Epstein-Barr Virus–Positive Hodgkin Lymphoma–Like Earlobe Lymphoid Infiltrate: Case Report

Keiji Tanese, MD, PhD,† Rei Haratoh, MD,*† Kozo Yamamoto, MD,† Akiko Wakabayashi, MD,*† Rie Irie, MD, PhD,‡ and Syunichi Miyakawa, MD, PhD*†*

Abstract: Hodgkin lymphoma (HL) is a malignancy of the lymph system usually occurring on the lymph nodes. A 57-year-old Japanese woman presented with the chief complaint of an enlarging tumor of the left ear. An excisional biopsy was taken, and histological examination showed a mixed infiltration of cells, including Reed–Sternberg cells and their mononuclear forms against a background of small lymphocytes. Reed–Sternberg cells were $CD15^+$, $CD20^+$, $CD30^+$, Ki-67^+, MUM-1^+, $CD45^-$, EMA^-, and Epstein-Barr virus–encoded small RNA was detected by in situ hybridization. We diagnosed this tumor as a skin infiltration with a lymphocyte-rich classical HL pattern. Skin involvement of HL is most often a secondary phenomenon representing a rare late manifestation of disease dissemination; however, we could not detect any evidence of systemic lesion for 6 months after the initial presentation. A case of HL only involving the skin was reported by several past reports, which termed it primary cutaneous HL. But, it is still controversial whether HL initially occurs on the skin because a diagnostic gray zone exists between HL, some non-HL entities, and nonneoplastic lymphoid infiltrates. Clinical and histological features of this case suggest that the skin will become a primary site of HL.

Key Words: cutaneous Hodgkin lymphoma, primary cutaneous Hodgkin lymphoma, lymphomatoid papulosis, anaplastic large cell lymphoma, diffuse large B-cell lymphoma anaplastic variant

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INTRODUCTION

Hodgkin lymphoma (HL) is a malignancy of the lymph system usually occurring on the lymph nodes. Owing to the results provided by immunophenotypic and molecular studies, HL is now considered to be a germinal center–related B-cell lymphoma.[1] The diagnosis of it is based on the identification of characteristic multinucleated giant cells termed Reed–Sternberg cells (RS cells) within an inflammatory milieu.[2] The 2008 World Health Organization classification classifies HL histologically into nodular lymphocyte–predominant HL and classical Hodgkin lymphoma (CHL). CHL has been further classified into 4 subtypes: lymphocyte rich, nodular sclerosis, mixed cellularity, and lymphocyte depleted.[3]

The most common first clinical sign of HL is a painless enlargement of 1 or more lymph nodes above the diaphragm. Subsequently, the disease can spread to nearby lymph nodes and later may spread to the spleen, lungs, liver, or bone marrow.[4] However, skin involvement of HL is rare and occurs as a late manifestation of disease dissemination.[5,6] On the other hand, several studies have reported cutaneous HL cases in the absence of systemic evidence, defining it as primary cutaneous Hodgkin lymphoma (PCHL)[7–19]; however, it is still controversial whether PCHL exists as a distinct clinical entity mainly due to the diagnostic gray zone between HL, some non-HL entities, and nonneoplastic lymphoid infiltrates. Here, we report the case of a cutaneous nodule on the earlobe, which showed histological features of lymphocyte-rich CHL pattern. Systemic evaluation failed to detect evidence of other primary lesions for 6 months, suggesting a diagnosis of PCHL.

CASE REPORT

A 57-year-old Japanese woman presented at our clinic with the chief complaint of a reddish hard nodule on the left ear of 3-month duration in June 2007. She was healthy and there were no problems in her performance status. We first suspected a benign interstitial tumor and kept her under observation. Two months after the first visit, the tumor had become a firm, round, reddish mass with a diameter of 2 cm (Fig. 1A). No cervical lymph node swelling was observed. We suspected malignant interstitial tumors and excisional biopsy was performed. The cut appearance of the tumor was a whitish to yellowish firm subcutaneous mass (Fig. 1B). On gross histological examination, the tumor was a relatively circumscribed mass involving the dermis (Fig. 2A). There was mixed cell infiltration, including giant cells of 2 nuclei with acidophilic nucleoi and their mononuclear forms against a background of small lymphocytes (Figs. 2B, C). There were no apparent nuclear atypia or mitotic figures in those small lymphocytes. The giant cells were positive for CD15, CD30, and CD20 (Fig. 3). Number of giant cells expressing these markers was 39–46 per 10 high-power field. Also, Ki-67, multiple myeloma oncogen-1 (MUM-1), and in situ hybridization of Epstein-Barr virus–encoded small RNA (EBER) were positive, but CD45 and epithelial membrane antigen (EMA) were negative (Fig. 4). Surrounding infiltrating lymphocytes were CD3 positive, but CD15, CD30, CD20, Ki-67, and EBER were negative (Figs. 3, 4). Although we could not perform molecular genetic studies, we diagnosed skin infiltration with a CHL lymphocyte-rich pattern pathologically. Systemic evaluation was performed by brain, neck, chest, and abdominal computed tomography scan; abdominal echogram; and Ga scintigram; however,

From the *Department of Dermatology, School of Medicine, Keio University, Tokyo, Japan; Departments of †Dermatology; and ‡Pathology, Kawasaki Municipal Hospital, Kawasaki, Japan.
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Reprints: Keiji Tanese, MD, PhD, Department of Dermatology, School of Medicine, Keio University, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan (e-mail: tanese@2001.jukuin.keio.ac.jp).

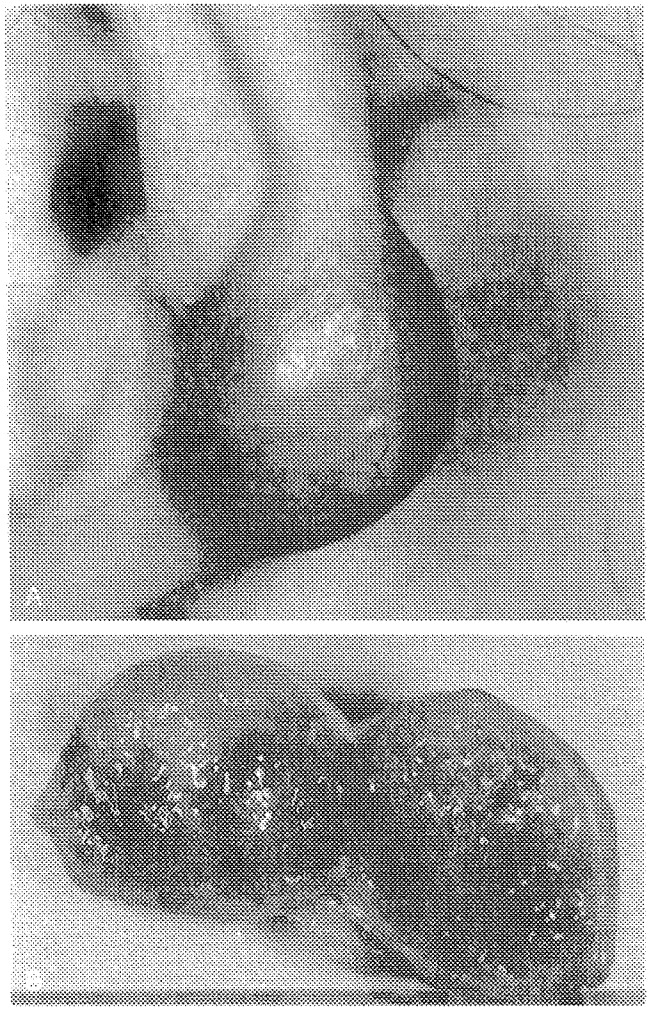

FIGURE 1. A, The tumor was a firm, round, reddish mass with a diameter of 2 cm. B, Cut appearance of the tumor was a whitish to yellowish firm subcutaneous mass.

we could not detect any primary lymph nodes. One month after biopsy, a new lesion occurred in the left earlobe. A second biopsy revealed the same histological features as the first excisional biopsy (Fig. 5). Meticulous systemic evaluation was performed again. However, computed tomography scan, abdominal echogram, and Ga scintigram failed to detect malignant intestinal lesion, and only positron emission tomography revealed a slight fluorodeoxyglucose (FDG) accumulation on the left side parotid lymph node (Fig. 6). Also, we still could not detect lymph node swelling by palpation. Although we could not perform a lymph node biopsy, we regarded this as a metastatic lesion of the HL rather than the primary site because FDG accumulation was weak compared with the tumor on the left ear. According to the Ann Arbor classification, the stage of this case was 2EA; thereafter, 4 cycles of ABVD (adriamycin, bleomycin, vincristine, and dacarbazine) therapy were performed in combination with irradiation. Almost 1½ years after the initial visit, there was no sign of recurrence or metastasis.

DISCUSSION

HL is a malignancy usually occurring on the lymph nodes. Without treatment, HL has a moderately aggressive clinical course. As treatment, chemotherapy is combined with irradiation, showing long-term survival. The most frequent cutaneous findings in HL are nonspecific eruptions, such as eczema, prurigo, erythema nodosum,[18] asteatosis, herpes zoster, and ichthyosis.[20] HL involving the skin is extremely unusual, being reported in 0.5%–7.5% of patients, and most often, it is secondary to retrograde lymphatic spread from the involved lymph nodes.[13,21,22]

A case suggestive of PCHL was first reported by Doesekker in 1919,[7] and subsequently, several cases of PCHL were reported, defining it as a variant of HL taking a relatively indolent clinical course.[8–19] However, some early cases reported as PCHL before immunohistochemical and genetic studies were available, which would probably be diagnosed today as other non-HLs or lymphoproliferative diseases. For instance, 2 of the 3 patients with PCHL described by Szur et al[13] are more suggestive of lymphomatoid papulosis than PCHL in the present diagnostic entity; however, the recent progression of immunohistochemistry and molecular biology has contributed to the differential diagnosis between HL and these diseases. Sioutos et al[14] described 5 cases of PCHL using immunohistochemical analysis in 1994; subsequently, several PCHL cases diagnosed by immunohistochemical and molecular biological techniques have been reported.[15–19] Nevertheless, it is still controversial whether PCHL exists as a distinct clinical entity because the borders between HL; nonneoplastic lymphoid infiltrates; and other non-HL entities, such as lymphomatoid papulosis, anaplastic large cell lymphoma, and some variants of diffuse large B-cell lymphoma, are not always clear. Especially, the finding of a few RS-like giant cells may be seen in many reactive conditions and non-HLs. Therefore, careful consideration and exclusion of other cutaneous lymphoid tumors are necessary to diagnose PCHL.

In our case, we first ruled out reactive conditions such as cutaneous lymphocytoma, a bite reaction, or other pseudolymphomas because the tumor showed recurrence 1 month after the initial biopsy. FDG accumulation on the left side parotid lymph node in positron emission tomography, suggestive of metastasis, will also support the feature of lymphoma.

Thereafter, we performed differential diagnosis between HL and other non-HL entities (Table 1). Lesions of lymphomatoid papulosis show a prominent mixed inflammatory background with numerous neutrophils and eosinophils. In anaplastic large cell lymphoma, sheets of atypical cells have frequently been found in routine hematoxylin and eosin staining.[23] In our case, there were relatively few giant cells, morphologically consistent with RS cells, against a background of small round lymphocytes. These giant cells were Ki-67$^+$ and the surrounding lymphocytes were Ki-67$^-$, suggesting that the surrounding cells were reactively infiltrated cells. The giant cells were CD30$^+$, CD15$^+$, CD20$^+$, MUM-1$^+$, CD45$^-$, and EMA$^-$. Expression of the CD30 molecule by RS cells is seen in more than 98% of CHLs, although its expression is also frequently detected in anaplastic large cell lymphoma and lymphomatoid papulosis. CD15 is another valuable marker of RS cells and is detected in about 80% of patients with CHL.[24] CD15 is characteristic of, but not specific to, RS cells because it can be detected in B-cell and T-cell lymphomas and in nonlymphoid tumors; therefore, the

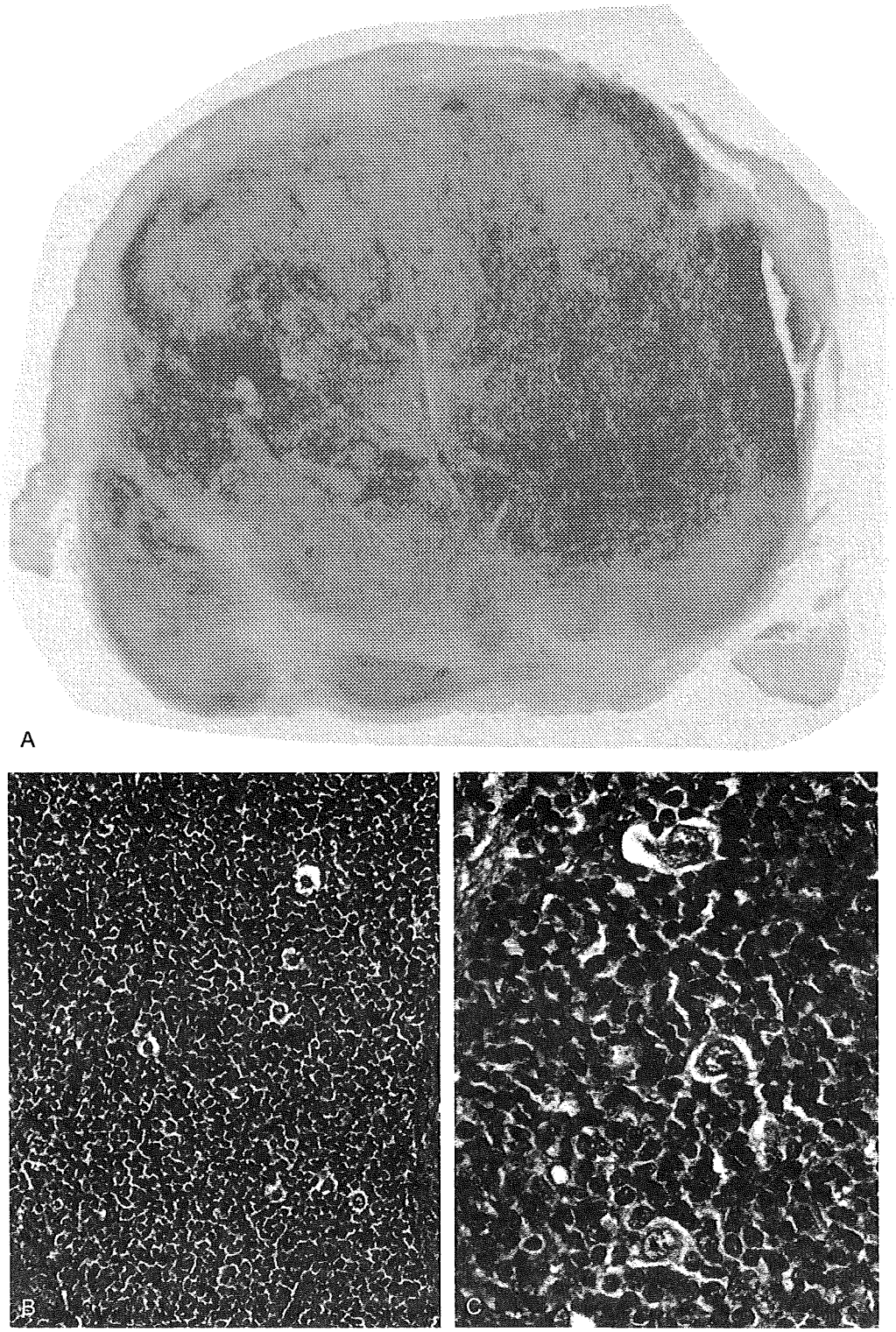

FIGURE 2. Histological findings. Hematoxylin–eosin staining. A, Tumor was a relatively circumscribed mass involving the dermis. B, Mixed infiltration of cells, including giant cells against a background of small lymphocytes (×200). C, Giant cells were composed of 2 nuclei with acidophilic nucleoi. Their mononuclear forms were also observed (×400).

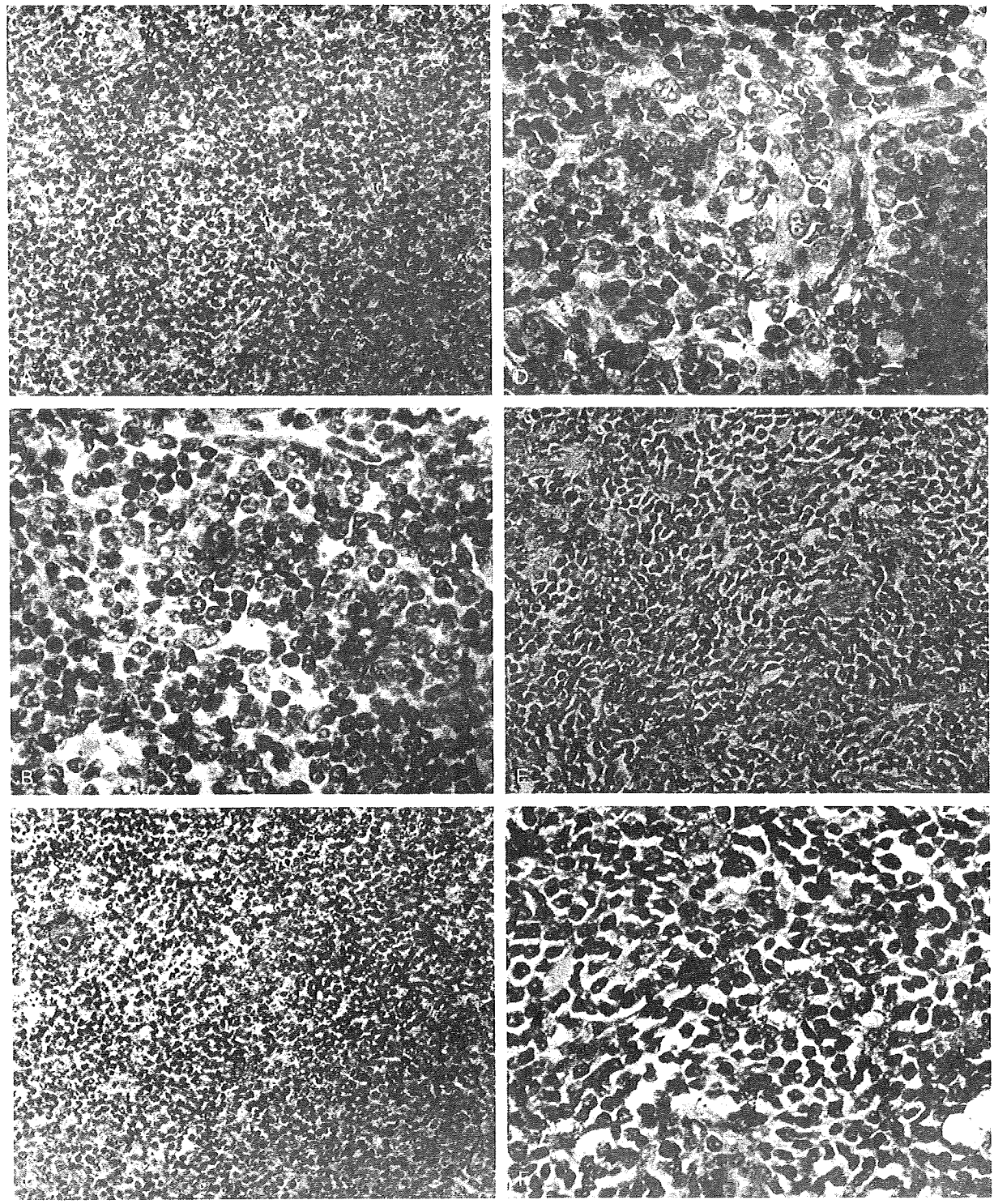

FIGURE 3. Immunohistochemical findings. A, Immunohistochemical findings of CD15 (×200). Scattered infiltration of CD15-positive giant cells against a background of CD15-negative small lymphocytes. B, High-power view of immunohistochemical findings of CD15 (×400). CD15 is expressed on cell surface of giant cells. The number of CD15-positive giant cells was 39 per 10 HPF. C, Immunohistochemical findings of CD30 (×200). Scattered infiltration of CD30-positive giant cells against a background of small lymphocytes. D, High-power view of immunohistochemical findings of CD30 (×400). The number of CD30-positive giant cells was 46 per 10 HPF. E, Immunohistochemical findings of CD20 (×200). Scattered infiltration of CD20-positive giant cells. F, High-power view of immunohistochemical findings of CD20 (×400). The number of CD20-positive giant cells was 45 per 10 HPF. HPF, high-power field.

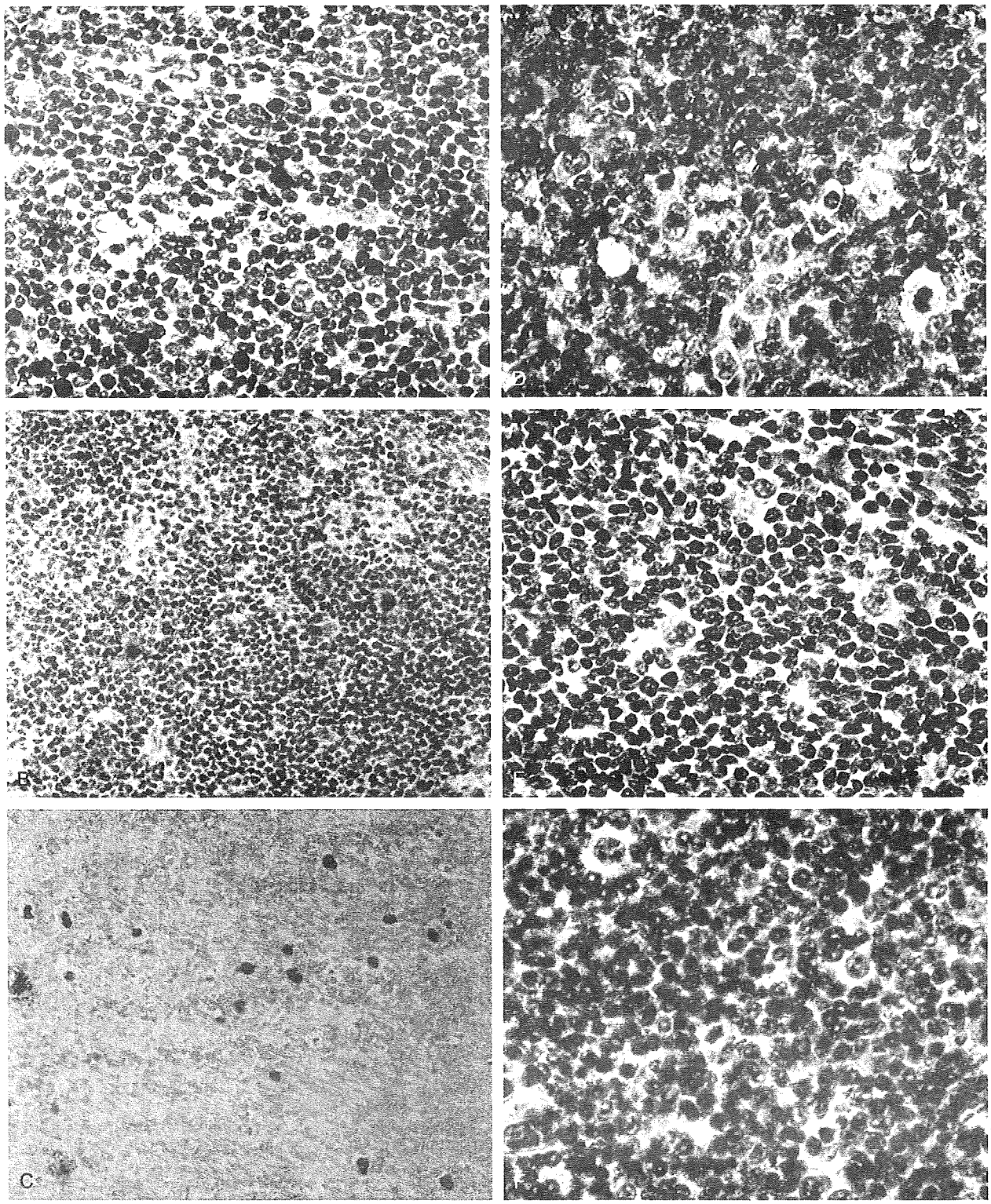

FIGURE 4. Immunohistochemical findings and in situ hybridization analysis. A, Immunohistochemical analysis of Ki-67 (×200). Ki-67 was positive in giant cells. B, Expression of MUM-1 was positive in giant cells (×200). C, Detection of EBER with in situ hybridization was observed in giant cells (×200). D, Expression of CD45 was observed in the surrounding small lymphocytes (×200). E, Expression of CD3 was observed in the surrounding small lymphocytes (×200). F, Expression of EMA was negative in all cells (×200).

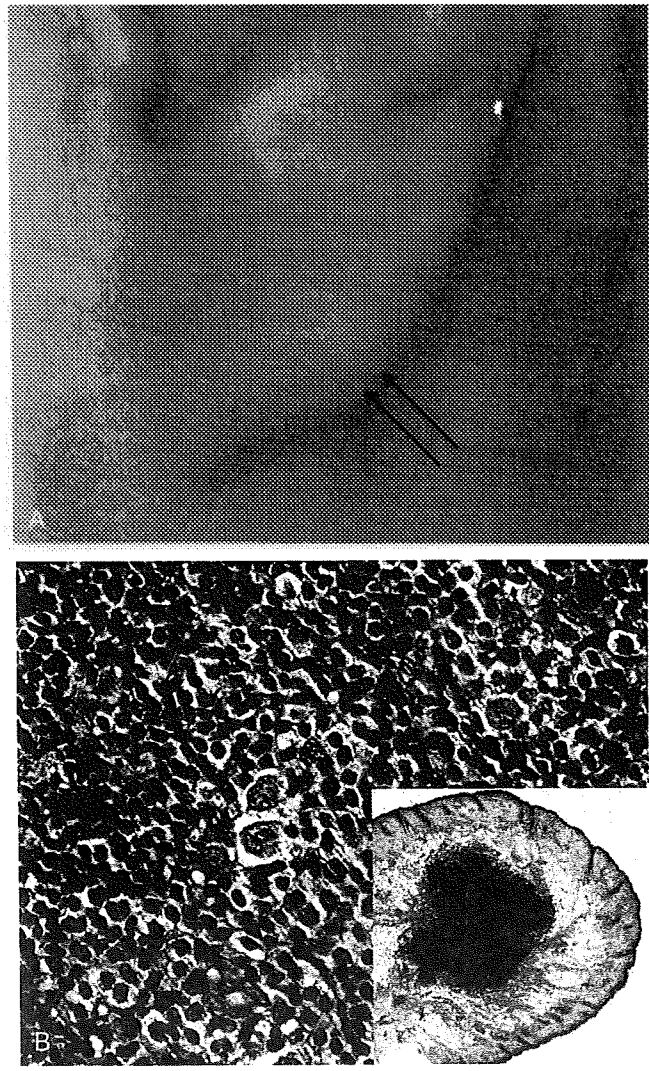

FIGURE 5. Clinical findings of the subcutaneous mass, which occurred 1 month after the initial biopsy. A, Small subcutaneous mass was detected in the left earlobe (arrow). B, Histological findings. Hematoxylin–eosin staining. Mixed infiltration of the cells, including giant cells of 2 nuclei with acidophilic nucleoi and their mononuclear forms were observed against a background of small lymphocytes (inset is low-power view).

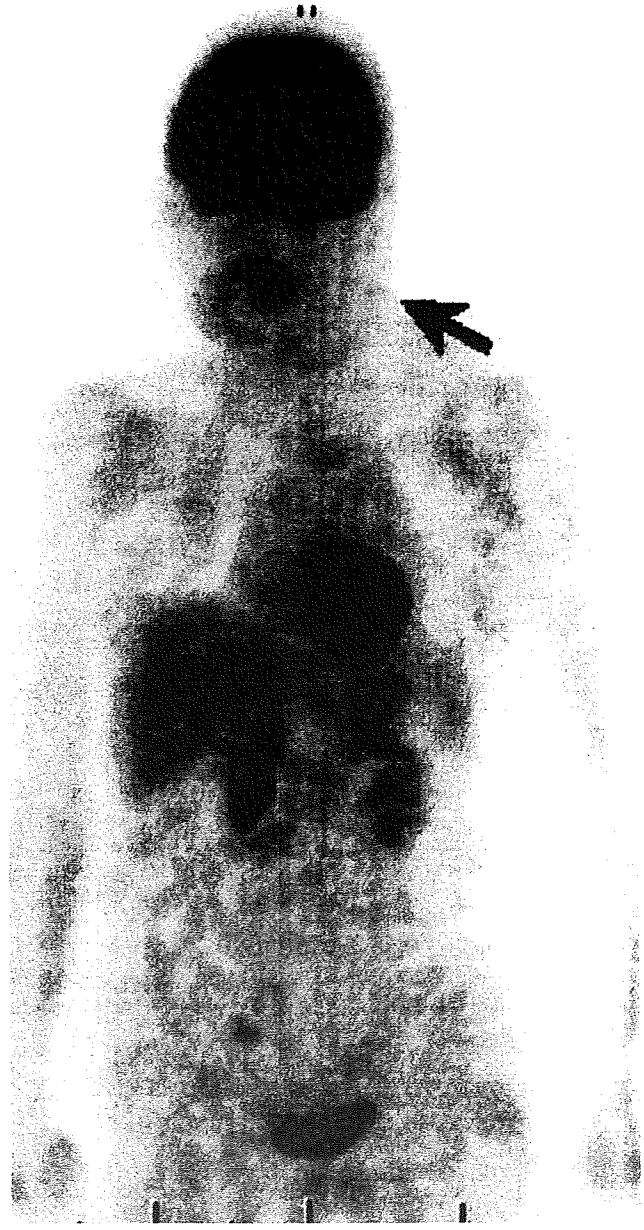

FIGURE 6. Positron emission tomography findings. Slight FDG accumulation was detected on the left side parotid lymph node (arrow). FDG signals expected in different tissues are nonspecific signal. Other radiological analysis performed at the same time could not detect malignant intestinal lesions.

"CD30$^+$, CD15$^+$ phenotype" of RS cells, as seen in this case, can also be present in lymphomatoid papulosis and anaplastic large cell lymphomas. On further analysis, giant cells lacked the expressions of CD45 and EMA in our case. RS cells in CHL usually lack CD45 and EMA expressions,[25,26] whereas RS-like cells in lymphomatoid papulosis and anaplastic large cell lymphomas usually express T-cell–associated antigens and EMA and CD45.[24] Our case also showed the expression of EBER in giant cells. About 50% HL cases have been shown to be positive for Epstein–Barr virus (EBV) using the highly sensitive technique of in situ hybridization for EBER transcripts, whereas almost all reported cases of cutaneous anaplastic large cell lymphomas were EBV negative[27]; however, a case of recurrent cutaneous anaplastic large cell lymphoma that was EBV positive has also been reported and the existence of EBV seems not to be a distinct marker.[28] On the other hand, the expression of CD20 in the giant cells of this case suggests that the tumor cells originate from B-cell lineage. Furthermore, expression of MUM-1 in giant cell will support the evidence that these cells are originated from germinal center B cells.

TABLE 1. CHL Lymphocyte-Rich Pattern and Non-HLs: Pathological Differential Diagnosis

Type of Lymphoma	Morphology	Immunophenotype
CHL lymphocyte rich	Scattered invasion of RS cells within an inflammatory milieu of lymphocyte	RS cells are $CD15^{+/-}$, $CD20^{+/-}$, $CD30^{+}$, $CD45^{+/-}$, EMA^{-}, $EBER^{+/-}$, MUM-1^{+}, and Ki-67^{+}. Surrounding lymphocytes are $CD3^{+}$and $CD45^{+}$
Lymphomatoid papulosis	Atypical cells with prominent mixed inflammatory background with numerous neutrophils and eosinophils	Tumor cells are $CD30^{+}$, $CD45^{+/-}$, $CD15^{+/-}$, $CD20^{-}$, Ki-67^{+}, and EMA^{-}
Anaplastic large cell lymphoma	Sheet-like proliferation of atypical cells	Atypical tumor cells are $CD30^{-}$, $CD45^{+/-}$, $CD15^{+/-}$, and $CD20^{-}$. In most cases $EBER^{-}$and EMA^{-}
Diffuse large B-cell lymphoma, anaplastic variant	Sinusoidal or cohesive growth pattern of atypical cells	Atypical tumor cells are $CD30^{-}$, $CD45^{-/+}$, $CD15^{-/+}$, $CD20^{+}$, $EBER^{+/-}$, MUM-$1^{+/-}$, and Ki-67^{+}
Present case	Scattered infiltration of giant cells within the inflammatory milieu of lymphocytes	Giant cells are $CD15^{+}$, $CD20^{+}$, $CD30^{+}$, $CD45^{-}$, EMA^{-}, $EBER^{-}$, MUM-1^{+}, and Ki-67^{+}. Surrounding lymphocytes are $CD3^{+}$and $CD45^{+}$

These results strongly distinguish diseases of T-cell–originated lymphomatoid papulosis and anaplastic large cell lymphomas. CD20 is found in 30%–40% of CHL cases.[29]

The differential diagnosis between diffuse large B-cell lymphoma, anaplastic variant, conventionally termed anaplastic large B-cell lymphoma, and CHL seems to be more confusing in this case. In diffuse large B-cell lymphoma, CD20 will be positive in almost all cases. Also, the frequent detection of EBER and expression of CD30 (30%) and CD15 (5%) have been reported in RS-like giant cells. Expression of MUM-1 in giant cells of this case will support the diagnosis of CHL, but expression of MUM-1 is also detected in some cases of diffuse large B-cell lymphoma. Furthermore, diffuse large B-cell lymphoma frequently lacks the expression of CD45[29]; therefore, we could not completely rule out the possibility of diffuse large B-cell lymphoma, anaplastic variant, from the immunohistochemical findings. However, diffuse large B-cell lymphoma, anaplastic variant, usually shows a sinusoidal or cohesive growth pattern of atypical cells.[30] Because our case showed scattered infiltration of giant cells, we suppose that the histological findings of this case are more likely to have a CHL lymphocyte-rich pattern.

Another possibility is that the cutaneous HL lesion in our case was a metastasis from an occult primary nodal HL. Because both primary and secondary cutaneous HLs show similar immunophenotypes,[31] immunohistochemistry is unlikely to distinguish primary lesion from metastatic lesion. In our case, FDG accumulation in the lymph nodes of the parotid gland was seen 6 months after the initial arrival; therefore, the possible existence of a primary occult nodal tumor preceding the skin lesions cannot be totally refuted. However, the cutaneous spread of HL usually occurs in the advanced stage. In most instances, the skin adjacent to the involved lymphoid tissue is infiltrated via retrograde lymphatic spread or by direct extension through the infiltration of soft tissue.[22] In this case, the clinical stage was 2EA and in a comparatively early stage. Also, FDG accumulation in lymph nodes was so weak that it could be ignored, considering the size of the tumor on the left ear. As in our case, the subsequent extension of PCHL to the draining regional lymph nodes was noted in 2 of the 5 patients reported by Sioutos et al.[14]

After careful consideration, this case showed that the skin will possibly become a primary site of HL. Further accumulation and analysis of similar cases are necessary to elucidate whether PCHL exists as a distinct clinical entity.

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CED
Clinical and Experimental Dermatology

Malignant eccrine spiradenoma: case report and review of the literature, including 15 Japanese cases

K. Tanese, T. Sato and A. Ishiko

Department of Dermatology, School of Medicine, Keio University, Tokyo, Japan

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Summary

Malignant eccrine spiradenoma (MES) is an extremely rare cutaneous malignant tumour. An 86-year-old man presented at our hospital with an enlarging tumour on the dorsum of the left hand. An excisional biopsy was taken and histological examination showed a solid island of cells of two distinct types: cells with abundant cytoplasm and oval vesicular nuclei, and small round cells with less cytoplasm and hyperchromatic nuclei with a high frequency of mitosis. We diagnosed this tumour as MES. Although we did not perform further treatment because of the patient's age, there was no sign of recurrence or metastasis in the 2 years of follow-up after excisional biopsy. We reviewed cases of malignant eccrine spiradenoma in the English and Japanese literature and found that 'sarcomatous' or 'squamous' change in histopathology was significantly correlated with a poorer prognosis. It is therefore important for treatment planning to evaluate the entire specimen histologically.

Malignant eccrine spiradenoma (MES) is an extremely rare and rapidly growing cutaneous malignant tumour. We present a patient with an MES and review the literature on this tumour.

Report

An 86-year-old man presented at our clinic with a tumour on the back of his left hand. His personal and family medical histories were unremarkable. He had noticed a reddish plaque > 10 years previously, which had suddenly begun enlarging without pain in the last 3 years.

On physical examination, the tumour was found to be an asymmetrical, irregular and mushroom-shaped reddish mass with a diameter of 30 mm. The tumour surface was eroded and it bled easily (Fig. 1). There was no swelling of the axial lymph nodes. We suspected

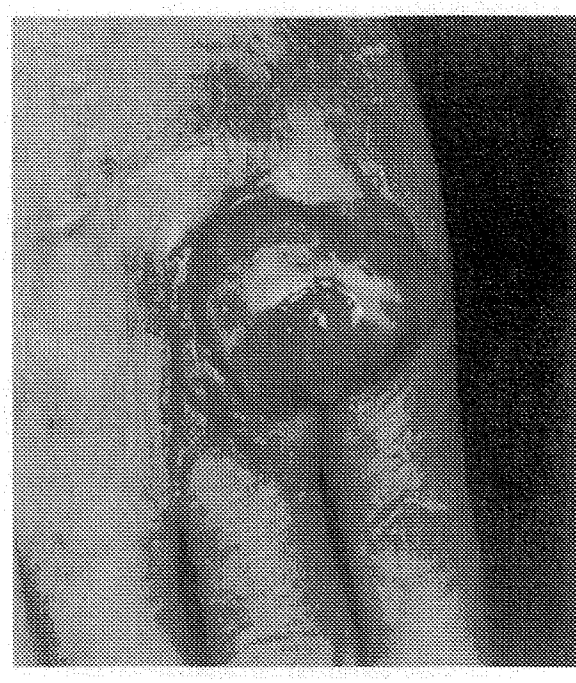

Figure 1 Asymmetrical mushroom-shaped reddish mass with a diameter of 30 mm, and an eroded surface was eroded that bled easily.

Correspondence: Dr Keiji Tanese, 35 Shinanomachi, Shinjyuku-ku, Tokyo, 160-8582, Japan.
E-mail: tanese@2001.jukuin.keio.ac.jp

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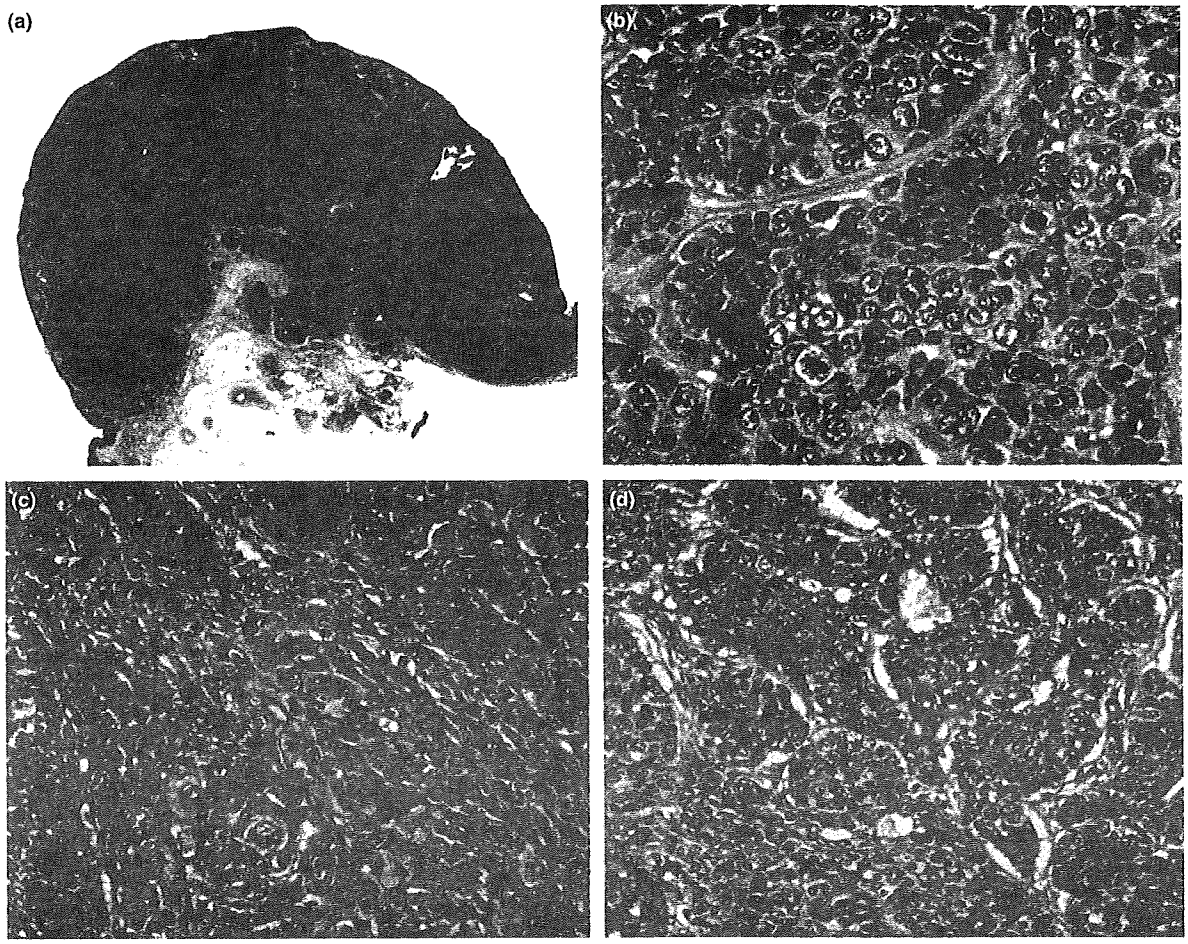

Figure 2 (a) The tumour was exophytic and composed of solid islands of tumour cells with lobular, nested and trabecular growth patterns; original magnification × 1. (b) Two distinct populations could be seen: large cells with abundant cytoplasm and oval vesicular nuclei and small lymphocyte-like cells with less cytoplasm and hyperchromatic nuclei, with a high number of mitotic figures; original magnification × 400. (c) Tumour cells showed interstitial invasion to the surrounding stroma; original magnification × 200, and (d) some ductal differentiation was seen. Haematoxylin and eosin; original magnification × 200.

amelanotic melanoma or a malignant adnexal skin tumour.

An excisional biopsy was taken, and histological examination showed that the tumour was exophytic and composed of solid islands of tumour cells, which showed lobular, nested and trabecular growth patterns. The overlying epidermis was almost intact but some connection with the tumour island was observed. The tumour was composed of two distinct cell populations: large cells with abundant cytoplasm and oval vesicular nuclei, and small lymphocyte-like cells with less cytoplasm and hyperchromatic nuclei showing nuclear atypia and a high frequency of atypical mitosis. Some ductal differentiation, small invaginations of hyalinized stroma and necrosis, and interstitial invasions of the tumour cells were seen focally. No area of the specimen showed sarcomatous or squamous changes (Fig. 2).

Immunhistochemical examination showed that the tumour cells were positive for the antibodies 34βE12 (cytokeratins 1, 5, 10 and 14), AE1 + AE3 (cytokeratins 1–8, 10, 14–16 and 19), CAM 5.2 (cytokeratins 8 and 18), epithelial membrane antigen (EMA), p53 and MIB-1 (Fig. 3). From these findings, we diagnosed this tumour as MES. Further adjuvant therapies were not performed because of the patient's age. After almost 2 years of follow-up since the initial operation, there was no sign of recurrence or metastasis.

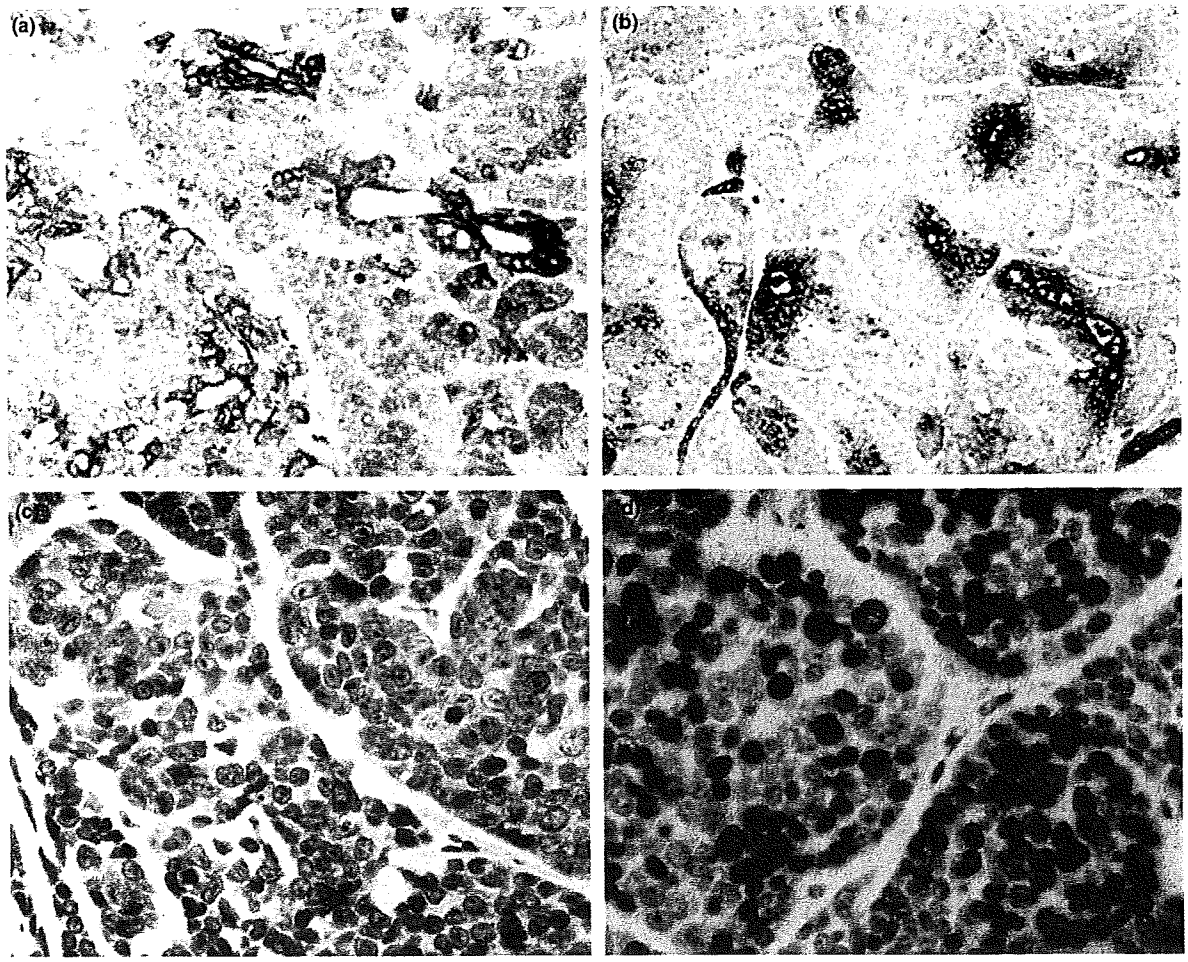

Figure 3 (a-d) Results of staining with (a) CAM 5.2 (cytokeratins 8 and 18), (b) epithelial membrane antigen, (c) p53 and (d) MIB-1. Original magnification (a,b) × 200; (c,d) × 400.

MES is a sweat-gland tumour that can arise on the skin surface. MES was first described by Dabska in 1972[1] as a lesion arising from malignant transformation of a benign eccrine spiradenoma. Its presentation is that of a rapidly growing lesion on a background of a persistent, chronic skin lesion. It may develop *de novo* or more often arise from a pre-existing benign eccrine spiradenoma.

MES is rare, with only 68 cases reported in the English literature, including 2 Japanese cases, 1 Korean, 1 Taiwanese and 1 African American case.[2–8] In addition, 15 other cases have been reported in the Japanese literature (Table 1).[9,10] To identify any differences in the clinical features and prognosis between the 19 East Asian and the 63 white cases, we summarized the age at diagnosis, gender, location and prognosis. We found that MES tends to involve elderly people regardless of the ethnic group, but it has a slight female predominance and is more likely to involve the head and neck in East Asians, although the difference between East Asians and white patients was not significant (χ^2 test).

Histopathologically, MES shows solid islands of tumour cells with a basaloid pattern. Prominent glandular differentiation may be seen at low magnification and there may be squamous or sarcomatous areas in the lesion.[8] The diagnosis depends on finding a contiguous benign spiradenoma, as the malignant component itself may lack the distinguishing features of spiradenoma. Malignant change is usually accompanied by the transgression of tumour cells through the capsule into the adjacent stroma. Tumour cells are highly pleomorphic and exhibit numerous mitotic figures. They express cytokeratins, epitherial membrane antigen and p53.[3] In our case, the pathological features matched these

Table 1 Cases of malignant eccrine spiradenoma reported in the Japanese literature.

Patient no.	Age, years	Gender	Site	Size (mm)	Recurrence	Metastasis	Treatment	Status
1	32	F	Head & neck	60 × 40	Yes	Liver	Resection	Died of disease
2	53	F	Limb	N/A	No	None	Resection	Alive, no evidence of disease
3	62	M	Head & neck	About 10	No	Lymph node	Resection, radiotherapy	Alive with disease
4	82	M	Head & neck	120 × 120	N/A	N/A	Resection	N/A
5	66	F	Head & neck	60 × 60	No	None	Resection	Alive, no evidence of disease
6	66	F	Head & neck	60 × 50	N/A	N/A	Resection	N/A
7	78	F	Trunk	90 × 70	No	None	Resection	Alive, no evidence of disease
8	76	F	Trunk	30 × 60	N/A	N/A	Resection	N/A
9	61	F	Trunk	N/A but multiple	Yes	Lymph node	Resection	N/A
10	86	M	Limb	35 × 35	Yes	N/A	Resection	N/A
11	62	F	Trunk	120 × 10	Yes	Lymph node, bone, liver	Resection, radiotherapy	Died of disease
12	58	M	Limb	30 × 30	Yes	Lymph node	Resection	Died of disease
13	90	F	Head & neck	50 × 50	No	None	Resection	Alive, no evidence of disease
14	53	M	Trunk	15 × 15	No	None	Resection	Alive, no evidence of disease
15	53	F	Trunk	60 × 40	No	Lymph node, bone, lung	Resection, radiotherapy, Immunotherapy	Died of disease
Our case	86	M	Limb	30 × 30	No	None	Resection	Alive, no evidence of disease

N/A, not applicable. Patients 1–14 were reported by Takeuchi *et al.*[9] and patient 15 by Kamel *et al.*[10] Patient 15 showed squamous changes.

characteristics of MES and the somewhat long clinical course before diagnosis may account for the pre-existing benign eccrine spiradenoma.

Because of the limited number of case reports, the management of MES is not evidence-based. In a review of 84 previous cases, 28 (33%) had metastasis and 15 (18%) died. Most cases were treated with wide local excision as the primary treatment. In addition, 10 patients underwent lymphadenectomy for lymph-node metastasis, 6 (60%) of whom were reported as free of disease. Hence, lymphadenectomy may have some effectiveness for lymph-node metastasis, but when visceral metastasis occurs, the treatment becomes difficult; all 15 reported cases with visceral metastasis died.

Several secondary therapies have been reported,[2] but their effectiveness is doubtful. There was a disappointing outcome for 6/10 patients (60%) who underwent radiation, 3/6 (50%) who underwent chemotherapy and the single patient who underwent immunotherapy. These data suggest that for visceral metastasis, adjuvant therapy has poor effectiveness and the prognosis is extremely poor. On the other hand, there are many case reports of a good clinical course without lymphadenectomy or secondary therapy; 35/84 'local excision only' patients (42%) were reported as disease-free. Our patient has also shown no recurrence during a 2-year follow-up without any other secondary therapy. We therefore speculate that there may be two groups of MES (aggressive and nonaggressive), and found in the literature that some cases of MES with sarcomatous or squamous changes in histopathological examination showed clinically aggressive behaviour.[8]

There were 13 reported cases of MES that showed sarcomatous or squamous change. Although such changes are slightly more common in female patients, there were no significant aetiological differences, including age, tumour size and location, between MES with or without sarcomatous and squamous changes. We then statistically analysed the 84 MES cases to identify whether such changes in histopathology can be a prognostic factor. Of 13 cases, 8 (61%)

Table 2 Comparison of pathological findings and prognosis of malignant eccrine spiradenoma in reported cases.

	Outcome		
Pathological findings	Fatal	Survived	Total
With sarcomatous or squamous change	7	6	13
Without sarcomatous or squamous change	8	63	70
Total	15	69	84

showed metastasis and 7 (53%) died. Of 15 fatal cases, 7 showed such changes in histopathology. Using the χ^2 test, patients with sarcomatous or squamous change in histopathological examinations had a significantly higher incidence of death ($P < 0.005$) (Table 2). Although the pathogenesis and mechanisms of the sarcomatous or squamous changes in MES are still not precisely known, this result suggests that histological alteration of MES promotes the metastasis of the tumour. Therefore, we conclude that the presence of sarcomatous or squamous change is a critical prognostic factor and it is important to evaluate the presence of such areas in the whole specimen.

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ORIGINAL ARTICLE

Prevalence of joint replacement surgery in rheumatoid arthritis patients: cross-sectional analysis in a large observational cohort in Japan

Jinju Nishino · Sakae Tanaka · Toshihiro Matsui · Toshihito Mori · Keita Nishimura · Yoshito Eto · Atsushi Kaneko · Koichiro Saisho · Masayuki Yasuda · Noriyuki Chiba · Yasuhiko Yoshinaga · Yukihiko Saeki · Atsuhito Seki · Shigeto Tohma

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Abstract The purpose of this study was to describe the prevalence of total joint arthroplasty (TJA) in Japanese rheumatoid arthritis (RA) patients undergoing conventional drug treatment in a large observational cohort in Japan. A total of 5,177 RA patients were studied for the prevalence of TJA, who were enrolled in the NinJa database during the fiscal year of 2006. The cases of 2,695 RA patients with more than ten years of disease duration were extracted and subjected to further analysis. The prevalence of TJA increased in accordance with the disease duration, and the prevalence was markedly increased after ten years. Among the 2,695 patients with more than ten years of disease duration, 1,431 TJAs were performed in 645 (24.6%) patients. The patients with TJA had higher disease activity than those without TJA. In this cross-sectional study, TJAs were performed in approximately a quarter of the Japanese RA patients with more than ten years of disease duration. The result showed that patients with higher disease activity required TJA.

J. Nishino (✉)
Nishino Clinic Orthopedics and Rheumatology,
2-9-15 Nishigaoka, Kita-ku, Tokyo 115-0056, Japan
e-mail: west@kitanet.ne.jp

S. Tanaka
Department of Orthopaedic Surgery, Faculty of Medicine,
The University of Tokyo, Tokyo, Japan

T. Matsui
Department of Rheumatology, Sagamihara National Hospital,
National Hospital Organization (NHO), Kanagawa, Japan

T. Mori
Department of Orthopaedic Surgery, Sagamihara National Hospital, National Hospital Organization (NHO),
Kanagawa, Japan

K. Nishimura
Department of Orthopaedic Surgery,
Teikyo University School of Medicine,
Tokyo, Japan

Y. Eto · A. Kaneko
Department of Orthopedics, Nagoya Medical Center,
NHO, Nagoya, Japan

K. Saisho
Department of Rheumatology, Miyakonojo Hospital,
NHO, Miyazaki, Japan

M. Yasuda
Department of Rheumatology, Beppu Medical Center,
NHO, Oita, Japan

N. Chiba
Department of Rheumatology, Morioka National Hospital,
NHO, Iwate, Japan

Y. Yoshinaga
Rheumatic Disease Center, Kurashiki Medical Center,
Kurashiki, Japan

Y. Saeki
Department of Rheumatology and Clinical Research,
NHO National Osaka Minami Medical Center,
Osaka, Japan

A. Seki
Division of Orthopedics,
National Center for Child Medical Health and Development,
Tokyo, Japan

S. Tohma
Department of Rheumatology,
Clinical Research Center for Allergy and Rheumatology,
Sagamihara Hospital, NHO, Kanagawa, Japan

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Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disorder which leads to varying degrees of functional impairment and disability. In spite of the recent progress in pharmacological treatment, some patients develop progressive joint destruction and eventually require surgery [1–4]. Total joint arthroplasty (TJA) is a highly successful procedure to treat destroyed joints in RA patients, and the effectiveness of the procedure on overall function and quality of life is now well established. Recently, the importance of certain inflammatory cytokines in the pathology of RA has become clear, and biologic agents targeting tumor necrosis factor-α (TNF-α) are commonly used in clinical practice. There is accumulating evidence that the progression of joint erosion can be ameliorated by early and aggressive treatment using disease-modifying antirheumatic drugs (DMARDs) and biological agents [5–8], and therefore clinical remission and prevention of joint destruction have become realistic goals of RA treatment. Such a drastic evolution of the applicability of drug therapy now compels us to reconsider the proper indication and timing of surgical intervention in RA patients. To do this, it is important to verify the prevalence of TJA in RA patients undergoing conventional drug treatment. Previous studies have revealed that there are variations in the rates of TJA by region, sex, race and socioeconomic status [9–11]. However, a nationwide survey of the prevalence of TJA in Japanese RA patients has not been performed, in part because of the absence of a national registry of procedures or a national health insurance program in Japan. Since 2002, we have developed a nationwide observational cohort database of rheumatic diseases, NinJa (National Database of Rheumatic Diseases by iR-net in Japan), which is located in 33 institutions located throughout the country. The aim of the current study was to analyze the prevalence of TJA in RA patients on conventional drug treatment strategies in Japan by cross-sectional analysis using the NinJa database.

Methods

Data source

The data source employed in this study was a nationwide observational cohort database of rheumatic diseases in Japan, NinJa, which was previously described in detail [12]. The NinJa project was reviewed and approved by the National Hospital Organization research ethics committees, and all the patients participating in the study provided written informed consent. NinJa has been performing data collection from patients since 2002 in 33 institutions located throughout the country. All the patients included in the present study fulfilled the 1,987 classification criteria of the American College of Rheumatology. The data consist of two components; one is the patient information collected over the course of the year [outcome, death, hospitalization, operation, number of TJAs in large joints (hip, knee, shoulder, and elbow), malignancy, and tuberculosis], and the other is the information collected on an arbitrary day in daily clinical practice [the count of tender joints (TJC) and swollen joints (SJC), a modified health assessment questionnaire (MHAQ), patient's global and pain visual analog scales (VASs), doctor's VAS, ESR, CRP, disease activity score (DAS)28-ESR, DAS28-CRP, use of corticosteroids, DMARDs, and nonsteroidal anti-inflammatory drugs].

Patients

To analyze the prevalence of TJA in large joints in RA patients, we examined the data for 5,177 RA patients who were enrolled in the NinJa database during the fiscal year of 2006 (from April 2006 to March 2007). The numbers of patients with disease durations of 0–5 years, 6–10 years, 11–15 years, 16–20 years, 21–25 years, 26–30 years and more than 31 years who had undergone TJA were counted, and the prevalences of TJAs were calculated.

Analyses and statistics

Descriptive statistics were employed to analyze clinical information, demographic factors and other test data. Continuous variables were expressed as means and SD. Differences between groups were examined using a one-way analysis of variance (ANOVA) for continuous variables, or a χ^2 test for categorized data when appropriate.

Results

The prevalence of TJA in Japanese RA patients

The proportion of operated patients increased in accordance with the duration of the disease (Fig. 1). The prevalences of TJA in the groups with disease durations of 0–5 years, 6–10 years, 11–15 years, 16–20 years, 21–25 years, 26–30 years and more than 31 years were 2.9%, 9.3%, 19.9%, 24,1%, 24.7%, 30.7% and 30.5%, respectively (Fig. 1). The prevalence of TJA increased in accordance with the disease duration. In particular, the prevalence markedly increased after ten years, and 5.5% of

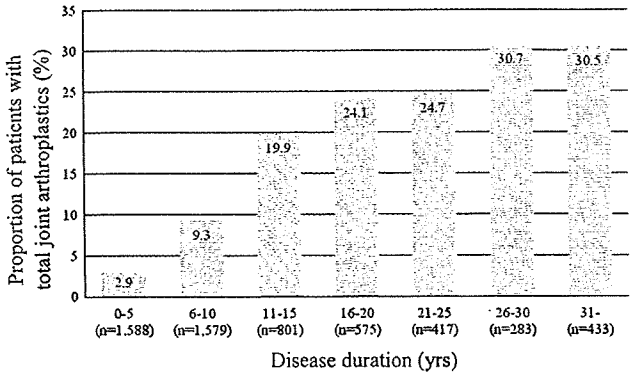

Fig. 1 The relationship between the prevalence of joint replacement surgery and (the patient's) disease duration

the patients underwent TJA within ten years and 24.6% after more than ten years of disease duration, which was significantly different ($P < 0.001$).

Since the prevalence of TJA in patients markedly and significantly increased after ten years of disease, 2,695 patients with more than ten years of disease (patients >10 years) were subjected to further analysis (Fig. 1). There were 2,347 (87.1%) women and 348 men (12.9%). The mean age was 63.8 years old with a standard deviation (SD) of 10.8 (range 23–92), and the mean disease duration was 20.8 (SD 9.3) years (range 10–65). Of the 2,695 patients >10 years disease duration, 645 (24.6%) patients underwent 1,431 TJA in total. The number of surgeries performed per patient was one in 254 patients (39.3%), two in 240 patients (37.3%), three in 74 patients (11.5%), four in 67 patients (10.5%) and five in nine patients (1.4%). More than 60% of the patients who underwent TJA received two or more arthroplasty surgeries, and the mean number of surgeries per patient was 1.87. The patients with TJA exhibited a significantly longer disease duration, more TJC, and a higher proportion of oral corticosteroid usage than those without TJA. DAS28 was significantly higher in the patients with TJA than in those without (4.66 vs. 4.01, $P < 0.001$). In addition, ESR, CRP, MHAQ and pain VAS were significantly higher in the patients with TJA. In contrast, there was no significant difference in the age of disease onset, gender, SJC, DMARD frequency, or the daily dose of oral corticosteroid between the groups. These results indicate that the patients with a higher level of disease activity and poorer control of the disease require TJA.

Discussion

In this cross-sectional study, we analyzed the prevalence of TJA in 5,177 Japanese RA patients who were enrolled in the NinJa database during 2006. The prevalence of TJA increased in accordance with the disease duration, and 5.5% of patients underwent TJA within ten years and 24.6% after more than ten years of disease duration. More than 60% of the operated patients with more than ten years of disease duration underwent more than one TJA, and the mean number of surgeries per patient was 1.87. These results are in line with the results of previous studies performed in European countries and in the USA [2–4, 13, 14]. Kapetanovic et al. reported that 24% of RA patients underwent primary TJA, and 46% of them underwent an additional arthroplasty during 16–20 years of follow-up [3]. Wolfe reported that 17.8% of RA patients underwent total joint arthroplasty over a mean disease duration of 15.9 years, and 25% of the patients seen within two years of disease onset were predicted to have a total joint arthroplasty within 23.1 years [4]. They also reported that the mean number of surgeries per patient was 1.85. Da Silva reported that 28.6% of RA patients underwent reconstructive surgery and Verstappen reported 27% in the Utrecht RA cohort [2, 14]. Based on a cross-sectional study, Hakala reported that 20% of RA patients required surgery within a decade of disease duration [13].

There are certain differences between this study and other reports. We were unable to find a gender difference in the prevalence of TJA, although some studies have reported that female RA patients have significantly more surgeries than male patients [2, 15]. Massardo et al. reported that the risk of joint surgery for RA-related joint disease was 1.4 times higher for women than men, mainly because of the greater frequency of small joint (hand and foot) surgery in women [15]. Da Silva also reported that female RA patients had more small joint surgeries than male patients [2]. James et al. commented that female gender was a risk factor for hand or foot joint surgery (the odds ratio being 3.2) [16]. We only analyzed the prevalence of large joint TJA and not that of small joint surgeries in the current study, which may explain the discrepancy between our results and the previous findings. James et al. also showed that a gender difference was not found in the prevalence of major surgeries (hip, knee, shoulder elbow replacement and spine surgery) (male 6.9% vs. female 7.3%) [16].

We were unable to find a correlation between the age of RA onset and the prevalence of surgery either. Consistent with our results, Kapetanovic also failed to show a gender or age difference between patients with and without large joint TJA [3]. This may be because surgeons avoid joint-sacrificing procedures such as TJA and instead select joint-preserving procedures, such as synovectomy, for younger patients. James et al. also reported that the ratio of intermediate to minor surgery (75.7%) in the patients with earlier onset (<45 years old) was larger than that (40.6%) in the patients with later onset (>60 years old) [16].

Table 1 Demographic and disease characteristics for patients with more than 10 years of disease duration with or without total joint arthroplasty (TJA)

	Patients with TJA ($n = 645$)	Patients without TJA ($n = 2,050$)	P value
Age (years)	65.1 (9.9)	63.4 (11.0)	0.001
Gender	F = 586	F = 1,761	0.346
	M = 58	M = 289	
Age of disease onset (years)	42.6 (12.1)	43.2 (12.9)	0.334
Disease duration (years)	22.5 (9.6)	20.2 (9.1)	0.001
DAS28·ESR	4.66 (1.19)	4.01 (1.2)	0.001
Tender joint count	6.3 (7.5)	4.4 (6.3)	0.001
Swollen joint count	3.3 (4.7)	3.0 (4.0)	0.075
Pain VAS (cm)	4.8 (2.5)	3.7 (2.5)	0.001
MHAQ	1.30 (0.80)	0.72 (0.71)	0.001
ESR (mm/hr)	53 (31)	38 (26)	0.001
CRP (mg/L)	16.4 (20.9)	10.6 (15.0)	0.001
DMARDs usage (%)	82.8	84.8	0.168
Oral corticosteroid usage (%)	74.2	65.4	0.001
Daily amount of oral corticosteroid (mg/day)	4.8 (3.4)	4.7 (2.6)	0.29

Data are presented as means (SD)

We found that the patients who had undergone TJA had higher disease activity. The mean DAS28 score of patients with TJA was 4.66, while the DAS28 of those without TJA was 4.01, which was significantly different ($P < 0.001$) (Table 1). The same was true of the other components such as pain VAS, MHAQ, ESR, CRP and the daily amount of oral corticosteroid (Table 1). These results suggest that patients who underwent TJA were under poorer disease control and suffered joint deterioration.

RA involves multiple joints, and therefore the indication of TJA should be determined not only by the function of the affected joints in isolation, but also the general status of the patient, including the functional conditions of other joints. We found a significant difference in MHAQ between patients >10 years with TJA and without TJA (1.3 vs. 0.72, $P < 0.001$). Consistent with our results, several previous reports have suggested that MHAQ is a risk factor for surgery in RA patients [3, 17–19]. As Anderson indicated in his review, "a patient who presents with a desire to improve his or her function is usually better motivated and will profit more by surgical intervention than an individual who is solely driven by an attempt to alleviate pain." [1].

Improved control of RA disease activity with new treatment strategies, such as early intervention using biologics combined with conventional DMARDs, is expected to reduce the extent of affected joints and the degree of joint destruction, and hence reduce the number of patients undergoing major joint surgery such as TJA. Several authors have in fact recently reported a decrease in orthopedic surgery with this type of aggressive treatment strategy [2, 20–23].

The limitation of the present study is that it is a retrospective cross-sectional analysis, and a prospective longitudinal study will need to be performed to identify the predictors of TJA in Japanese RA patients. In addition, most of the patients included in this study had been subjected to conventional treatment strategy and not to the early aggressive treatment recently recommended. However, the details of TJAs done under a conventional drug treatment strategy that are shown here provide basic data for discussing and ultimately constructing a new point of view concerning orthopedic intervention in the era of new drug treatment strategies in Japan.

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High frequency of acid α-glucosidase pseudodeficiency complicates newborn screening for glycogen storage disease type II in the Japanese population

Shingo Kumamoto [a], Tatsuya Katafuchi [a], Kimitoshi Nakamura [b], Fumio Endo [b], Eri Oda [c], Torayuki Okuyama [c], Marian A. Kroos [d], Arnold J.J. Reuser [d], Toshika Okumiya [a,*]

[a] Department of Analytical Biochemistry, School of Health Sciences, Kumamoto University, 4-24-1, Kuhonji, Kumamoto 862-0976, Japan
[b] Department of Pediatrics, School of Medicine, Kumamoto University, Kumamoto, Japan
[c] Department of Laboratory Medicine, National Center for Child Health and Development, Tokyo, Japan
[d] Department of Clinical Genetics, Erasmus MC, Rotterdam, The Netherlands

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ABSTRACT

To investigate the feasibility of newborn screening for glycogen storage disease type II (GSDII; Pompe disease or acid maltase deficiency) in the Japanese population, we assayed the acid α-glucosidase activity in dried blood spots from 715 Japanese newborns and 18 previously diagnosed patients using a fluorometric procedure. The enzyme activity of apparently healthy newborns showed a bimodal distribution. The median activity of the minor group (31 individuals, 4.3% of the samples) was 6.5 times lower than that of the major group. Four of the 715 control samples (0.56%) fell in the patient range. We then analyzed genomic DNA, extracted from the same blood spots, for the occurrence of two sequence variants, c.1726G>A and c.2065G>A, known to cause "pseudodeficiency". This analysis revealed that 27 of 28 individuals homozygous for c.[1726A; 2065A] belonged to the minor group. One c.[1726A; 2065A] homozygote had just slightly higher activity. Twelve of the 18 patients with GSDII either had one (9 cases) or two (3 cases) c.[1726A; 2065A] alleles. The frequency of this allele was double in the patient compared to the control group (0.42 *vs* 0.19) at the expense of a lower frequency of the c.[1726G; 2065G] and c.[1726G; 2065A] alleles (0.58 *vs* 0.71 and 0 *vs* 0.1). These findings illustrate that c.[1726A; 2065A] homozygosity among apparently healthy individuals (3.9 per 100) complicates newborn screening for GSDII in Japan, and further that one or more pathogenic mutations are associated with the c.[1726A; 2065A] allele.

Enzyme replacement therapy for lysosomal storage diseases has focused attention on the need for early diagnosis in order to optimize the therapeutic outcome. Along this line several initiatives have been taken to develop methods for newborn screening. Most methods are based on the direct measurement of lysosomal enzyme activities in dried blood spots (DBSs) [1–5]. Other procedures include antibodies to increase the specificity of the assay, or to determine the amount of enzyme protein rather than activity, or to probe lysosomal disease markers [6–9]. Multiplex assays with the measurement of several lysosomal enzyme activities are aimed to improve the cost effectiveness of newborn screening [10–13].

From several clinical trials since 1999, the picture emerges that patients with glycogen storage disease type II (GSDII) can benefit from enzyme replacement therapy [14–18]. GSDII, also known as Pompe disease or acid maltase deficiency (OMIM No. 232300) is an autosomal recessive disorder of glycogen metabolism resulting from a generalized deficiency of the lysosomal enzyme acid α-glucosidase (AαGlu[1]; EC 3.2.1.20/3). The enzyme deficiency causes intralysosomal glycogen storage in numerous tissues, but predominantly in muscle. The disorder exhibits a broad clinical spectrum with regard to age of onset, cardiac involvement and progression of skeletal muscle dysfunction. The effect of therapy in severely affected infants is readily recognized by regression of the cardiomegaly, prolonged survival and acquirement of motor skills. Beneficial effects of enzyme replacement therapy in children, adolescents and adults with GSDII also have been reported and are promising, but the crucial outcomes of larger clinical trials is still to be awaited as well as the long term effects [17–20]. Further, it appears that infants with rather well preserved muscle morphology respond better to therapy than those who are diagnosed late and have severe muscle damage at start of treatment. Early diagnosis seems a must in GSDII to optimize any form of therapeutic intervention [21].

Recently, we have established an assay procedure for the reliable diagnosis of GSDII in mixed leukocytes whereby acarbose is used to inhibit the interfering α-glucosidase activity of

* Corresponding author. Fax: +81 96 373 5492.
E-mail address: okumiyat@kumamoto-u.ac.jp (T. Okumiya).

[1] *Abbreviations used:* AαGlu, acid α-glucosidase; GSDII, glycogen storage disease type II; 4MU-αGlc, 4-methylumbelliferyl α-D-glucopyranoside; DBS, dried blood spot.

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maltase–glucoamylase [22]. Of note, acarbose was introduced earlier to eliminate the interfering maltase-glucoamylase activity in DBS assays [10] and is more suitable for that purpose than maltose [3]. Given the recent interest in newborn screening and the awareness that the diagnosis of GSDII in Japan and in other Asian populations might be complicated by the existence of a "pseudodeficiency" allele [23,24], we performed a pilot experiment investigating the feasibility of newborn screening in Japan. "Pseudodeficiency" of AαGlu is associated with two SNPs, c.1726G>A (p.G576S) and c.2065G>A (p.E689K) that have a different distribution in Asian compared with Caucasian populations [23–25]. Substitution p.E689K caused by c.2065G>A characterizes the "GAA4" allozyme, which is found in Chinese and Japanese populations with frequencies of 0.27–0.28 and 0.27–0.31, respectively, and reduces the AαGlu activity by 50% at most [26–28; JSNP, http://snp.ims.u-tokyo.ac.jp/map/cgi-bin/aa_XM.cgi?NM_000152.2]. On the contrary, substitution p.G576S caused by c.1726G>A reduces the activity to such extent that it falls into the patient range [24]. Recently, it was shown that the structural changes brought about by each of the two substitutions are small and do not affect the active site of AαGlu [23].

Here, we present the results of the experiment in which we measured the AαGlu activity in DBSs with a fluorometric procedure while we performed in parallel haplotype analysis on DNA extracted from the same spot. Based on our findings we conclude that the high frequency of the "pseudodeficiency" allele in the Japanese population complicates the finding of an enzymatic screening procedure that is both sensitive and specific.

Subjects, materials and methods

Subjects and DBS collection

Seven hundred and fifteen Japanese newborns (second to fifth day postpartum) and 18 Japanese patients with GSDII were enrolled in this study. The patient group included one patient with classic infantile form, 6 with juvenile form, 10 with adult form and one with unknown phenotype. The DBSs on filter paper were obtained with the standard heel-stick for collecting newborn screening samples, or prepared by drop-wise addition of EDTA-blood samples on the filter paper (filter paper #510AD01, Advantec, Tokyo, Japan) that is routinely used for newborn screening in Japan. DBSs were dried at room temperature for at least 3 h but no more than 16 h, and were subsequently stored at −20 °C in sealed plastic bags until use. Written informed consent was obtained from all subjects, and all samples from these subjects were prepared and analyzed in accordance with the protocols approved by the institutional responsible committee.

Chemicals and reagents

4-Methylumbelliferyl α-D-glucopyranoside (4MU-αGlc) was purchased from Sigma-Aldrich (St. Louis, MO). Acarbose, 4-methylumbelliferone and Proteinase K were from Toronto Research Chemicals (North York, Canada), Nacalai Tesque (Osaka, Japan) and Roche (Basel, Switzerland), respectively. Ampdirect™ Plus with NovaTaq™ Hot Start DNA polymerase was obtained from Simadzu (Kyoto, Japan). Other chemicals were of reagent grade and from Sigma–Aldrich or Nacalai Tesque.

Enzymatic assay

A 3.2-mm diameter disk was punched out from the DBS on the filter paper and incubated in a well of a 96-well clear microwell-plate (Corning, New York, NY) with 100 μL distilled water by mixing gently for 1 h at room temperature. The water extract was used for the enzymatic assay. The disk was recovered for DNA extraction and genotype analysis. The AαGlu activity was measured fluorometrically with 4MU-αGlc as substrate according to our previous report with minor changes [22]. Briefly, 20 μL of the extract was added to 40 μL of the substrate solution containing 2.0 mmol/L 4MU-αGlc in 0.2 mol/L citrate/0.4 mol/L sodium-phosphate buffer, at pH 4.0 with 4.5 μmol/L acarbose (3.0 μmol/L in final concentration), in a 96-well black microwell-plate (PerkinElmer, Boston, MA). The reaction mixture was incubated at 37 °C for 24 h, and the reaction was stopped by addition of 190 μL of 0.2 mol/L glycine-NaOH buffer at pH 10.7 containing 0.1% Triton X100. The fluorescence intensity was measured with the CORONA spectrofluorometer (MTP-600F, Colona Electric, Hitachinaka, Japan) at excitation and emission wave-lengths of 360 nm and 450 nm, respectively, and corrected for substrate blank. We used a stock solution of 100 μmol/L 4-methylumbelliferone in 20 mmol/L sodium-phosphate buffer (pH 7.0) to calibrate the measurement of liberated 4-methylumbelliferone. The enzyme activity was expressed as pico moles 4-methylumbelliferone released per hour per 3.2 mm diameter disk (pmol/h/disk). Each assay was performed in duplicate. The measured values per group are expressed as means ± SD unless otherwise indicated.

Disk clean-up and genotype analysis

After extraction with distilled water for assay of enzyme activity, the disk was recovered, washed with a 100 μL solution of 0.1% Triton X100 in water and incubated in 100 μL digestion buffer containing 0.2 mg/ml Proteinase K, 0.5% sodium dodecyl sulfate, 5 mM EDTA, 400 mM NaCl and 20 mM Tris–HCl (pH 8.0), in a 1.5 ml reaction tube, at 55 °C for 1 h. The reaction was terminated by heating for 10 min on a heat block at 95 °C. The disk was rinsed twice with 500 μL of 10 mM Tris–HCl containing 1 mM EDTA (pH 8.0), once with 400 μL isopropanol, and then dried on a heat block at 70 °C for 60 min. The cleaned-up disk was stored at 4 °C until use for genotype analysis. Genotype analysis was performed by PCR-based detection (amplification refractory mutation system; ARMS). Each cleaned-up disk was cut into 4 pieces with scissors or with a scalpel, and the pieces were placed into 4 PCR tubes each containing 10 μL Ampdirect™ Plus (including PCR buffer and dNTPs), 0.5 units NovaTaq™ Hot Start DNA polymerase, and a set of specific primers (each 0.5 μmol/L), in a total volume of 20 μL reaction mixture. We designed 4 different oligonucleotide primers for 4 PCR sets including either, 5′-TACAACCTGCACAACCTCAACG-3′ (F1) or 5′-TACAACCTGCACAACCTCAACA-3′ (F2) as forward primer and either, 5′-GGCCTGCTGGGCCGACTC-3′ (R1) or 5′-GGCCTGCTGGGCCGACTT-3′ (R2) as reverse primer for the amplification of 4 different *GAA* alleles characterized by different SNPs: the combination F1 + R1 for c.[1726G; 2065G], F1 + R2 for c.[1726G; 2065A], F2 + R1 for c.[1726A; 2065G], and F2 + R2 for c.[1726A; 2065A]. Each oligonucleotide primer was designed to have a one-base mismatch nucleotide at the −4 base position from the 3′ terminal end to improve the selectivity for allele detection. PCR was performed under the following conditions; an initial denaturation at 96 °C for 10 min; 40 cycles amplification with denaturation at 96 °C for 20 s, annealing at 64 °C for 20 s and extension at 72 °C for 90 s; and extra extension at 72 °C for 7 min. The PCR products (1209 bp fragments) were separated by 2% agarose gel electrophoresis and visualized with ethidium bromide staining. This method allows determining 10 different diplotypes, which were constructed from the combination of the 4 haplotypes (Tables 1 and 2). To confirm the reliability of the present method, DNA sequencing analysis was performed according to the procedure described elsewhere [24] for all diplotypes from 18 individuals.

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