Composite B-cell lymphoma and extranodal natural killer/T-cell lymphoma presenting with distinct clone evolution and tumor microenvironment: A report of two cases

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Abstract. Composite lymphoma containing both B-cell lymphoma and extranodal natural killer/T-cell lymphoma (ENKTL) is extremely rare. Clonal evolution and tumor microenvironment (TME) features have not been reported previously. The present study reports one case of the heterochronous occurrence of follicular lymphoma (FL) and ENKTL and one case of the simultaneous occurrence of diffuse large B-cell lymphoma (DLBCL) and ENKTL. The results of targeted DNA sequencing demonstrated the genetic features and dynamic clonal evolution of each component. Notably, in the case of ENKTL secondary from FL, predominant clones harbored in ENKTL were already present at the initial diagnosis of FL and expanded to full clonal prevalence afterward in ENKTL. In the case of synchronous DLBCL and ENKTL, predominant clones in ENKTL were exclusive to those clones dominating in DLBCL. Multiple immunofluorescence and CIBERSORT analyses depicted TME features and showed that $CD68^+$ macrophages were abundant in FL, DLBCL and ENKTL. The present report could broaden our understanding of composite lymphoma.

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Introduction

Composite lymphoma is defined as the presence of two or more distinct lymphomas synchronously or heterochronously occurring in the same patient (1). Most of the reported cases are composite aggressive B-cell lymphomas and indolent B-cell lymphomas, including diffuse large B-cell lymphoma (DLBCL) combined with follicular lymphoma (FL) or margin zone lymphoma (MZL). However, composite B-cell lymphoma and extranodal natural killer/T-cell lymphoma (ENKTL) are extremely rare. To the best of our knowledge, only two cases of composite DLBCL and ENKTL have been reported, while no previous case of composite FL and ENKTL has been reported (2,3). Nagai *et al* (2) documented the first case of synchronous ENKTL and DLBCL. In this instance, the patient underwent a transbronchial lung biopsy and a gastroscopic biopsy, which revealed histopathological results consistent with ENKTL and DLBCL, respectively. Kawai *et al* (3) reported another case where the patient first had a biopsy of the nasal cavity, which showed ENKTL, followed by a uterine cervix biopsy that indicated DLBCL. Both cases presented as synchronous composite lymphoma. The present study reports one case of the heterochronous occurrence of FL and ENKTL, and one case of the simultaneous occurrence of DLBCL and ENKTL. Although the diagnosis of composite lymphoma depends on pathological analysis, including immunohistochemical staining and *in situ* hybridization, clonal evolution analysis can provide clearer insights into the origins of the two different lymphomas present.

Case report

Case 1. Heterochronous occurrence of FL and ENKTL (Fig. 1). In June 2019, a 60-year-old male patient was admitted to the Cancer Center, Union Hospital (Wuhan, China) with

multiple enlarged lymph nodes. The patient received a diagnosis of FL , stage IV (Fig. 1A). The FL was classified as grade 1-2 according to the World Health Organization Classification 2016 (4), and the immunohistochemical results showed that the samples were positive for BCL2, CD10 and CD20. Additionally, the patient was Epstein-Barr encoding region (EBER) *in situ* hybridization (ISH)-negative (Fig. 1C). The patient received four cycles of R-CHOP chemotherapy (375 mg/m^2 rituximab on day 1, 750 mg/m^2 cyclophosphamide on day 2, 50 mg/m^2 Adriamycin on day 2, 2 mg vincristine on day 2 and 100 mg prednisone on days 2-6) and four cycles of R-FC (375 mg/m^2 rituximab on day 1, 25 mg/m^2 fludarabine on days 2-4 and 250 mg/m^2 cyclophosphamide on days 2-4) to achieve complete remission (CR). The FL treatment was ended in January 2020. In March 2020, the patient presented with a painful erythematous ulcerated plaque on the right upper leg. A biopsy of the lesion was performed in May 2020, and the pathological diagnosis was ENKTL, with EBER ISH positivity (Fig. 1D). Positron emission tomography-computed tomography (PET-CT) scans showed hypermetabolism in the right lower extremity skin and lumbar spine (Fig. 1B), and bone marrow puncture and exfoliative cytology examinations showed abnormal cells. Therefore, the patient was diagnosed with ENKTL stage IV and received six cycles of PP-GEMOX (200 mg programmed cell death protein 1 inhibitor sintilimab on day 1, 2,500 U/m^2 pegaspargase on day 1, 1,000 mg/m^2 gemcitabine on day 1 and 130 mg/m^2 oxaliplatin on day 1) and achieved CR, thereafter receiving maintenance treatment with sintilimab (200 mg, every 3 weeks) for 1 year. The patient remained disease-free for >40 months after this second CR.

To further elucidate the pathogenesis, targeted DNA sequencing (Table S1) and bulk RNA sequencing (Data S1) were performed in these two paired samples. The results demonstrated that the predominant mutated genes were CREBBP, CIITA, EPHA2, BUB1B, CARD11 and TNFRSF14 in the FL specimen, while the predominant mutated genes were KMT2D, STAT3, MGA, SYK, FAT1 and FAT4 in the ENKTL specimen. There was no overlapping mutated gene between the FL and ENKTL specimens. Clone evolution analysis was also performed (Data S1). As shown in Fig. 1E, clones 0 and 3, harbored predominantly in ENKTL samples, were already present at the diagnosis of FL and expanded to full clonal prevalence when ENKTL was later diagnosed.

To better understand the nature of the tumor microenvironment (TME), CIBERSORT analysis was applied based on bulk RNA sequencing. As shown in Fig. 1F, the results of the CIBERSORT (immunedeconv v2.1.0) analysis showed that the composite lymphoma of FL and ENKTL had individual distinct TME compositions.

Case 2. Synchronous occurrence of DLBCL and ENKT (Fig. 2). In January 2021, a 51-year-old male patient with a recent diagnosis of DLBCL was referred to Cancer Center, Union Hospital. The patient had undergone a partial ileectomy and ileal-ileal side-to-end anastomosis due to ileum perforation. PET-CT revealed pathological FDG uptake in the nasal cavity, nasopharynx, liver, subcapsular region of liver, right mesenteric nodes and right external iliac nodes (Fig. 2A). The postoperative pathological diagnosis was EBV-positive DLBCL, not otherwise specified (NOS), with immunohistochemical staining results as follows: CD20$^+$, CD5$^-$, CD10$^-$, BCL2$^+$, BCL6$^+$, MUM1$^+$, MYC$^+$ (30%), p53$^-$, and EBER ISH$^+$ (Fig. 2C). The patient was therefore diagnosed as EBV-positive DLBCL stage IV according to the results of pathology and PET/CT scan. The patient received R-CHOP chemotherapy (doses as aforementioned) as the first-line treatment. After four cycles of R-CHOP, an interim PET-CT revealed that most of the lesions had disappeared, but the lesions of the nasal cavity, nasopharynx and subcapsular region of the liver persisted. Notably, fluorodeoxyglucose uptake in the nasal and nasopharyngeal lesions was markedly increased [interim maximum standardized uptake value (SUV_{max}), 16.8; baseline SUV_{max}, 7.9] (Fig. 2B). A biopsy of the nasal and nasopharyngeal lesions was performed in May 2021, and the pathological result revealed ENKTL, nasal type. Immunohistochemical results showed that GranB, CD3, CD56 and EBER ISH staining was positive, while CD20 staining was negative (Fig. 2D). Therefore, the patient was re-diagnosed with composite DLBCL and ENKTL, and treated with pegaspargase-based chemotherapy plus sintilimab (2,500 IU/m^2 pegaspargase on day 1 and 200 mg sintilimab on day 1, every 3 weeks). After six cycles of immunochemotherapy and radiation to the nasal cavity and nasopharynx (54 Gy/27 fractions), the patient achieved CR and received maintenance treatment with sintilimab (200 mg, every 3 weeks) for 1 year. The patient has remained disease-free for >30 months by far.

The results of targeted DNA sequencing demonstrated that the predominant mutated genes were AR, BCL7H, CD79A, P2RY8, PTPRO, REL, RELN and TRAF3 in the DLBCL specimen, while the predominant mutated genes were PRDM1, ABL2, B2M, CREBBP, DDX3X, FAT4, FLT1 and STAT3 in the ENKTL specimen. There was no overlapping mutated gene between the DLBCL and ENKTL specimens. Clone evolution analysis revealed that this case of synchronous composite lymphoma exhibited a pattern of clonal dynamics that was markedly different from that of case 1 (heterochronous composite lymphoma). The ancestral clones of both the DLBCL and ENKTL samples were characterized using CSF3R and MSH6 mutations. However, the clonal lineages harboring PRDM1, DDX3X and STAT3 mutations (clusters 1 and 2) in the ENKTL sample were exclusive to the clonal lineages dominating in the DLBCL sample (clusters 0 and 3) (Fig. 2E). The results suggested that the two lymphomas may have evolved from totally divergent clones.

To better understand the TME of this type of heterochronous composite lymphoma, bulk RNA sequencing was also conducted in these two paired samples (Fig. 2F). Results of CIBERSORT analysis showed that the TME of synchronous composite lymphoma can vary considerably between different subtypes.

Discussion

Composite lymphoma containing both ENKTL and B-cell lymphoma is extremely rare. The two cases reported in the present study broaden our understanding of composite lymphoma and allow some speculation on such exceptional events.

The definite mechanisms behind the development of two synchronous or heterochronous lymphoid neoplasms are not well established due to the complexity and rarity of composite lymphoma, and the diverse causes of different subtypes of

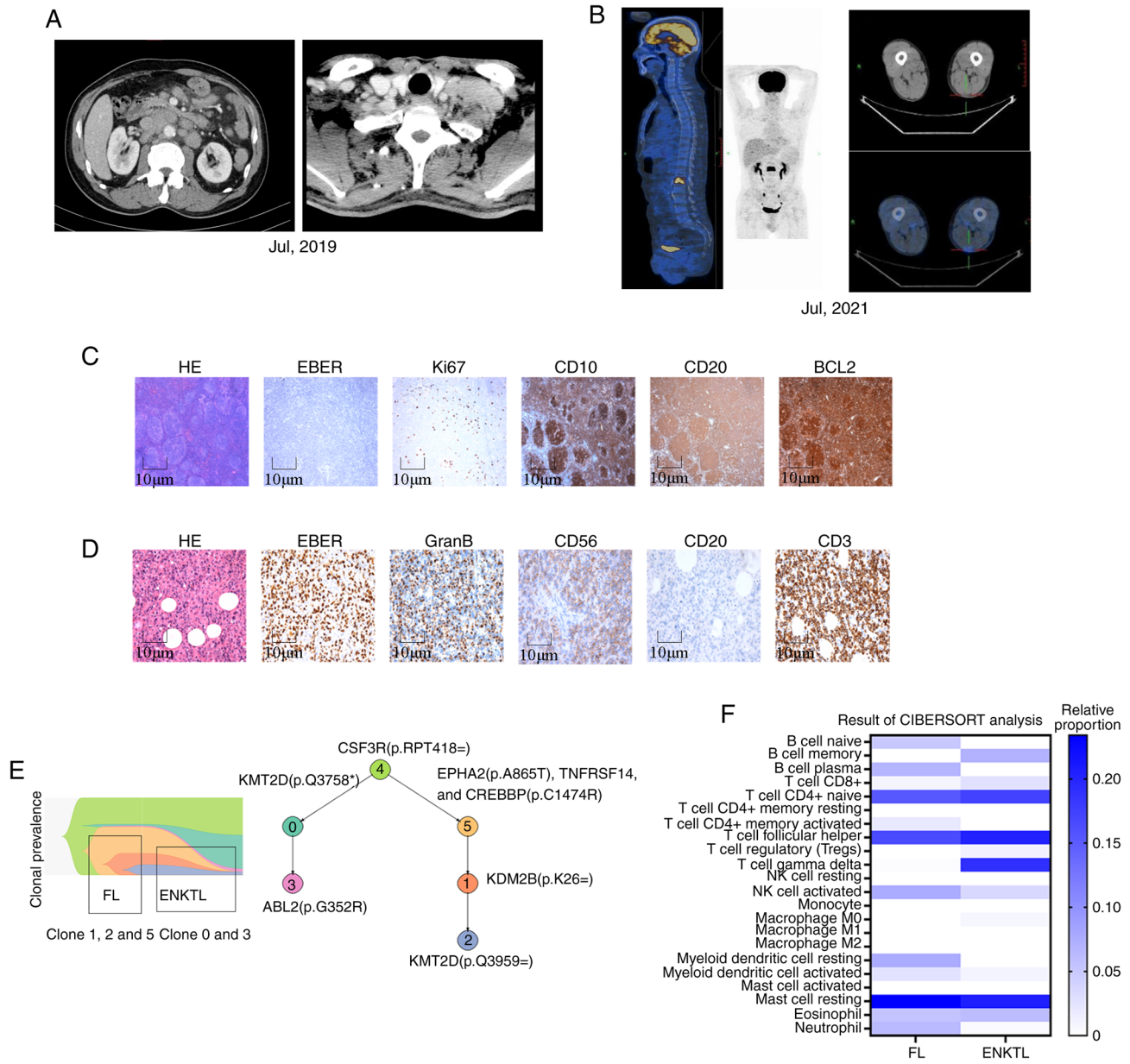

Figure 1. Results in case 1. (A) Histological manifestations of FL based on a biopsy of the left supraclavicular lymph node (x400 magnification). (B) Histological manifestations of ENKTL based on a biopsy of the lower limb mass (x400 magnification). (C) CT images captured at the initial diagnosis of FL. (D) Positron emission tomography-CT images captured at the initial diagnosis ENKTL. (E) Result of the clone evolution analysis based on targeted DNA sequencing. (F) Result of CIBERSORT analysis of immune cell composition based on bulk RNA sequencing. The values ranging from 0 to 0.2 represent the relative proportion or fraction of each immune cell type within the sample, as estimated by the CIBERSORT algorithm. NK, natural killer; ENKTL, extranodal NK/T-cell lymphoma; CT, computed tomography; FL, follicular lymphoma; HE, hematoxylin and eosin; EBER, Epstein-Barr encoding region; GranB, grazyme B.

lymphoma. One possibility is immune dysfunction, which may induce mixed neoplastic clones (5). EBV infection is closely linked to the development of lymphoma, particularly in the subtypes of ENKTL and EBV-positive DLBCL, which reflects immune dysregulation. It is worth noting that EBV infection may not always be present in both parts of composite lymphoma (6,7). In some cases, previous immunochemotherapy for malignancies could result in immunosuppression, thus contributing to a subsequent EBV infection and the development of lymphoma.

The present study examined EBV infection in two cases. In the first case, EBV infection was only found in ENKTL but not in FL. We speculated that the immunochemotherapy for the patient's first FL could have caused immunosuppression, which led to a subsequent EBV infection and the development of ENKTL. In the second case, EBV infection was found in both DLBCL and ENKTL. It was concluded that the virus infected both B-cells and NK/T cells simultaneously, then transformed the B cells and NK/T cells independently, forming two independent neoplastic clones.

The clonal association between the two distinct lymphoma components is a major concern in composite lymphoma. A number of studies have reported contradicting findings, namely that these two components may be clonally related or unrelated (8-10). However, the clonal association in composite DLBCL/FL and ENKTL has not been analyzed by sequencing

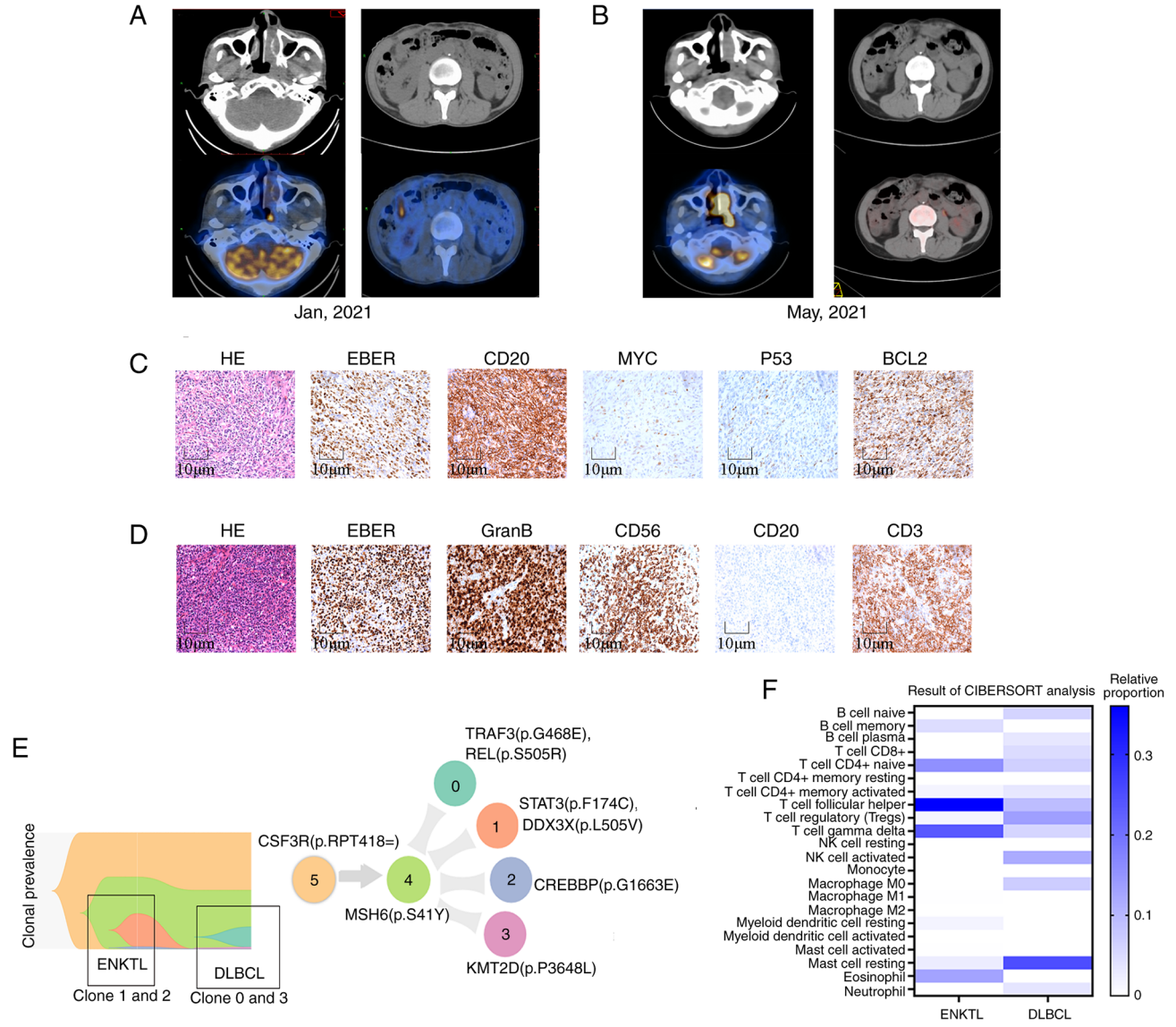

Figure 2. Results in case 2. (A) Histological manifestations of DLBCL based on surgical resection of the ileum (x400 magnification). (B) Histological manifestations of ENKTL based on a nasal biopsy (x400 magnification). (C) PET-CT images captured at the initial diagnosis of DLBCL. (D) PET-CT images captured during the interim of DLBCL treatment and the initial diagnosis of ENKTL. (E) Result of clone evolution analysis based on targeted DNA sequencing. (F) Result of CIBERSORT analysis of immune cell composition based on bulk RNA sequencing. The values ranging from 0 to 0.2 represent the relative proportion or fraction of each immune cell type within the sample, as estimated by the CIBERSORT algorithm. NK, natural killer; ENKTL, extranodal NK/T-cell lymphoma; PET-CT, positron emission tomography-computed tomography; DLBCL, diffuse large B-cell lymphoma; HE, hematoxylin and eosin; EBER, Epstein-Barr encoding region; GranB, grazyme B.

due to its rarity. The present sequencing results demonstrated that composite B-cell lymphoma and ENKTL could evolve from divergent clones.

The composition of the TME can vary widely in synchronous or heterochronous composite lymphomas, affecting the treatment and prognosis of patients. Notably, abundant $CD68^+$ macrophages were observed in the FL, DLBCL and ENKTL samples of the present two patients. $CD68^+$ macrophages are closely associated with the poor prognosis of lymphoma, involving tumor growth and drug resistance (11,12). Targeting macrophages could be a desirable treatment option.

In summary, composite B-cell lymphoma and ENKTL lymphoma are extremely rare, with potentially distinct clonal evolution models and TMEs. Understanding their genetic and TME features guides accurate treatment; however, further studies are needed to confirm the findings and interpretations of the present study.

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Availability of data and materials

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

in the present study may be found in the National Center for Biotechnology Information BioProject database under accession number PRJNA1249522 or at the following URL: https://www.ncbi.nlm.nih.gov/sra/PRJNA1249522.

Authors' contributions

QW, QL and LZ had the original idea and designed the study. WY, XC, FZ and HP advised on patient treatment strategies, and obtained and analyzed data. WY and FZ interpreted clinical data. QW and QL wrote the draft. WY, XC, HP and LZ assisted in revising the manuscript. All authors have read and approved the final manuscript. WY, XC, FZ, HP and LZ confirm the authenticity of all the raw data.

Ethics approval and consent to participate

This study was approved by the Ethical Committees of Union Hospital, Tongji Medical College, Huazhong University of Science and Technology (Wuhan, China; approval no. 2024-0986). This study was conducted in accordance with the principles of the Declaration of Helsinki.

Patient consent for publication

Written consent was obtained from the patients for their information to be published.

Competing interests

The authors declare that they have no competing interests.

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