

Extranodal NK/T Cell Lymphoma Accompanied by Heavy Eosinophilic Infiltration and Peripheral Blood Eosinophilia, Involving Skeletal Muscles

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The patient was a 52-year-old female with swelling in both lower legs and peripheral blood eosinophilia. Biopsy specimen revealed the heavy infiltration of eosinophils with sparse small lymphocytes showing mild atypia. The diagnosis was Kimura disease. The symptoms including eosinophilia were relieved by steroid treatment. At 17 months from initial biopsy, the patient developed swelling of the buttock. At 25 months, fever and dyspnea with multiple lung nodules developed. Wedge resection revealed multiple aggregates of CD3(+), CD56(+), Epstein-Barr virus(+) large atypical lymphocytes with necrosis. The patient was finally diagnosed with extranodal NK/T cell lymphoma (NKTL). Epstein-Barr virus *in situ* hybridization retrospectively performed on the previous biopsies demonstrated Epstein-Barr virus infection in small CD3(+) lymphocytes. The patient expired after 26 months despite chemotherapy. Blood eosinophilia correlated well with disease activity during the clinical course. This case shows not only unusual histologic features, which hampered the correct diagnosis, but also a unique clinical manifestation of NKTL.

Key Words: Lymphoma, extranodal NK/T cell; Angiolymphoid hyperplasia with eosinophilia; Eosinophilia; Epstein-Barr virus infections

Extranodal natural killer/T cell lymphoma, nasal type (NKTL), is a malignant neoplasm of mostly NK cells and, rarely, cytotoxic T cells, with a strong association with Epstein-Barr virus (EBV) and poor prognosis. Although it frequently occurs in the nasal cavity and adjacent structures, various extra-nasal sites are often primarily involved including skin, soft tissue, gastrointestinal tract and testis,¹ some of which are barely suspicious for malignancy at initial presentation. Reactive inflammatory cells and a near-absence of cytological atypia of tumor cells often mask the neoplastic features of the lesion and can, therefore, mislead or delay a pathologic diagnosis. Thus, the prompt recognition of this aggressive tumor is of great importance to avoid delay of appropriate treatment. Herein, we report a unique NKTL case, primarily occurring in the skeletal muscles of both lower legs, that was characterized by heavy infiltration of eosinophils with a scarcity of tumor cells with a little cytologic atypia in tissues and peripheral blood eosinophilia that correlated with disease activity.

CASE REPORT

A 52-year-old female visited our orthopedic clinic and was admitted with swelling and pain in both lower legs suggestive of compartment syndrome. The symptoms had developed about 1-year previously and had gradually increased. The patient had been diagnosed with diabetes mellitus and hypertension, and had been treated for 10 years. Laboratory testing showed peripheral blood eosinophilia of up to 22.2% on complete blood count (CBC) (Fig. 1), while no other specific abnormalities were found including lactate dehydrogenase, serum IgE levels and stool examination for parasitic ova. Magnetic resonance imaging (MRI) revealed swelling with enhancement in both posterior compartments, particularly of the soleus and gastrocnemius muscles, which was suggestive of inflammatory or infiltrative disease (Fig. 2A). Bilateral fasciotomy and muscle biopsy was performed. Grossly, the lesions were soft tissue mainly composed of muscular and fibroadipose tissue with poor circumscription. Microscopic examination showed diffuse infiltration of inflammatory cells through the fibromuscular tissue predominate-

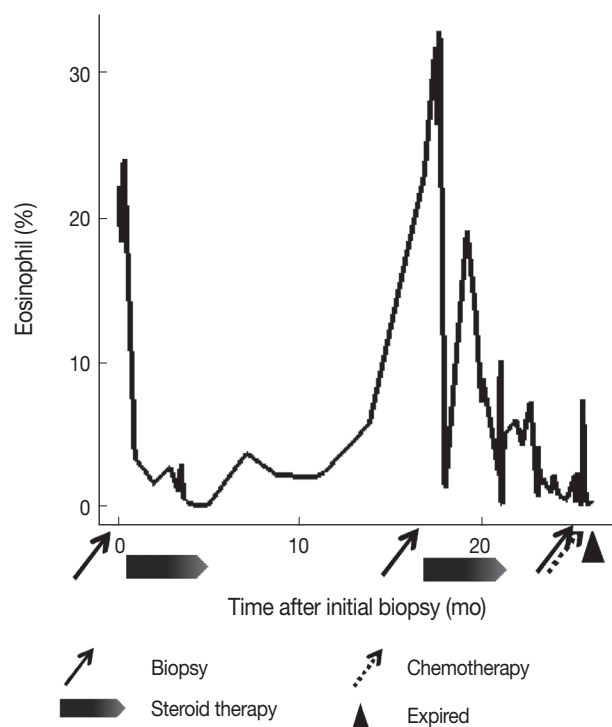


Fig. 1. Peripheral blood eosinophilia during disease course. Eosinophil percentage on complete blood count correlated with clinical course and disease activity.

ed by an extensive number of eosinophils admixed with relatively a small number of lymphocytes with little cytologic atypia (Fig. 2B, C). A few vague granulomas and mild vascular proliferation were observed. There were no lymphoid follicles. On immunohistochemistry, small CD3(+) cells and CD20(+) cells were observed to be admixed with each other with prevalence of CD3(+) cells over CD20(+) cells. None of the lymphoid cells expressed CD56. Ki-67 immunostaining showed scattered positive cells and the proliferation index was generally less than 10%. No histological evidence of parasitic, bacterial or fungal infection was seen at that time and there was no suspicion of malignancy. Therefore, the lesions were regarded as reactive, with heavy soft tissue infiltration of eosinophils, which led to the misdiagnosis of Kimura disease despite the absence of lymphoid follicles. Retrograde slide review with EBV *in situ* hybridization for EBV-encoded RNA revealed scattered EBV(+) small lymphoid cells (Fig. 2D), which were suspected to be CD3(+) and granzyme B(+) cells. Although eosinophilia on peripheral blood persisted after the biopsy, it subsided immediately after initiation of prednisolone therapy, and the leg lesion gradually decreased in size and softened. Prednisolone medication was tapered and discontinued after 5 months, during which time the

eosinophil percentage on CBC was consistently less than 5% (Fig. 1). At 17 months from initial biopsy, left buttock swelling developed rapidly and MRI revealed well-enhanced lesions in both gluteus maximus muscles. The buttock biopsy showed diffuse and massive infiltration of eosinophils and small lymphoid cells, with mild atypism throughout muscular and adjacent fibroadipose tissue in a similar pattern to the first biopsy, again leading to pathologic diagnosis of involvement of Kimura disease. Eosinophil percentage on CBC was again increased up to 31.7% (Fig. 1). Retrograde slide review with CD56 immunostaining and EBV *in situ* hybridization revealed some EBV(+) CD3(+) cells, which only weakly expressed CD56. Although symptoms were temporarily relieved by the restart of prednisolone medication, new lesions intermittently developed including right forearm, facial and orbital muscles, requiring intravenous and oral steroid therapy with limited response. At 25 months, sudden fever and dyspnea developed. Computed tomography scan revealed multiple nodules in both lung fields. Diagnostic wedge resection was performed for the lung lesion. Microscopically, there was infiltration and mass formation of atypical medium to large lymphoid cells in the lung parenchyma with multifocal necrosis and frequent apoptotic bodies (Fig. 2E). Eosinophil infiltration was very rare. CD56 and CD3 were strongly positive in the cell membrane and cytoplasm of atypical lymphoid cells, respectively (Fig. 2F). Moreover, CD30 (Ki-1), granzyme B, latent membrane protein-1 were also expressed in atypical cells. EBV *in situ* hybridization was positive in most of the neoplastic cells (Fig. 2F). Monoclonal T cell proliferation was not observed in polymerase chain reaction (PCR) analysis for T cell receptor gamma gene rearrangement. These pathologic features were typical for NKTL. Despite the chemotherapy with cyclophosphamide, doxorubicin, vincristine, prednisone and intrathecal methotrexate, the disease progressed and the patient expired at 26 months from initial biopsy.

DISCUSSION

The variability in clinical and histological presentations of NKTL often makes it difficult to differentiate this malignant disease from various non-neoplastic diseases.²⁻⁴ Although reactive cell infiltration including eosinophils and macrophages is known to be a possible feature of NKTL,¹ the extensive infiltration of eosinophils masking neoplastic lymphoid cells observed in the present case has not been previously described. Moreover, the intermingled CD3(+) small lymphoid cells had only mild

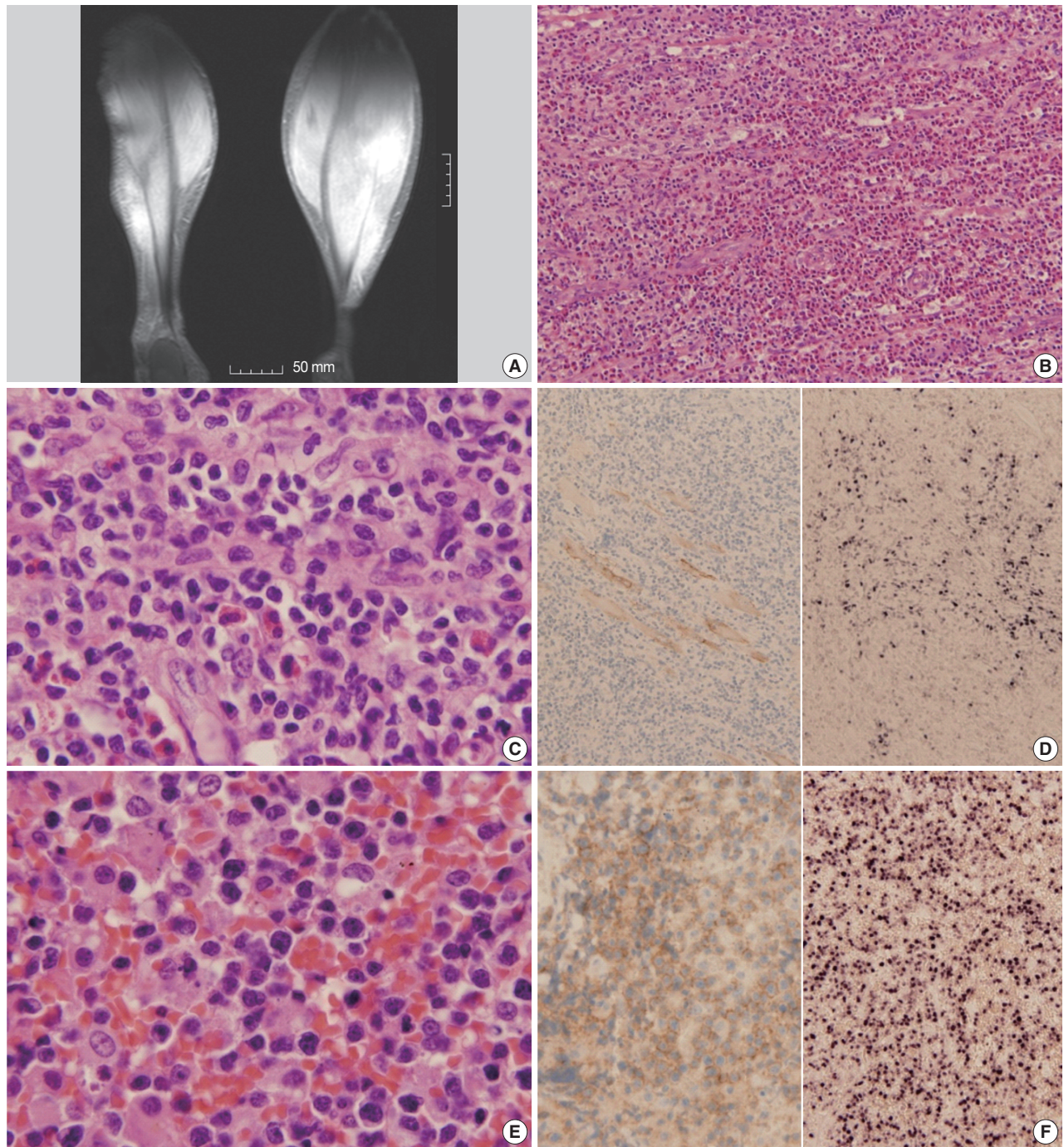


Fig. 2. Imaging and pathologic findings of leg (A-D) and lung (E, F) lesions. (A) Magnetic resonance imaging shows diffuse swelling of bilateral calf muscle. (B) Heavy eosinophilic infiltration is observed masking lymphoid cells in leg lesions. (C) Small lymphoid cells show little atypia admixed with eosinophils. (D) No CD56(+) lymphoid cells are evident on immunohistochemistry (left panel). Retrograde review of the slides with Epstein-Barr virus (EBV) *in situ* hybridization for EBV-encoded RNA (EBER) reveals some scattered EBV(+) small lymphoid cells (right panel). (E) Lung nodule is composed of medium to large atypical lymphoid cells with frequent apoptosis. (F) Immunostaining for CD56 is positive in the cytoplasmic membrane of atypical cells (left panel). Most of the atypical cells are positive for EBER on EBV *in situ* hybridization (right panel).

cytologic atypia, and immunohistochemical staining for CD56 was negative at initial presentation, which led to the exclusion of NKTL as a possible differential diagnosis. It is obvious that

CD56 immunostaining can sift out most NKTL from suspicious cases. However, CD56-negative NKTL certainly exists and, therefore, EBV *in situ* hybridization should be applied to

all suspicious cases with unusual histology. Another important way of compensating for the incompleteness of diagnosis is steady clinical follow-up in readiness for rebiopsy. Indeed, in the present patient, later biopsies revealed more conventional neoplastic features. The number of EBV(+) cells steadily increased from leg and buttock muscle biopsies to the lung lesion, and eosinophil infiltration concomitantly decreased. Moreover, the neoplastic cells exhibited evident transformed features with increased cell size and marked atypism in the lung lesions but not in the previous muscle lesions. Immunophenotype of neoplastic cells evolved from CD56(-)/CD30(-) in the leg lesions and CD56(weak)/CD30(-) in the buttock lesions, finally to CD56(+)/CD30(+) in the lung lesions. Therefore, the present case might represent a kind of typical example of NKTL with pathologic progression in terms of histology and immunophenotype. Otherwise, there might be the possibility that the patient had suffered from chronic EBV-associated T or NK cell lymphoproliferative disorder, which finally progressed to overt NKTL. However, based on the facts that the patient had no previous medical history suggesting lymphoproliferative disorder and that putative EBV(+) cells in initial leg lesions expressed CD3 and granzyme B, although double immunohistochemistry and *in situ* hybridization was not performed, it is more plausible that the muscle lesions represented an unusual pathologic manifestation of NKTL. Constant clinical suspicion is required for follow up of the disease course even after a lesion has been diagnosed as non-malignant.

NKTL arising outside the nasal cavity or non-upper aerodigestive tract seems to be more aggressive.^{1,5} Although some cases were characterized by granulomatous myositis-like features in a similar clinical setting,² the predominant feature in the present case was not granulomatous reaction but an extremely heavy eosinophilic infiltration, which led to a misdiagnosis as Kimura disease, despite the absence of lymphoid follicular hyperplasia, which is an important diagnostic feature of Kimura disease. It is a chronic inflammatory disease of unknown etiology characterized by heavy eosinophilic infiltration and frequent peripheral blood eosinophilia.⁶ However, Kimura disease is generally not associated with EBV,⁶ which is the characteristic point of the present case. Although one case of EBV(+) Kimura disease in an elderly male was reported, EBV DNA was only detected by PCR, and EBV *in situ* hybridization was not performed.⁷ Therefore, which cells and how many cells were infected with EBV were not clear in this previous case. Moreover, considering that EBV is latently infected in a minority of memory B cell after primary infection, the possibility that the EBV

genome detected in their case might be from EBV latently infected B cells in an aged person cannot be excluded, and the role of EBV in the pathogenesis of Kimura disease in the previous case remains to be clarified.

It is noteworthy that the eosinophil percentage in the peripheral blood presently appeared to correlate with disease activity, showing fluctuation in accordance with surgical excision or steroid treatment (Fig. 1). Therefore, it might be possible to use eosinophil count for disease monitoring in patients with a similar situation. Immunologically, eosinophils are responsible for elimination of foreign material, especially parasites, and are recruited by particular cytokines and chemokines such as interleukin (IL)-5. Among lymphoid malignancies, eosinophil infiltration is well-known in classical Hodgkin lymphoma, peripheral T cell lymphoma (not otherwise specified), angioimmunoblastic T cell lymphoma and adult T cell leukemia/lymphoma,¹ where neoplastic cells express cytokines and chemokines to recruit eosinophils such as IL-5 and CCL17.⁸ While IL-5 is mainly produced and secreted by T cells of Th2 type, B cells and Hodgkin-Reed-Sternberg cells are able to produce IL-5 when infected by EBV.⁹ In the present case, it is conceivable that neoplastic NK cell-induced changes in a microenvironment of cytokines and chemokines influenced the unusually massive eosinophil recruitment and blood eosinophilia. Indeed, there have been several reports that NK cells or their specific subsets produce IL-5 and influence eosinophilic infiltration.¹⁰⁻¹² Especially, NK cells of intermediate differentiation stage or NK2 cell subset have been suggested to express IL-5 based on *in vitro* study.¹³⁻¹⁵ These observations suggest that the present case might be a neoplastic counterpart of the NK2 cell subset, which are known as NK cells grown in IL-4 producing IL-5 and IL-13, in contrast to NK1 cells grown in IL-12 producing IL-10 and interferon- γ . Although NKTL is thought to be a collection of somewhat heterogeneous subgroups,⁵ the subclassification of NKTL based on immunological subsets has not been performed. In this context, the present case provides a new viewpoint for understanding NKTL.

In summary, the present case demonstrates an atypical presentation of NKTL in both clinical and histological features. The peculiar feature of the present case was heavy eosinophilic infiltration with a small number of tumor cells, as well as peripheral blood eosinophilia, which roughly reflected disease activity. Therefore, consistent suspicion and attention are needed for the correct diagnosis of NKTL, which would lead to prompt and optimal treatment for patients.

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