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Case Study

Bilateral Conjunctival Lesions in Blastic Plasmacytoid Dendritic Cell Neoplasm

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The purpose of this study is to report on a patient who developed conjunctival lesions of blastic plasmacytoid dendritic cell neoplasm (BPDCN) after radiation to skin lesions of the same pathological type. A 79-year-old man developed salmon-pink lesions in the lower fornix of the conjunctiva of both eyes and biopsy revealed BPDCN. One and a half years previously, he noticed an erythematous plaque with a 30-mm diameter, which later became multiple, on the left chest, and the biopsy revealed BPDCN. The bone marrow was negative for CD56-positive cells, but fluorescence-activated cell sorting analysis of peripheral blood cells revealed a group of cells positive for CD4 and CD56, or CD4 and CD123. The monocyte fraction, in an increased percentage of white blood cell counts, did contain atypical cells positive for the three markers. Whole-body 2-[^{18}F]fluoro-2-deoxy-D-glucose positron emission tomography combined with computed tomography demonstrated no abnormal uptake lesions. He received 30 Gy of radiation to the chest lesions, and later, the same dose of radiation to novel skin lesions on the back, waist, and chest, and also to pharyngeal lesions. In conclusion, the conjunctiva could be involved with BPDCN and pathological differential diagnosis by biopsy is mandatory to establish the correct diagnosis. 〔*J Clin Exp Hematopathol 51(1) : 49-55, 2011*〕

Keywords: blastic plasmacytoid dendritic cell neoplasm (BPDCN), conjunctiva, skin (cutaneous), pharynx (pharyngeal), 2-[^{18}F]fluoro-2-deoxy-D-glucose positron emission tomography combined with computed tomography (FDG-PET/CT)

INTRODUCTION

Ocular adnexa is the term that describes the supporting tissues for eye globes in the orbital space, such as lacrimal glands, lacrimal sacs, conjunctiva, eyelids, and extraocular muscles, and is a major site for the development of malignant lymphoma.[1] The conjunctiva, as part of the ocular adnexa, is the primary or the secondary site of lymphoma involvement. The most frequent pathological type of primary lymphoma, occurring in the conjunctiva, is extranodal marginal zone B-cell lymphoma of the mucosa-associated lymphoid tissue (MALT), or MALT lymphoma.[1-4] Indeed, the conjunctiva has been shown to have mucosa (conjunctiva)-associated lymphoid tissue, which can be acquired in the normal condition.[5] Other less common types of primary lymphoma in the conjunctiva, which have been reported so far, include T-cell lymphoma[6] and Burkitt lymphoma.[7] Benign lymphoid hyperplasia[8-10] and leukemic infiltration[11-15] also manifest as conjunctival masses. All conjunctival lesions, including lymphoma, leukemic infiltration, and benign lymphoid hyperplasia, present the same clinical feature called salmon-pink lesions.

In this study, we report on a patient who developed bilateral conjunctival lesions of blastic plasmacytoid dendritic cell neoplasm (BPDCN) after radiation to original skin lesions of the same pathological type.

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CASE REPORT

A 79-year-old man was referred for probable involvement of malignant lymphoma in the conjunctiva of both eyes. One and a half years previously, he noticed an erythematous plaque with a 30-mm diameter on the left chest (Fig. 1A), and biopsy revealed BPDCN (Fig. 2). In the following three months, he gradually developed multiple chest skin lesions (Fig. 1B) and thus a bone marrow biopsy was carried out to

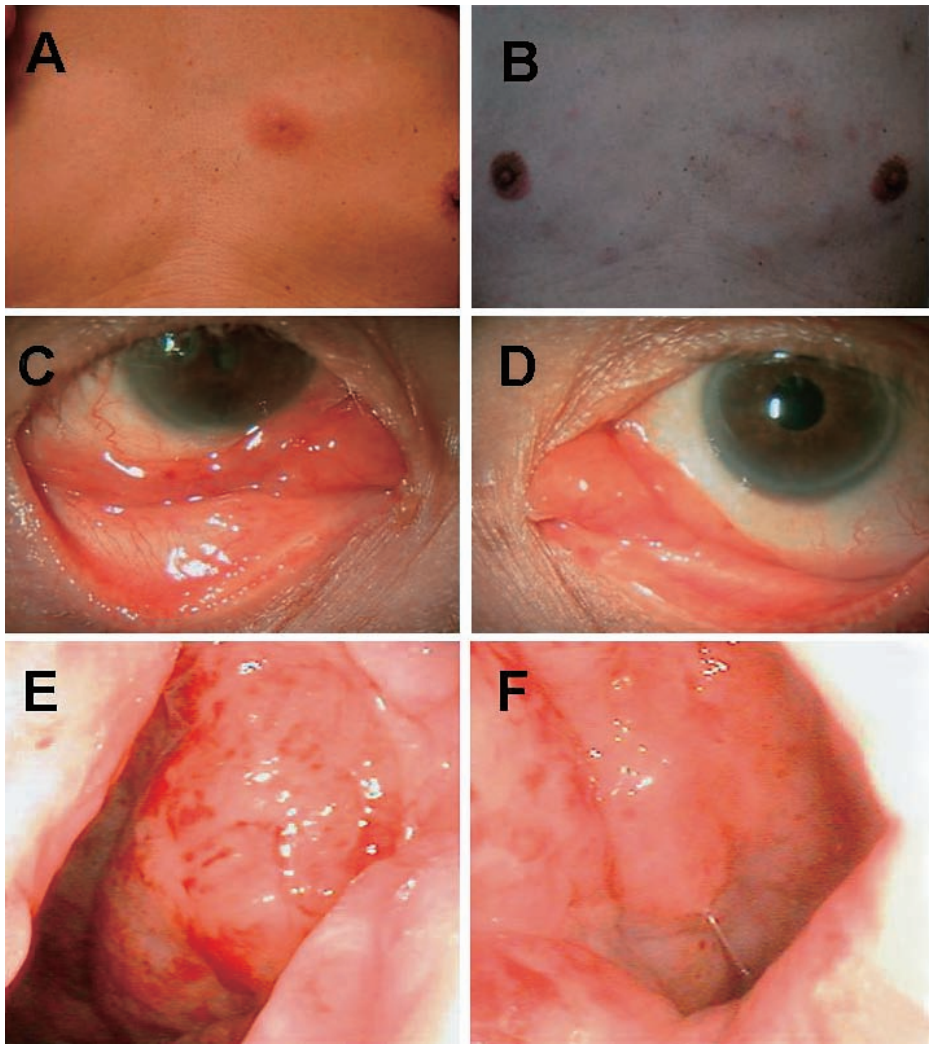


Fig. 1. A solitary round erythematous plaque with 30-mm diameter on the left chest skin (*1A*) at initial presentation and multiple skin lesions on the chest (*1B*) after a few months of follow-up. The conjunctival salmon-pink lesions involving the lower bulbar conjunctiva and lacrimal caruncle in both eyes (*1C*: right eye, and *1D*: left eye) a year later. Tumorous elevation on the right upper pharynx (*1E*) and the left upper pharynx (*1F*), near the orifice of the Eustachian tube, two months after conjunctival biopsy.

reveal hypercellular marrow but without CD56-positive cells. The proportion of monocytes (9.8%) among the bone marrow cells was within the normal range. Complete blood cell counts showed $6.31\times10^3/\mu$L of white blood cells with an increased proportion (16%) of monocytes, $4.16\times10^6/\mu$L of red blood cells, and $160\times10^3/\mu$L of platelets. The monocyte fraction contained atypical cells positive for CD4, CD56, and CD123 but negative for BDCA-2. The blood chemistry revealed liver and kidney functions in the normal ranges. Fluorescence-activated cell sorting (flow cytometric) analysis of peripheral blood cells at that time revealed a group of cells positive for both CD4 and CD56, and positive for both CD4 and CD123 (Fig. 3). Whole-body 2-[^{18}F]fluoro-2-deoxy-D-glucose positron emission tomography combined with computed tomography (FDG-PET/CT) demonstrated no lesion with abnormal uptake except for somewhat higher uptake in the bone marrow (Fig. 4). He received 30 Gy (in 10 fractions) of radiation to the chest lesions. Two months and 6 months later, he received 30 Gy of radiation to novel skin lesions on the back and those on the waist and chest, respectively.

Half a year previously, he noticed enlargement of the lacrimal caruncles of both eyes. Since the lacrimal caruncular lesions in both eyes extended gradually to the lower conjunctival fornix (Fig. 1C and 1D), the patient underwent biopsy of the lesions in both eyes, which revealed BPDCN (Fig. 2).

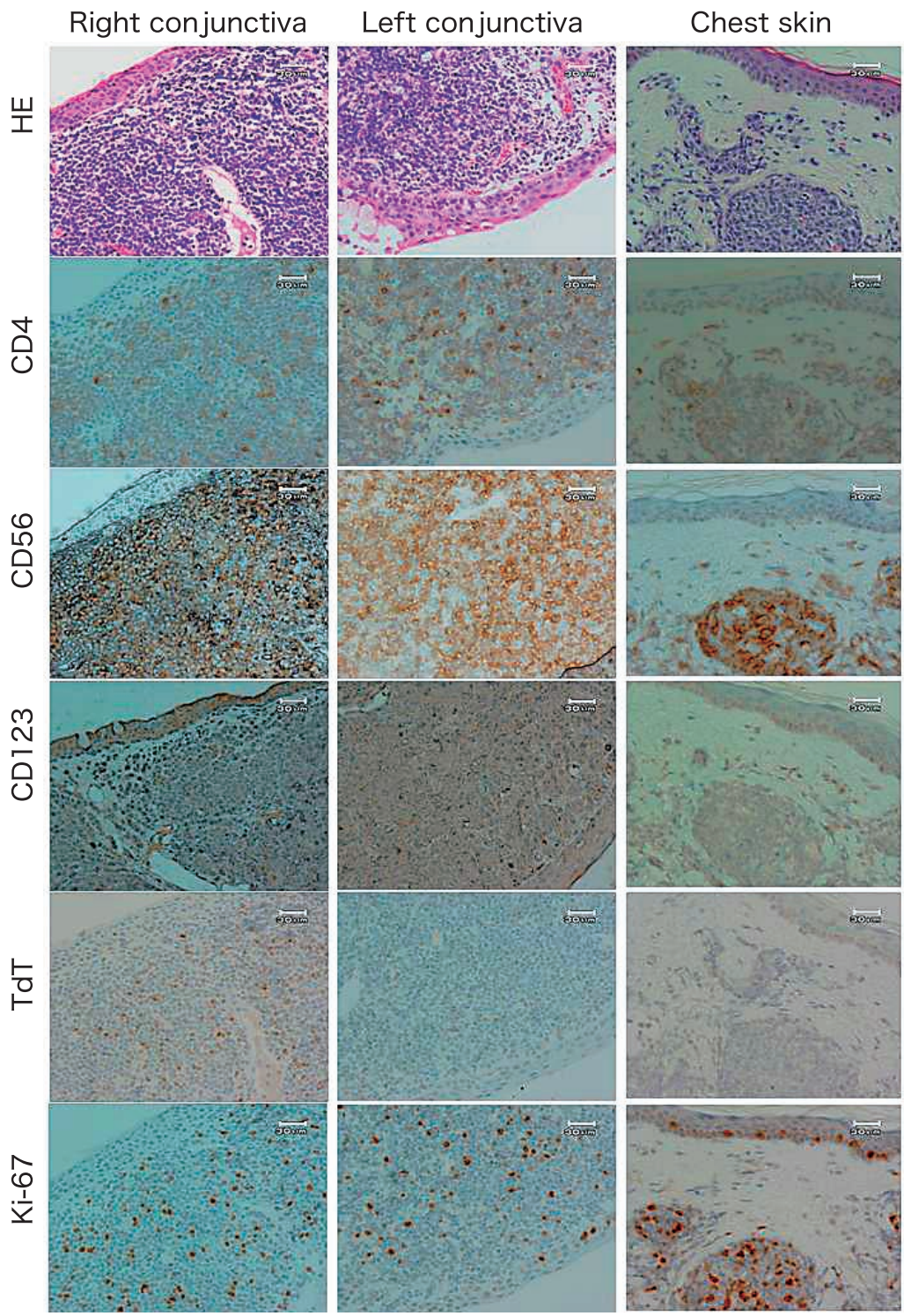
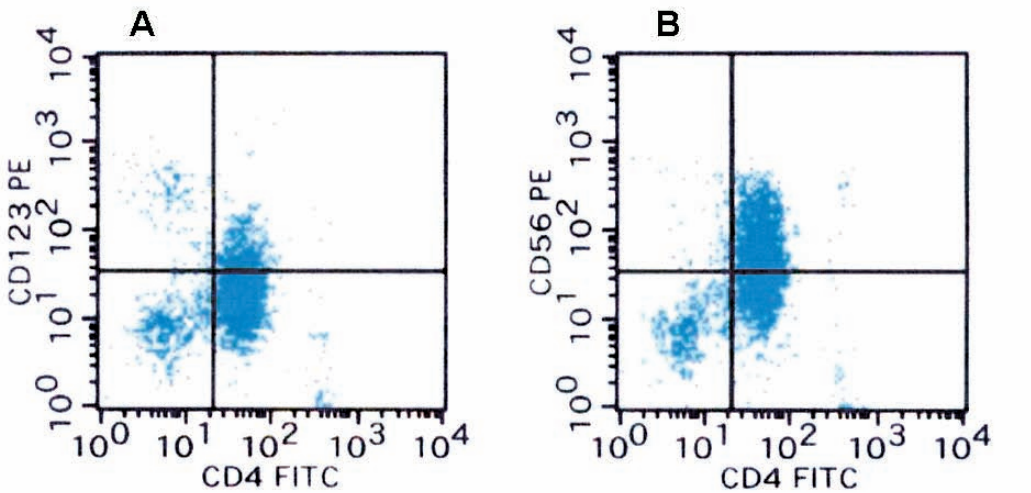


Fig. 2. Hematoxylin-eosin staining (*top row*) and immunohistochemical staining in the cutaneous (*right column*), right (*left column*), and left (*middle column*) conjunctival lesions. All three lesions are positive for CD4 and CD56, but negative for CD123. The neoplastic cells only in the right conjunctiva, but not in the skin and the left conjunctiva, are also positive for terminal deoxynucleotidyl transferase (TdT). The cells are positive for Ki-67 in all the lesions. *Bar* = 30 μm.



Quad	Events	% Gated
UL	89	3.39
UR	375	14.30
LL	284	10.83
LR	1874	71.47

Quad	Events	% Gated
UL	56	2.03
UR	1334	48.35
LL	290	10.51
LR	1079	39.11

Fig. 3. Fluorescence-activated cell sorting (flow cytometric) analysis of peripheral blood cells 3 months after the onset of the skin lesion on the left chest. ***Panel A***, double staining for CD4 with fluorescein isothiocyanate (*FITC*) and CD123 with phycoerythrin (*PE*); ***Panel B***, double staining for CD4 with fluorescein isothiocyanate (*FITC*) and CD56 with phycoerythrin (*PE*). The percentage of cells positive for CD4 and CD123 in the upper right (*UR*) quadrant (*3A*) is about 14% while the percentage of cells positive for CD4 and CD56 in the upper right (*UR*) quadrant (*3B*) is about 48%.

The best-corrected visual acuity was 1.0 in the left eye and no light perception in the right eye. The left eye had clear media with intraocular lens implantation and normal fundus. The right eye exhibited phthisis after the patient lost its vision by unsuccessful surgeries for retinal detachment 26 years previously. He underwent cataract surgery in the left eye 10 years previously. He had brachytherapy for prostatic adenocarcinoma 5 years previously.

A month after the biopsy of the conjunctiva, he noticed hearing disturbance as a howling sound or stuffiness of the ear on the left side. He basically had poor hearing on the right side. Pharyngeal endoscopy revealed tumorous elevation around the orifice of the Eustachian tube on both sides (Fig. 1E and 1F). Pharyngeal biopsy revealed BPDCN infiltration. Complete blood cell counts showed $6.24\times10^3/\mu$L of white blood cells with an increased proportion (30%) of monocytes, $3.98\times10^6/\mu$L of red blood cells, and $134\times10^3/\mu$L of platelets. Repeat FDG-PET/CT showed abnormal uptake in the left elbow skin and higher uptake in the bilateral upper pharynx compared with that at the initial scan (Fig. 4). Radiation was applied to the pharyngeal lesions and the new skin lesion.

METHODS

The tissues were fixed for 3 hr with formaldehyde and embedded in paraffin. The paraffin sections were deparaffinized and stained with hematoxylin-eosin and also by immunohistochemistry. Tissue sections were processed by standardized heating pretreatment (heating at 100°C for 30 min) for antigen retrieval prior to entering the immunohistochemical procedures.[10] In brief, the sections were incubated with 3% hydrogen peroxide for 5 min to inactivate endogenous peroxidase and blocked with 10% normal goat serum for 10 min. The sections were then incubated with primary antibodies overnight at 4°C, washed with 0.05% Tween 20-containing phosphate buffered saline three times, incubated with the secondary antibody at room temperature for 30-60 min, and washed. The color was developed with diaminobenzidine and the nuclei were counterstained with hematoxylin.

The standard primary antibodies used in this study were CD20 (mouse monoclonal, 1 : 200 dilution, Novocastra, Leica Microsystems, Wetzlar, Germany), CD3ε (mouse monoclonal, 1 : 50 dilution, Novocastra), CD4 (mouse monoclonal, pre-diluted, Nichirei, Tokyo, Japan), CD56 (mouse monoclonal, 1 : 25 dilution, Novocastra), CD123 (mouse

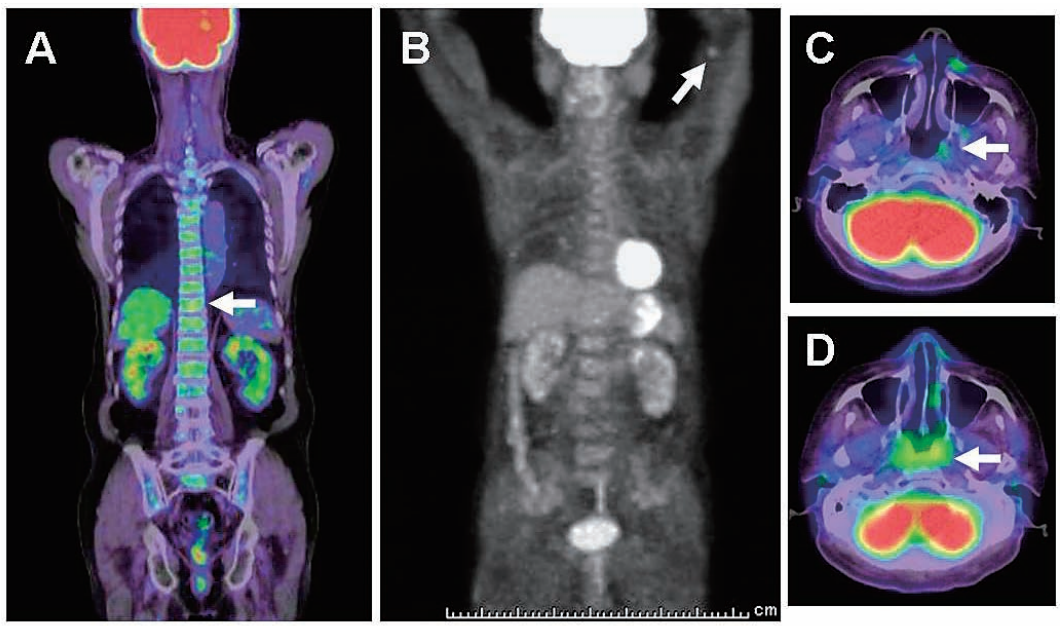


Fig. 4. Whole-body 2-[^{18}F]fluoro-2-deoxy-D-glucose positron emission tomography combined with computed tomography (FDG-PET/CT). On the initial scan after the skin biopsy, no lesion with abnormal uptake was noted except for somewhat higher uptake in the bone marrow (*arrow* in *4A*). On the second scan after the conjunctival biopsy, a year and three months after the initial scan, abnormal uptake was noted on the left elbow skin (*arrow* in *4B*), and higher uptake in the bilateral upper pharynx (*arrow* in *4D*) compared with that at the initial scan (*4C*).

monoclonal, 1 : 50 dilution, Dendritics, Lyon, France), Ki-67 (rabbit polyclonal, 1 : 5,000 dilution, Novocastra), and terminal deoxynucleotidyl transferase (TdT, mouse monoclonal, 1 : 20 dilution, Novocastra).

PATHOLOGICAL RESULTS

The skin biopsy specimen (Fig. 2) demonstrated that medium-sized lymphoid cells, with irregularly shaped nuclei, finely dispersed chromatin, and indistinct nucleoli, showed diffuse infiltration or formed nodular nests in the dermis (Fig. 2). Perivascular infiltration was present, but epidermal infiltration was absent. The lymphoid cells were positive for CD4 and CD56, but gave negative results for CD123, TdT, CD3, or CD20 by immunohistochemistry, leading to the diagnosis of BPDCN. The excisional biopsy specimens in the conjunctiva of both eyes (Fig. 2) showed that lymphoid cells with nuclear atypia and fine chromatin aggregated diffusely beneath the conjunctival epithelium (Fig. 2). The lymphoid cells were positive for CD4 and CD56, but negative for CD123, CD3, or CD20. TdT was positive in the right conjunctival lesion while negative in the left conjunctival lesion. On pharyngeal biopsy, diffuse monotonous cells under the pharyngeal epithelium were positive for CD4, CD56, and Ki-67, but negative for CD3, CD20, or cytokeratin AE1/AE3 (data not shown). Myeloperoxidase and lysozyme immunohistochemical staining was absent in tumor cells of all the tissues.

DISCUSSION

Blastic plasmacytoid dendritic cell neoplasm (BPDCN), defined as a new entity in the new 2008 World Health Organization (WHO) classification of tumors of hematopoietic and lymphoid tissues, frequently manifests as solitary or multiple skin lesions in the initial phase, and later develops leukemic dissemination with poor prognosis.[16,17] The neoplastic cells are derived from plasmacytoid dendritic cells, which account for less than 0.1% of peripheral blood mononuclear cells and function as type 1 interferon-producing cells. The plasmacytoid dendritic cells can differentiate into dendritic cells upon activation and might play a role in antigen presentation. Therefore, the entity represents a hematopoietic neoplasm and is not considered as lymphoma in the most recent classification.[18]

Histopathologically, the BPDCN is characterized by subcutaneous infiltration with medium-sized blastoid cells. The diagnosis of the BPDCN is confirmed by immunohistochemical results : neoplastic cells are positive for CD4, CD56 [140 kDa neural cell adhesion molecule (N-CAM) isoform], and CD123 (interleukin-3 receptor α-chain), while negative for CD20 and CD3, which are lineage-specific markers of B-cells and T-cells, respectively. The three markers are not always positive, as demonstrated in the present case where the tumor cells were positive for CD4 and CD56, but negative for CD123. The immunostaining for CD123 would be inter-

preted as weakly positive in the present case. TdT, a marker for precursor hematopoietic cells, which reinforces the BPDCN diagnosis, was positive in the right conjunctival lesion while negative in the left conjunctival lesion and the cutaneous lesion of this patient.

The conjunctival lesions (salmon-pink lesions) in the present patient appeared clinically similar to B-cell and T-cell lymphomas[1-4,6,7] as well as benign lymphoid hyperplasia.[8-10] Leukemic infiltration of the conjunctiva is rare but does occur to present the same clinical picture as lymphoma.[11-15] In the older literature, three patients have been described in separate case reports to develop multiple skin lesions and a conjunctival lesion in the course of acute myelomonocytic leukemia.[11-13] The manifestations in these three patients were similar to those in the present patient, and thus, might have been diagnosed as BPDCN using the current criteria.

BPDCN is known to develop leukemic dissemination as a feature of myelomonocytic leukemia in the late phase of the disease, leading to poor prognosis.[16-18] As such, we immunocytochemically stained the bone marrow biopsy specimen to identify CD56-positive cells and also analyzed peripheral blood cells with flow cytometry to search for CD4-, CD56-, and CD123-positive cells. The bone marrow did not contain CD56-positive cells while the peripheral blood cells contained a group of cells positive for CD4 and CD56 or for CD4 and CD123 in a large percentage of the white blood cells compared with the normal condition. Furthermore, the ratio of monocytes, in the background of the normal level of the white blood cell counts, was elevated in the initial phase and gradually increased during the follow-up. The monocyte fraction did indeed contain atypical cells that were positive for the three markers (CD4, CD56, and CD123) but negative for BDCA-2, C-type lectin uniquely expressed by human plasmacytoid dendritic cells. Unfortunately, karyotype analysis was not performed. This state of the present patient might be interpreted as smoldering myelomonocytic leukemia. The absence of immunostaining for myeloperoxidase and lysozyme in tumor cells of all the tissues supports the diagnosis of BPDCN, but not acute myelomonocytic leukemia, as the primary disease.

Under these circumstances, chemotherapy was not chosen as a treatment option and palliative radiation was applied to the skin lesions and the pharyngeal lesions, given the patient's old age and the basically poor prognosis of the disease. Radiation was not given to the residual conjunctival lesions since the lesions were almost totally excised.[3] The present patient is unique in that he maintained a state of so-called smoldering leukemia for years. In addition, the skin lesions, although developing on multiple sites of the body, took a waxing-and-waning course. He might have myelodysplasia, which would be reflected in somewhat higher uptake in the bone marrow by FDG-PET/CT. Thus, the main approach is palliative treatment, such as local radiation, and observation to check for overt leukemic conversion.

In conclusion, this study is the first to describe conjunctival involvement with BPDCN. Since lymphoma lesions of any histopathological type as well as benign lymphoid hyperplasia manifest clinically as salmon-pink lesions, excisional biopsy is mandatory to establish the correct diagnosis. It remains unknown why the BPDCN cells in this patient showed conjunctival infiltration as well as pharyngeal infiltration. The BPDCN cells might acquire a propensity for mucosal involvement after radiotherapy to the skin lesions.

REFERENCES

1 Matsuo T, Ichimura K, Okada H, Shinagawa K, Fukushima K, *et al.*: Clonal analysis of bilateral, recurrent, or systemically multifocal ocular adnexal lymphoma. J Clin Exp Hematopathol 50:27-38, 2010

2 Hardman-Lea S, Kerr-Muir M, Wotherspoon AC, Green WT, Morell A, *et al.*: Mucosa-associated lymphoid tissue lymphoma of the conjunctiva. Arch Ophthalmol 112:1207-1212, 1994

3 Matsuo T, Yoshino T: Long-term follow-up results of observation or radiation for conjunctival malignant lymphoma. Ophthalmology 111:1233-1237, 2004

4 Matsuo T, Ichimura K, Yoshino T: Spontaneous regression of bilateral conjunctival extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue. J Clin Exp Hematopathol 47:79-81, 2007

5 Wotherspoon AC, Hardman-Lea S, Isaacson PG: Mucosa-associated lymphoid tissue (MALT) in the human conjunctiva. J Pathol 174:33-37, 1994

6 Al-Muammar A, Hodge WG, Farmer J: Conjunctival T-cell lymphoma. A clinicopathologic case report. Ophthalmology 113:459-461, 2006

7 Weisenthal RW, Streeten BW, Dubansky AS, Hutchison RE, Pecora JL: Burkitt lymphoma presenting as a conjunctival mass. Ophthalmology 102:129-134, 1995

8 Shields CL, Shields JA, Carvalho C, Rundle P, Smith AF: Conjunctival lymphoid tumors: clinical analysis of 117 cases and relationship to systemic lymphoma. Ophthalmology 108:979-984, 2001

9 Goto Y, Ohaki Y, Ibaraki N: A clinicopathologic case report of inflammatory pseudotumors involving the conjunctiva and lung. Jpn J Ophthalmol 48:573-577, 2004

10 Matsuo T, Ichimura K, Sato Y, Tanimoto Y, Kiura K, *et al.*: Immunoglobulin G4 (IgG4)-positive or -negative ocular adnexal benign lymphoid lesions in relation to systemic involvement. J Clin Exp Hematopathol 50:129-142, 2010

11 Font RL, Mackay B, Tang R: Acute monocytic leukemia recurring as bilateral perilimbal infiltrates. Immunohistochemical and ultrastructural confirmation. Ophthalmology 92:1681-1685, 1985

12 Lee DA, Su WPD: Acute myelomonocytic leukemia cutis presenting as a conjunctival lesion. Int J Dermatol 24:369-370, 1985

13 Tsumura T, Sakaguchi M, Shiotani N, Sugita A, Nakahara T: A case of acute myelomonocytic leukemia with subconjunctival tumor. Jpn J Ophthalmol 35:226-231, 1991

14 Cook BE Jr, Bartley GB: Acute lymphoblastic leukemia manifesting in an adult as a conjunctival mass. Am J Ophthalmol 124:104-105, 1997

15 Lee SS, Robinson MR, Morris JC, Mirtsching BC, Shen D, *et al*. : Conjunctival involvement with T-cell prolymphocytic leukemia: report of a case and review of the literature. Surv Ophthalmol 49:525-536, 2004

16 Dalle S, Beylot-Barry M, Bagot M, Lipsker D, Machet L, *et al*. : Blastic plasmacytoid dendritic cell neoplasm: is transplantation the treatment of choice ? Br J Dermatol 162:74-79, 2010

17 Cota C, Vale E, Viana I, Requena L, Ferrara G, *et al*. : Cutaneous manifestations of blastic plasmacytoid dendritic cell neoplasm - morphologic and phenotypic variability in a series of 33 patients. Am J Surg Pathol 34:75-87, 2010

18 Jegalian AG, Facchetti F, Jaffe ES: Plasmacytoid dendritic cells: physiologic roles and pathologic states. Adv Anat Pathol 16:392-404, 2009