# Case Report

# Natural Killer Cell Malignancy associated with Epstein-Barr Virus and Hemophagocytic Syndrome

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*Natural killer cell malignancy is a rare and aggressive lymphoid neoplasm encompassing extranodal NK/T-cell lymphoma, nasal-type (ENKLN) and aggressive NK-cell lymphoma/leukemia (ANKL).*

*A case of cutaneous ENKLN and a case of ANKL in Thai patients are reported. Both patients developed hemophagocytic syndrome and shortly succumbed to death.*

*The cells in cutaneous ENKLN are small to medium in size with minimal cytoplasm, round nuclei, irregular nuclear membrane, and fine chromatin with inconspicuous nucleoli. While that of ANKL are medium to large-sized mononuclear cells with moderate cytoplasm. Their nuclei are elongated to embryo-like with irregularly thickened nuclear membrane, fine chromatin, and small to occasional prominent nucleolus. Ancillary techniques studied on paraffin embedded tissues of both cases demonstrated that the neoplastic cells exhibit cytoplasmic CD3+, CD56+ and cytotoxic granules + by immunohistochemistry, absence of T cell receptor gene rearrangement by PCR, and presence of Epstein-Barr virus mRNA (EBER) transcripts by in situ hybridization.*

*The authors reviewed the literature on natural killer cell neoplasm and compared the clinical characteristics, natural history, and association of Epstein-Barr virus infection with hemophagocytic syndrome.*

***Keywords:*** *Natural killer-cell malignancy, Epstein-Barr virus, Hemophagocytic syndrome*

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Natural killer (NK)-cell malignancy encompasses extranodal NK/T-cell lymphoma, nasal-type and aggressive NK-cell lymphoma/leukemia. Formerly known in the WHO classification 2001 as blastic NK-cell lymphoma, CD4-positive/CD56+ hematodermic neoplasm is now proposed to be derived from a precursor of specialized dendritic cells[1].

In Asian populations where NK-cell lymphoma is more common than other races, extranodal NK/T-cell lymphoma, nasal type accounts for up to 8.3% while aggressive NK-cell lymphoma accounts for up to 0.2% of all lymphomas[2].

The authors report the first two cases of NK-cell malignancy of Thai patients documented in the English literature consisting of one cutaneous ENKLN and another case of ANKL involving bone marrow. The neoplastic cells in both cases had different morphology with identical immunophenotypic features (cytoplasmic CD3 and membranous CD56); Epstein-Barr virus (EBV) encoded mRNA (EBER) positivity and absence of T-cell receptor (TCR) gene rearrangement. Both patients suffered from hemophagocytic syndrome (HPS) and eventually died in a few days.

The authors compared characteristic clinical, pathologic, and molecular features of the two entities and review the association of EBV to the disease and HPS.

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## Material and Method

The two cases diagnosed during 2005-2006

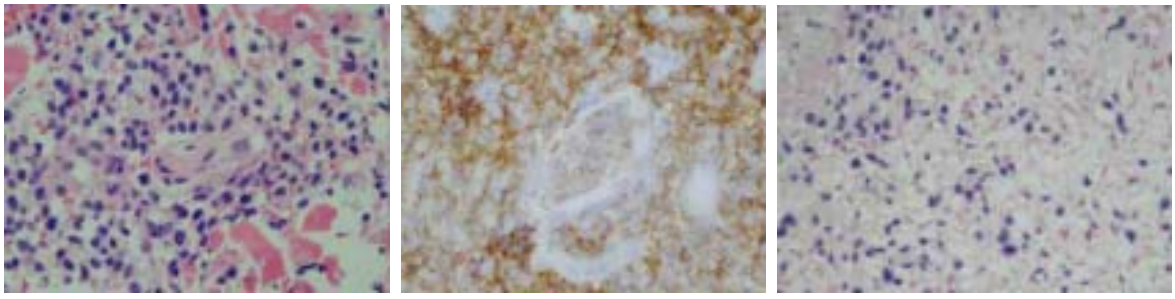
were retrieved from the archives of the Pathologic Units at Thammasat Chalerm Prakiate Hospital. Case 1, cutaneous ENKLN was diagnosed on skin tissue biopsied from chronic ulcer at the left leg. Case 2, ANKL was diagnosed on bone marrow biopsy specimen. Patients' records, histochemical and paraffin immunoperoxidase stained slides were reviewed. Immunophenotypic associated markers of the neoplastic cells included T-cell = CD3 (monoclonal, episilon chain, NCL-CD3-PS1, Novo Costra); B-cell = CD20 and CD79a; NK-cell = CD56 (monoclonal, SCLC123C3, Zymed); cytotoxic T-cell = CD8; cytotoxic T-cell or NK-cell = granzyme B (monoclonal, GrB-7, Dako); anaplastic lymphoma = anaplastic lymphoma kinase (ALK) and CD30; precursor cell = terminal deoxynucleotidyl transferase (TdT); myeloid cell = myeloperoxidase (MPO); and histiocyte marker = CD68. They were visualized by avidinbiotin complex technique. Additional molecular studies for TCR gene and EBV were performed. The DNA was extracted from deparaffinized tissues and measured by spectrophotometer. TCR-γ gene was amplified by multiplex polymerase chain reaction (PCR), run on polyacrelamide gel and examined under short wavelength UV light as described elsewhere[3]. The β-globin gene was also evaluated to assess the appropriateness of DNA extraction. *In situ* hybridization (ISH) for EBV encoded mRNA using peptide nucleic acid probes (Dako Cytomation) was detected on paraffin sections, as previously mentioned[4].

**Case Report**

***Case 1***-A 40-year-old female presented with a left thigh ulcer for 4 months. There was no febrile illness, hepatosplenomegaly, or lymphadenopathy. One month later to initial presentation, she developed left facial palsy that progressed to bilateral palsy. Cerebrospinal fluid (CSF) cytology, containing admixture of numerous small to medium-sized lymphoid cells (Fig. 2A), was reported as negative for malignancy without knowledge of chronic ulcer. Her symptoms transiently responded to a short course of steroid treatment. A few days before death, she presented with alteration of consciousness and pancytopenia (Hct 29.8%, WBC 9,000/µL, Plt 4,000/µL with normochromic, anisocytosis 1+, poikilocytosis 1+, spherocytosis 1+, polychromasia 1+). The skin biopsy from chronic ulcer, taken one day prior to death, revealed dense abnormal lymphoid infiltrate in the dermis and subcutis with perineural invasion, angiocentric, and periappendigeal pattern. Mild epidermotrophism was noted. The abnormal lymphoid cells were small to medium-sized, minimal cytoplasm and round nuclei with irregular nuclear membrane, fine chromatin with inconspicuous nucleoli (Fig. 1A). Paraffin immunoperoxidase study demonstrated that these cells expressed cytoplasmic CD3 and membranous CD56 (Fig. 1B) but not CD8, CD20, CD30, CD79a, TdT and MPO. PCR for TCR gene yielded absence of TCR gene rearrangement, thus indicating that the neoplastic cells had TCR gene in germline configuration. ISH for EBER was positive (Fig. 1C). Retrospective study on lymphoid cells in previous CSF marked with CD3 and CD56 (Fig. 2B) but not CD20. Eventually, the patient died 5 months after initial clinical onset due to severe bleeding. Available necropsy specimens from bone marrow and liver disclosed minimally infiltration by admixture of T-cells (CD3+) and B-cells (CD20+). There was some hemophagocytosis noted in the normal cellular marrow. The final diagnosis was cutaneous extranodal NK/T-cell lymphoma, nasal-type with CNS involvement (stage IV), associated with EBV and HPS.

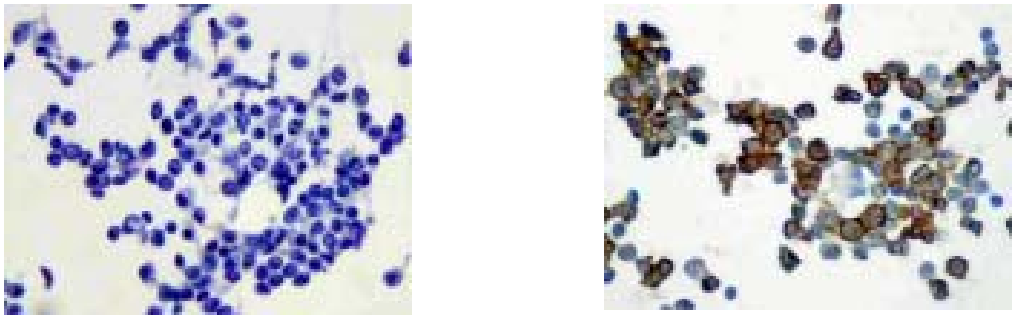
***Case 2***-A 34 year-old female presented with a high-grade fever for 1 month without specific symptoms, neither hepatosplenomegaly, nor lymphadenopathy. Septic workup was unremarkable. She was treated with acetaminophen and antibiotics. Two weeks prior to death, complete blood count revealed pancytopenia (Hb 7.9 g%, WBC 600/µL and platelet 36,000/µL) with lymphocyte predominant (L 95%, N 5%). There was no abnormal leukocyte seen on the peripheral blood smear. Bone marrow biopsy disclosed moderate hypocellularity with a few scattered medium to large-sized mononuclear cells of undetermined nature and some hemophagocytosis. These abnormal cells, accounting for 10% of cellular marrow, had a moderate amount of cytoplasm. The nuclei were elongated to embryo-like with irregularly thickened nuclear membrane, fine chromatin, and small to occasional prominent nucleolus (Fig. 3A). They expressed cytoplasmic CD3, membranous CD56 (Fig. 3.B) and granzyme-B and were negative for CD20, CD30 and ALK. Equivocal staining with CD68 and MPO were noted in rare atypical cells. Evaluation for TCR gene resulted in absence of monoclonal band. ISH for EBER was positive (Fig. 3C). The patient died 6 weeks after initial presentation. Postmortem examination was not allowed. The diagnosis was aggressive NK-cell lymphoma/leukemia involving bone marrow, associated with EBV and HPS.

Comparison of characteristic clinical, pathologic, and molecular features of the two entities is tabulated in Table 1.



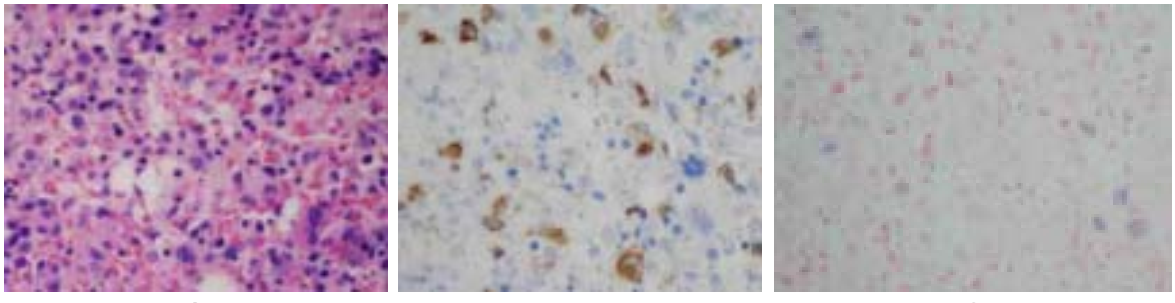
A B C

**Fig. 1** Skin biopsy of extranodal NK/T-cell lymphoma, nasal-type (Case 1)
A, Abnormal lymphoid cells infiltrating dermis, forming angiocentric pattern and perineural invasion, are small to medium-sized and exhibit irregular nuclear membrane, fine chromatin with inconspicuous nucleoli (H&E x 400)
These cells express membranous positivity for CD56 by paraffin immunostaining (B) and nuclear EBER by *in situ* hybridization (C)



A B

**Fig. 2** Cellular cerebrospinal fluid cytology (Case 1)
A, Admixture of small to medium-sized lymphoid cells (Papanicolaou x 400)
B, Immunostaining for CD56 reveals membranous positivity



A B C

**Fig. 3** Bone marrow biopsy of aggressive NK-cell lymphoma/leukemia (Case 2)
A, few scattered medium to large-sized mononuclear cells accounting for 10% of total cellularity
They have a moderate amount of cytoplasm and elongated to embryo-like nuclei with irregularly thickened nuclear membrane, fine chromatin and small nucleolus (H&E x400)
B, Membranous positivity for CD56
C, Expression of nuclear EBER by *in situ* hybridization

**Table 1.** Clinicopathologic features of case 1 and case 2

| Features | Case 1 ENKLN | Case 2 ANKL |
|---|---|---|
| Age (Yr) | 40 | 34 |
| Ill duration (months) | 5 | 1.5 |
| Primary organ | Skin | Bone marrow |
| Stage | IV with CNS involvement | Lymphoma with bone marrow involvement |
| Hemophagocytic syndrome | Presence | Presence |
| Cytomorphology | Numerous small to medium-sized, minimal amount of cytoplasm, round nuclei, irregular nuclear membrane, fine chromatin, inconspicuous nucleoli | Scattered medium to large-sized, moderate amount of cytoplasm, elongated to embryo-like nuclei, irregularly thickened nuclear membrane, fine chromatin, small to occasional prominent nucleolus |
| Immunoprofile | CD3+, CD56+, CD8-, CD20-, CD30-, CD79a-, TdT-, MPO- | CD3+, CD56+, granzyme-B+, CD20-, CD30-, ALK- |
| PCR for TCR gene rearrangement | Absence | Absence |
| ISH for EBER | Positive | Positive |

Note: ENKLN, extranodal NK/T-cell lymphoma, nasal type; ANKL, aggressive NK-cell lymphoma/leukemia; TdT, terminal deoxynucleotidyl transferase; ALK, anaplastic lymphoma kinase; MPO, myeloperoxidase; PCR for TCR, polymerase chain reaction for T-cell receptor; ISH for EBER, *in situ* hybridization for Epstein-Barr virus mRNA

## Discussion

Nasal-type NK/T-cell lymphoma often affects adults while ANKL has a younger median age of onset affecting mainly young adults.

ENKLN is commonly and prototypically arising at the nasal cavity, thus nasal NK/T-cell lymphoma may be used in the lymphoma arising at nasal region[5]. On the other hand, non-nasal extra-nodal NK/T-cell lymphoma has predilection for the skin, upper aerodigestive tract, testis, gastrointestinal tract, soft tissue, and spleen[6]. Skin lesions vary from generalized erythematous maculopapular rash to single or multiple nodules that commonly ulcerated[6]. In patients presenting with only skin lesions, a median survival of 27 months was reported, compared with 5 months for patients presenting with combined cutaneous and extracutaneous disease[7]. Systemic symptoms such as fever, malaise, and weight loss may be present, and some are accompanied by a HPS. Histologically, neoplastic NK cells are characterized by broad cytologic spectrum varying from small or medium to large size with oval or irregular nuclei. The large cells have moderate to large amount of granular cytoplasm and hyperchromatic nuclei with prominent nucleoli. Some may have had a mixed, inflammatory background. Designated NK/T (rather than NK)-cell lymphoma, because in most they cases appear to be EBV+ CD56+, rare cases and show an EBV+ CD56-, cytotoxic T-cell phenotype.

Aggressive NK-cell leukemia is characterized by a systemic proliferation of neoplastic NK-cells. The disease has an aggressive clinical course[8]. Fever with constitutional symptoms is the usual initial presentation. Leukemic blood picture with hepatosplenomegaly and lymphadenopathy are common. The authors postulated that in case 2, the hemophagocytic syndrome and a small number of neoplastic NK-cells in marrow might have contributed to the absence of malignant cells in the peripheral blood. In such a case, "leukemia/lymphoma" is preferred[9,10]. The typically involved bone marrow shows massive, focal or subtle infiltration by the neoplastic cells intermingled with hemophagocytic

cells. Admixture with apoptotic bodies and necrosis is common[8]. Like nasal-type NK/T-cell lymphoma, these cells have similar immunophenotype, genetics, EBV status, and frequent association with HPS[6,10].

ANKL has poor prognosis irrespective of treatment. Most patients die within 6 weeks to 3 months after initial presentation[6,10]. Some cases of nasal-type NK/T-cell lymphoma may present with localized disease and may be responsive to radiation and chemotherapy; however that of extra-nasal cavity is more aggressive with a short survival time and poor response to therapy[6].

Morphologically, ANKL contains a more monotonous neoplastic cellular population, ranging from large granular lymphocytes to large cells with prominent nucleoli but that of nasal-type NK/T-cell lymphoma vary from small or medium to large hyperchromatic cells. Rare patients with nasal-type NK/T-cell lymphoma may progress to aggressive, systemic disease indistinguishable from ANKL[11]. The genomic alteration patterns of ANKL were loss of 7p and 17p13.1 and gain of 1q occurred with significant frequency, whereas nasal-type NK/T-cell lymphoma were loss of 6q21-q22.1, 6q22.33-q23.2, 6q25.3, and 6q26-q7[12].

EBV RNA was positive in 50-94% of NK/T-cell lymphoma in extra-nasal sites, with 80% positive rate in tumor of skin and 100% in aggressive NK cell leukemia[13, 6]. Identification of single episomal form of EBV in neoplastic NK-cells suggested oncogenetic role of the virus on NK-cell lymphoma[14].

HPS is defined by increased proliferation and activation of benign macrophages with hemophagocytosis throughout the reticuloendothelial system when it occurs secondary to EBV-positive T-cell and NK-cell malignancy, is associated with rapidly fatal outcome. The inflammatory cytokines released from neoplastic NK-cell such as interferon-γ (INF-γ), macrophage colony-stimulating factor and interleukin-6 might directly activate histiocytes, resulting in the occurrence of hemophagocytic lymphohis- tiocytosis. INF-γ contributes to lymphomatogenesis by maintaining tumor cells survival in an autocrine fashion[15].

Although the two NK-neoplastic entities share identical immunophenotypes and germline configuration of TCR gene, they exhibit some different clinical settings and genomic, alteration patterns.

The rarity, aggressive and diverse clinical setting with the requirement of multiple investigative modalities not only mount the delay in establishing diagnosis but also impart highly fatal outcomes. Highly suspicious indices in the appropriate setting are the key to early recognition. Nevertheless, further understanding of neoplastic, NK-cell biology, EBV tumorigenesis, and HPS mechanism will bring about better treatment outcome.

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## *มะเร็งเม็ดเลือดขาวประเภท natural killer-cell และความสัมพันธ์ของ Epstein-Barr virus ต่อการเกิดโรคและภาวะแทรกซ้อน hemophagocytic syndrome*

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*มะเร็งเม็ดเลือดขาวประเภท natural killer-cell เป็นโรคที่พบได้น้อยและมีการดำเนินโรคที่รุนแรง มะเร็งในกลุ่มนี้มี 2 ชนิดคือ extranodal NK/T-cell lymphoma, nasal-type (ENKLN) และ aggressive NK-cell lymphoma leukemia (ANKL)*

*รายงานนี้บรรยายผู้ป่วย 2 ราย โดยรายแรกได้รับการวินิจฉัยว่าเป็น ENKLN ที่ผิวหนัง และอีกรายเป็น ANKL โดยผู้ป่วยทั้งสองรายมีภาวะแทรกซ้อน hemophagocytic syndrome ก่อนเสียชีวิต*

*ลักษณะเซลล์มะเร็งในเนื้อเยื่อผู้ป่วยกรณี ENKLN เป็นเซลล์ขนาดเล็กถึงกลาง มีไซโตพลาซึมปริมาณน้อย นิวเคลียสกลม เยื่อแผ่นนิวเคลียสขรุขระ โครมาตินละเอียดและ เห็นนิวคลีโอลัสไม่ชัดเจน ในขณะที่เซลล์มะเร็งกรณี ANKL เป็นเซลล์ขนาดกลางถึงใหญ่ มีไซโตพลาซึมปริมาณพอสมควร นิวเคลียสรูปร่างรียาวหรือคล้ายตัวอ่อน เยื่อแผ่นนิวเคลียสขรุขระและหนาบางไม่สม่ำเสมอ โครมาตินละเอียดและ นิวคลีโอลัสขนาดเล็กและบางครั้งเห็นได้เด่นชัด*

*เมื่อศึกษาเนื้อเยื่อเพิ่มเติมด้วยวิธีพิเศษพบว่าเซลล์มะเร็งจากทั้งสองกรณีแสดงลักษณะเดียวกัน ดังนี้ immunohistochemistry พบว่ามี cytoplasmic CD3+ และ membranous CD56+ โดยที่ PCR for T-cell receptor gene ไม่พบ rearrangement และ* in situ *hybridization for EBV mRNA (EBER) ให้ผลบวก*

*การรวบรวมข้อมูลจากการทบทวนบทความที่เกี่ยวข้องกับโรคมะเร็งเม็ดเลือดขาวชนิด natural killer-cell เพื่อเปรียบเทียบลักษณะทางคลินิก การดำเนินโรค และอธิบายความสัมพันธ์ของ Epstein-Barr virus ต่อการเกิดภาวะ hemo- phagocytic syndrome*