

Diagnostic challenge presented by extranodal NK/T cell lymphoma expressing CD20, CD30 and CD15: A case report

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Abstract. The atypical expression of immune phenotypes in lymphoma is often associated with a poor prognosis and presents diagnostic challenges. The present study reports on a rare extranodal NK/T cell lymphoma. In addition to typical morphology and immunohistochemical characteristics, these tumors strongly express CD20 and CD30 and partially express CD15, which is associated with aggressive clinical behavior. Differential diagnosis should be cautiously approached in extranodal NK/T cell lymphoma because the abovementioned markers are typically expressed in B cell lymphoma or Hodgkin's lymphoma. In addition to rigorous histological and comprehensive immunohistochemical staining, whole-body imaging and molecular testing can assist with diagnosis. In the present case, the patient died of multiple organ failure shortly after diagnosis. Lymphoma exhibits an atypical immunophenotype, thus emphasizing the importance of a thorough analysis of the interrelations among clinical, imaging and pathological features.

Introduction

Epstein-Barr virus-positive (EBV⁺) extranodal T and NK cell lymphoma (ENKTL) is a common peripheral T cell lymphoma in East Asia and South America (1). CD20 is typically considered to be a specific marker for B cells, playing a crucial role in B lymphocyte differentiation, signal transduction and cell cycle regulation. It also targets monoclonal antibody therapies against B cell lymphoma (2). Therefore, CD20 is an essential marker for the diagnosis and treatment of B cell

lymphoma. However, CD20 expression in T cell lymphomas is rare and can pose diagnostic challenges. CD20 expression in T cell lymphomas is primarily observed in peripheral T cell lymphoma and is rarely seen in ENKTL (3,4).

CD30, a tumor necrosis factor receptor superfamily member, was first discovered in Hodgkin's lymphoma (HL), but it is also expressed in normally activated B and T cells. CD30 is also expressed in hematological diseases such as anaplastic large-cell lymphoma, mediastinal large B cell lymphoma, mycosis fungoides, infectious mononucleosis and embryonal carcinoma (5-7). CD30 is expressed in 20-50% of ENKTL cases and, in some cases, up to 70% (8,9). Brentuximab vedotin targets CD30-positive cells in patients with lymphoma (9). Furthermore, CD15 is typically expressed in HL or chronic myeloid leukemia and, in rare cases, in peripheral T cell lymphoma not otherwise specified (PTCL-NOS). However, to the best of our knowledge, there have been no relevant reports in ENKTL.

The present case describes a rare case of ENKTL with high CD20 and CD30 expression and partial CD15 expression in tumor cells, which posed a significant diagnostic challenge. The patient died of multiple organ failure shortly after diagnosis. To the best of our knowledge, there have been no similar cases reported before. This study discusses the clinical and pathological features of the tumor, as well as the possible molecular mechanisms and therapeutic targets.

Case report

A patient with lymphadenopathy, a sore throat and malaise for 1 month was admitted to Lanzhou University Second Hospital (Lanzhou, China) in October 2020. A chest computed tomography (CT) scan revealed multiple enlarged lymph nodes in the mediastinum, hilum, armpit and neck, with no abnormalities in the nasopharynx (Fig. 1). Blood tests showed severe anemia with the following parameters: WBC count, $1.7 \times 10^9/l$ (normal range, $3.50-9.50 \times 10^9/l$); RBC count, $2.94 \times 10^{12}/l$ (normal range, $4.30-5.80 \times 10^{12}/l$); and HGB, 77 g/l (normal range, 130-175 g/l). An elevated ferritin count of >200,000 ng/ml (normal range, 30.00-400.00 ng/ml) and an elevated vitamin B12 count, of 1,960.00 pg/ml (normal range, 197.00-771.00 pg/ml) were also observed. A cervical lymph node biopsy was performed after

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admission. Tissues were fixed with 4% neutral formalin (12 h at 25°C) and embedded in paraffin. Consecutive tissue sections (4- μ m thick) were prepared and stained with hematoxylin and eosin (8 h at 25°C). The biopsy revealed destruction of the lymph node structure by tumor cells, which had diffused into the surrounding fibrous and adipose tissues. Furthermore, nerve invasion and vascular damage were observed. The tumor cells were medium- to large-sized heterotypic lymphocytes with moderate cytoplasm, irregular nuclei, small nucleoli and obvious coagulative necrosis. Immunohistochemical staining was performed on the Ventana automated staining system, BenchMark ULTRA (Roche Tissue Diagnostics). Antigens were detected using the assay kit ultraView Universal DAB Detection Kit (cat no. 760-500). After deparaffinisation on a BenchMark ULTRA, antigen repair was performed with repair solution at 99°C. The primary antibody was added dropwise and incubated at 37°C for 32 min, the horseradish peroxidase (HRP)-labeled secondary antibody was incubated for 8 min, and then DAB was used to develop the colours. Hematoxylin was used to return the blue lining. In all the processes, the buffer reaction buffer was used for rinsing, and finally the film was blocked by gradient alcohol. The cells strongly expressed CD20 (ready-to-use; clone L26; cat. no. kit-0001), CD3 (ready-to-use; clone MX036; cat. no. MAB-0740), CD43 (ready-to-use; clone MX099; cat. no. MAB-0892), CD30 (ready-to-use; clone MX080; cat. no. MAB-0868), TIA-1 (ready-to-use; clone 2G9A10F5; cat. no. MAB-0798) and Granzyme (ready-to-use; clone GZB01; cat. no. MAB-0352), and partially expressed CD15 (ready-to-use; clone MMA; cat. no. MAB-0779). The CD30-positive rate in large tumor cells was ~80%. In addition, >90% of cells were positive for Ki-67 (ready-to-use; clone SP6; cat. no. RMA-0542). The tumor cells were negative for CD79a (ready-to-use; clone MX076; cat. no. MAB-0864), PAX5 (ready-to-use; clone MX017; cat. no. MAB-0706), CD4 (ready-to-use; clone SP35; cat. no. RMA-0620), CD56 (ready-to-use; clone MX039; cat. no. MAB-0743), CD8 (ready-to-use; clone MX117; cat. no. MAB-1031) and ALK (ready-to-use; clone MX064; cat. no. MAB-0848) (all Fuzhou Maixin Biotech Co., Ltd.). *In situ* hybridization revealed EBV in the tumor cells (Fig. 2). Furthermore, the T cell receptor gene and immunoglobulin heavy chain exhibited a polyclonal pattern. A whole-body positron emission tomography-CT scan subsequently revealed a high metabolic area in the nasopharynx (Fig. 3), and the patient was diagnosed with ENKTL with an abnormal expression of CD30, CD15 and CD20 (Ann Arbor Stage IV) (10). Following the diagnosis, the patient underwent treatment with a CHOP chemotherapy regimen consisting of 1,200 mg cyclophosphamide, 40 mg doxorubicin and 2 mg vincristine on day 1, and 100 mg prednisone on days 1-5. However, after one cycle of chemotherapy, the patient died of multiple organ failure.

Discussion

The definition and diagnostic criteria for ENKTL in the 5th edition of the WHO classification of hematolymphoid neoplasms remain unchanged (11). ENKTL is classified into nasal and non-nasal types depending on the primary site of the lesion. The nasal type accounts for the majority (80%) of cases. The remaining 20% of the cases show lesions in the skin,

gastrointestinal tract, testes and other sites, and these have a worse prognosis than nasal presentations (12). ENKTL exhibits a diffuse growth pattern, with growth around blood vessels and their destruction. Common features of ENKTL include coagulative necrosis and apoptotic bodies. The cytological spectrum of tumor cells includes small, medium, large and anaplastic cells. The background may be accompanied by various inflammatory cells, such as small lymphocytes, plasma cells, tissue cells and eosinophils, even resembling inflammation (13,14). Tumor cells display a T cell or NK cell phenotype, with most cases expressing CD2, cytoplasmic CD3 ϵ and CD56, along with cytotoxic molecules such as TIA1, granzyme and perforin. Most tumor cells are EBV-positive on *in situ* hybridization (12).

Molecular studies have revealed that ENKTL exhibits complex molecular mechanisms (15-17). Recurrent mutations in genes associated with the JAK-STAT pathway (e.g., STAT3, JAK3, STAT5B), epigenetic regulators (e.g., BCOR, KMT2D, ARID1A, EP300), tumor suppressor genes (e.g., TP53, MGA) and RNA helicases (e.g., DDX3X) have been observed in ENKTL. Furthermore, structural activation of the JAK/STAT pathway through mutations and phosphorylation plays a crucial role in ENKTL pathogenesis and represents a potential therapeutic target. Abnormalities in other signaling pathways, such as NF- κ B and PDGFR, and alterations in genes such as BIRC5, MYC, RUNX3, AURKA and EZH2 are also potential therapeutic targets (15-17). Immune evasion has been highlighted as a key mechanism for ENKTL cell survival, which is possibly driven by LMP-1 or STAT3-mediated upregulation of PD-L1. Immune checkpoint inhibitors targeting the PD1/PD-L1 axis are promising for ENKTL treatment (15-17).

T cell lymphomas occasionally express B cell markers, such as CD20 or CD79a, with 5-8% of T cell lymphomas showing CD20-positivity, with the most common type bringing PTCL-NOS; some ENKTLs also show aberrant CD20 expression (18,19). Huang *et al* (4) reviewed 18 cases of ENKTL with CD20 expression, making it the most comprehensive case series to date. They noted that abnormal CD20 expression in ENKTL often occurs in elderly patients and is associated with a highly invasive clinical course and poor prognosis. Several hypotheses can explain this abnormal CD20 expression. One possible explanation is that during malignant transformation, T, B and NK cells, which share common progenitor cells, express markers typical to other cell types, suggesting that CD20 expression in T cell lymphoma is related to malignant transformation. Another possibility is that CD20 positivity results from abnormal antigen expression associated with T cell lymphoma. In some instances, CD20 expression might be lost upon recurrence, indicating that it could be a transient phenomenon. In addition, CD20-positive T cell subsets in the blood may contribute to abnormal CD20 expression in T cell lymphomas. Normal CD20⁺ T cell precursors have been confirmed to exist in the blood, and possibly account for the abnormal CD20 expression observed in some T cell lymphomas (20). Overall, the precise mechanism underlying CD20 expression in T cell lymphoma remains unclear, and further research is needed to elucidate this phenomenon.

When ENKTL abnormally expresses CD20, CD30 and CD15, it must be differentiated from HL, anaplastic large-cell lymphoma and diffuse large B cell lymphoma. Hodgkin's and Reed-Sternberg cells in HL are characterized by CD30 and CD15 expression, with heterogeneous CD20 staining.

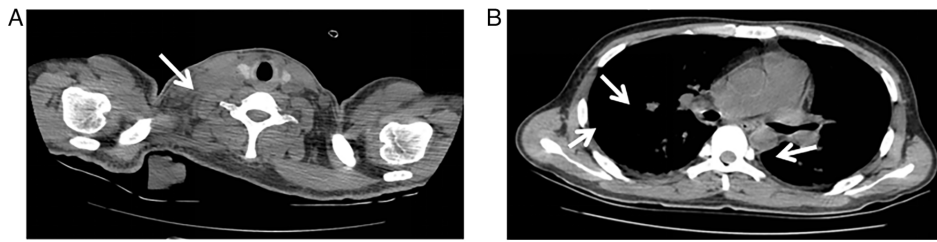


Figure 1. (A) Neck CT scan shows multiple enlarged lymph nodes on the right side of the neck, some of which appear fused; (B) Chest CT scan shows multiple nodules in both lungs with irregular and lobulated edges, accompanied with multiple enlarged lymph nodes in the mediastinum and bilateral hilum. CT, computed tomography.

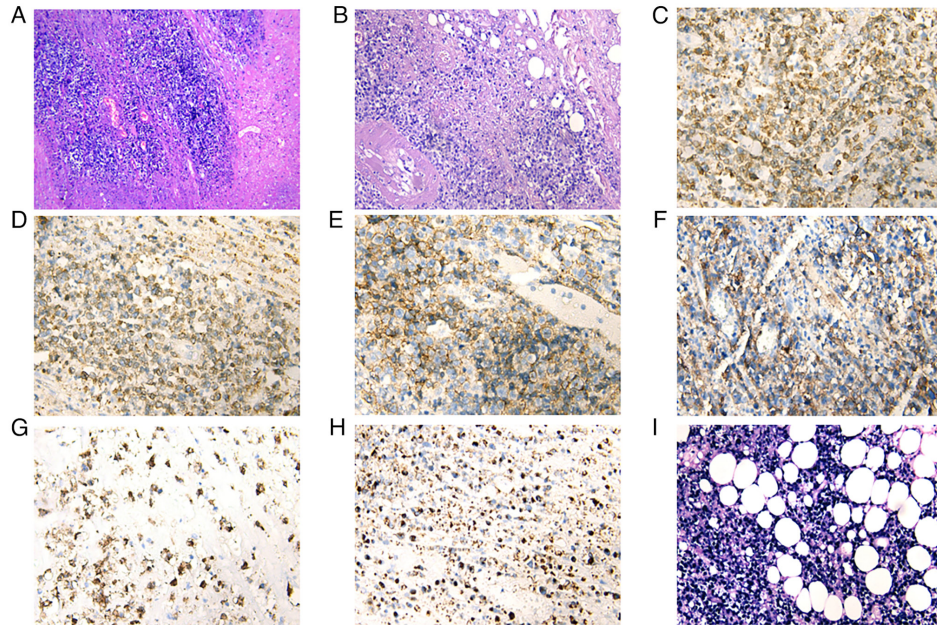


Figure 2. (A and B) Under the microscope, the lymph node structure appears to be destroyed by the tumor cells, which have infiltrated the surrounding fatty and fibrous tissue. The tumor cells comprise medium to large lymphocytes with irregular nuclei. Immunohistochemical analysis shows tumor cells positive for (C) CD3, (D) CD8, (E) CD30, (F) CD20, (G) CD15 and (H) TIA1. (I) Tumor cells positive for EBER on *in situ* hybridization. [(A) HE staining with magnification, x40; (B) HE staining with magnification, x100; (C-H) immunohistochemical staining with magnification, x200; (I) *In situ* hybridization for EBER, magnification, x200]. EBER, Epstein-Barr virus small-encoded RNA; HE, hematoxylin and eosin.

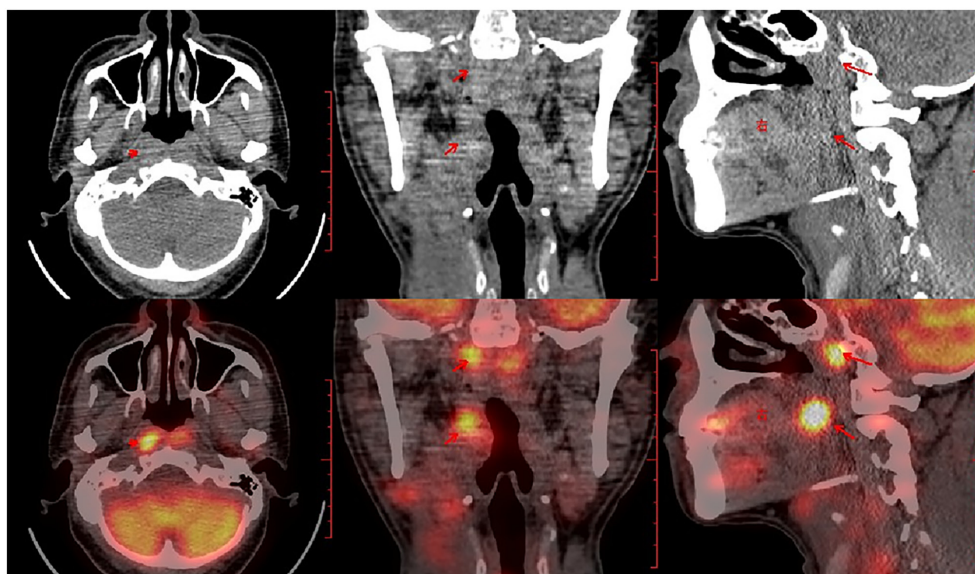


Figure 3. A Whole-body positron emission tomography-computed tomography scan reveals heightened fluorodeoxyglucose drug uptake in the posterior wall of the nasopharynx, the right pharyngeal recess, the right tonsil and the tip of the tongue (SUV_{max} , 17.6).

However, T cell atypia, necrosis and cytotoxic expression typically rule out HL (14). Further, detection of EBV is crucial for diagnosing both ENKTL and anaplastic large-cell lymphoma. In diffuse large B cell lymphoma, in addition to CD20, other B cell markers, such as CD79a and PAX-5, are also expressed.

Studies have shown that CD20 expression may be associated with a highly aggressive clinical course and a poor prognosis (3-8 months) (21,22). Treatment options for ENKTL include CHOP or regimens such as steroid, methotrexate, ifosfamide, L-asparaginase and etoposide (SMILE) (23). The efficacy of targeting CD20 in T cell lymphoma remains uncertain. Although rituximab, a CD20-targeted therapy, is widely used for CD20-positive B cell lymphomas, literature on its use for CD20-positive T cell lymphoma is limited. Some studies suggest that CD30 is a prognostic factor for overall survival or progression-free survival in T cell lymphoma (24). A recent study reported that ENKTL patients with high expression (>40%) exhibit improved overall survival compared with those with low or negative (0%) CD30 expression levels (24); therefore, the present study used 40% as a meaningful cut-off value for CD30 positive expression. Furthermore, CD30 expression has been reported to affect the survival of clinical subgroups of patient (24). However, some studies hypothesize that CD30 is unrelated to prognosis (8,9,25). Brentuximab vedotin, an antibody-drug conjugate targeting CD30, represents a significant advance in lymphoma treatment. It is approved for relapsed HL and ALCL and has shown effectiveness in other CD30-expressing lymphomas, such as PTCL-NOS (6).

To the best of our knowledge, this is the first report in the literature of ENKTL expressing CD20, CD30 and CD15, which is associated with aggressive clinical behavior. The present case revealed that differential diagnosis should be cautiously approached in ENKTL because the aforementioned markers are typically expressed in B cell lymphoma or HL. In addition to rigorous histological and comprehensive immunohistochemical staining, whole-body imaging and molecular testing can assist with diagnosis. Understanding the molecular mechanisms of CD30 and CD20 expression in ENKTL can also bring novel ideas for treatment.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributors

PZ and JY conceived the study idea and drafted the manuscript. PZ and JY carried out data collection. QZ, CX and

BZ performed the CT/PET scans. PZ and SY performed the biopsy. PZ and YL interpreted the data and revised the manuscript. PZ and JY confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

This retrospective study was not applicable for ethics approval and written informed consent to participate was provided.

Patient consent for publication

The patient provided written informed consent for the case study to be published.

Competing interests

The authors declare that they have no competing interests.

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