration (capacitance) and TEWL at the nonlesional forearm and lower leg of patients and normal volunteers in a comparison between extrinsic and intrinsic AD. Skin surface hydration was significantly lower in extrinsic AD than in normal control subjects (Fig. 1). There was no significant difference in the hydration level between intrinsic AD and healthy controls. Extrinsic AD tended to show lower values than intrinsic AD at both sites. TEWL, another assessment of the barrier function, was statistically higher in extrinsic AD than intrinsic AD and normal controls at the nonlesional forearm (Fig. 1). Thus, the skin barrier function was impaired in extrinsic AD and preserved in intrinsic AD, validating this clinical dichotomy.

Correlation between disease severity and pruritus in both extrinsic and intrinsic atopic dermatitis

In advance of analysing CPT, we also examined the correlation between the itch levels and SCORAD in the two types of AD. In both types, VAS scores on the lesional skin were correlated with SCORAD (Fig. 2), suggesting that both types of AD are associated with disease severity-dependent pruritus.

Significant correlation between electric current perception threshold (CPT) and skin surface hydration and between CPT and transepidermal water loss in intrinsic atopic dermatitis as well as in normal individuals

CPT for 5- and 250-Hz current stimuli was measured in patients with AD and normal volunteers. In all experiments, the results from 5- and 250-Hz current stimuli were virtually the same. Figure 3 shows the mean ± SD CPT in each group, and there was no significant difference between the groups in the nonlesional or lesional skin.

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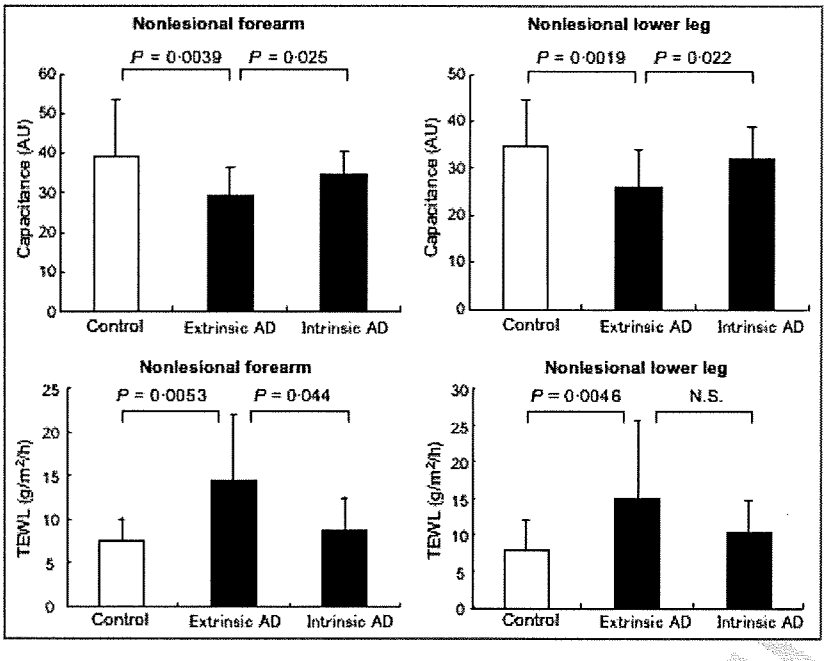

Fig 1. Skin surface hydration on nonlesional forearm and lower leg in extrinsic and intrinsic atopic dermatitis (AD) and healthy controls. Skin surface hydration is represented by capacitance in arbitrary units (AU). Results are shown as mean ± SD. N.S., not significant.

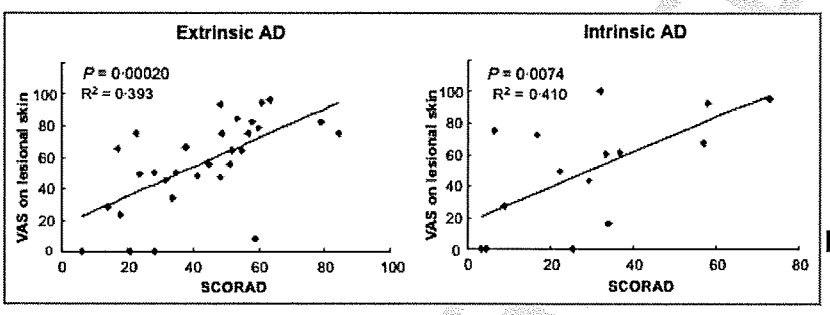

Fig 2. Relationship between SCORAD and visual analogue scale (VAS) score in extrinsic and intrinsic atopic dermatitis (AD).

When CPT was analysed in relation to skin surface hydration, an interesting finding was obtained. In normal subjects (control), CPT was significantly correlated with skin surface hydration (Fig. 4), suggesting that the water-poor cornified layer has a property to evoke pruritus in response to external stimuli. Similarly, the lesional skin of patients with intrinsic AD showed such a significant correlation between CPT and skin surface hydration. However, there was no correlation in extrinsic AD, as large individual variations of CPT were seen in the patients with extrinsic AD and low levels of skin surface hydration.

As to the relation of CPT to TEWL, there was no significant correlation between these two parameters in the lesional skin of patients with AD. However, CPT of nonlesional forearm, as assessed by 250-Hz sensitivity, was inversely correlated with TEWL in intrinsic AD as well as in controls (Fig. 5). The results suggest that intrinsic AD is associated with a normal stratum corneum and no excess elicitability of externally stimulated pruritus, while extrinsic AD does not show such a regular, surface hydration-related irritant perception.

Different electric current perception threshold levels in relation to pre-existing pruritus between extrinsic and intrinsic atopic dermatitis

It is possible that CPT is affected by the itch state in patients with AD. We therefore investigated the relationship between CPT and the pre-existing pruritus assessed by VAS. In the lesional skin of both types of AD there was no correlation between CPT and VAS (data not shown). In the nonlesional lower leg, however, CPT was significantly correlated with VAS in extrinsic but not intrinsic AD (Fig. 6), suggesting that the pre-existing pruritus rather downmodulates the sensitivity to external stimuli in extrinsic AD. The nonlesional forearm exhibited the same tendency but without statistical significance. Thus, the pruritic normal-appearing skin seems to be insensitive to further itchy stimuli in extrinsic AD.

Discussion

The precise concept of intrinsic AD in comparison with extrinsic AD has been a matter of controversy. Extrinsic AD seems

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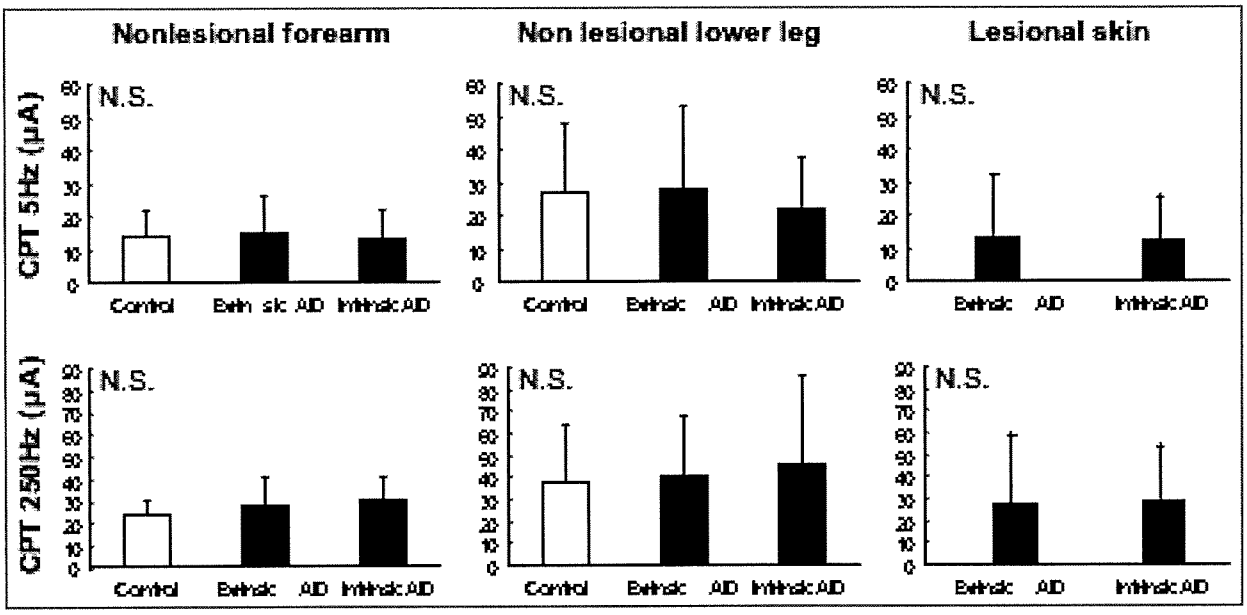

Fig 3. Electric current perception threshold (CPT) on nonlesional forearm or lower leg and lesional skin in extrinsic and intrinsic atopic dermatitis (AD). Results are shown as mean ± SD. N.S., not significant.

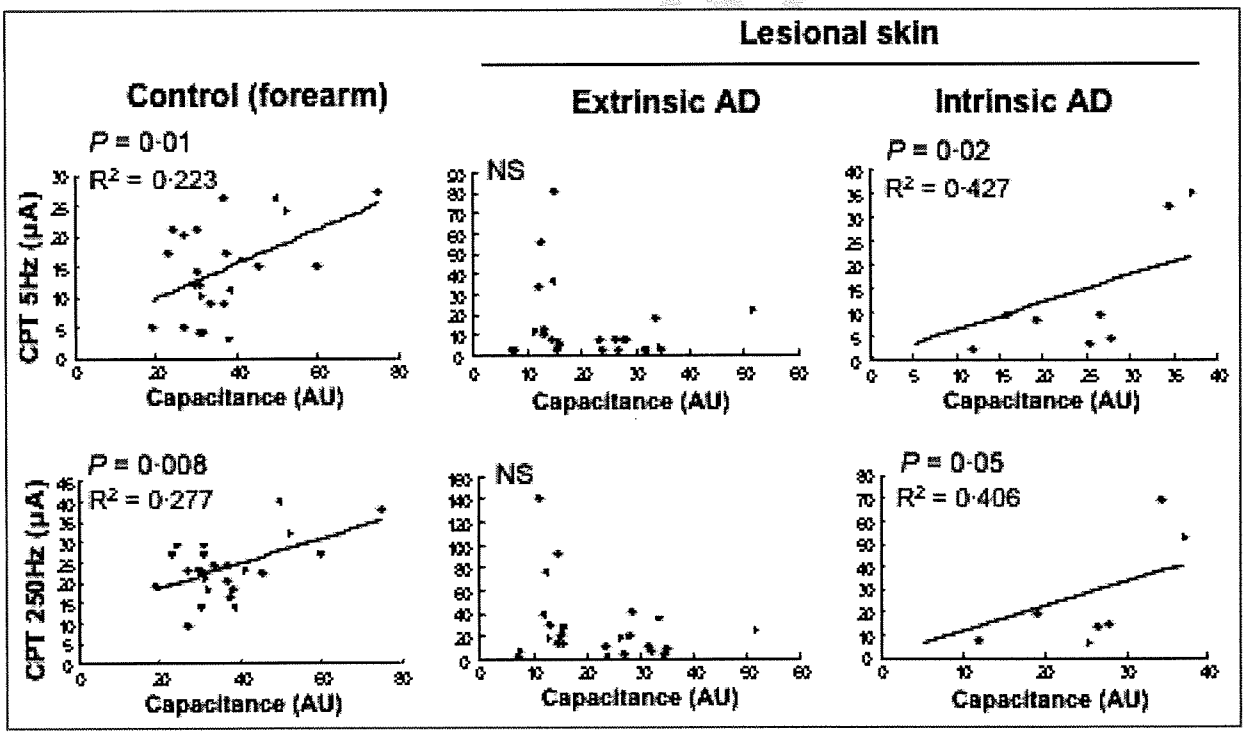

Fig 4. Relationship between skin surface hydration, represented by capacitance in arbitrary units (AU), and electric current perception threshold (CPT) in lesional skin of patients with extrinsic and intrinsic atopic dermatitis (AD) and in nonlesional forearm skin of healthy controls. N.S., not significant.

to be induced by sequential events, including impairment of stratum corneum, permeation of external substances, exposure of immunocompetent cells to the allergens, and T cell and IgE responses to the antigenic determinants.[1] Growing evidence has supported this mechanism underlying the extrinsic type. The recent finding that filaggrin gene mutations are a predisposing factor for AD has clearly demonstrated the presence of barrier impairment in patients with AD.[10,11] On the other hand, the pathophysiology of intrinsic AD remains obscure, and it may be difficult for clinicians to differentiate the two

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