

Diagnostic challenges in T-lymphoblastic lymphoma, early T-cell precursor acute lymphoblastic leukemia or mixed phenotype acute leukemia

A case report

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Abstract

Rationale: The diagnosis of hematological malignancies depends on laboratory analysis and often requires multiple experimental methods to judge, otherwise misdiagnosis is apt to happen. Lymph node biopsy immunohistochemistry (IHC) for T-lymphoblastic lymphoma (T-LBL) requires the establishment of antibody set screening. For identifying T-LBL and early T-cell precursor acute lymphoblastic leukemia (ETP-ALL) by lymph node biopsy and IHC, WHO has not yet proposed a better IHC antibody combination.

Patient concerns: Here we reported 1 case with tortuous diagnosis experience. Initially, a 51-year-old man was diagnosed as T-LBL by lymph node biopsy, but in another hospital acute myeloid leukemia (AML) was confirmed by bone marrow puncture. Finally, it was diagnosed as mixed phenotype acute leukemia (MPAL) through our comprehensive evaluation including bone marrow cell morphology, cytochemical staining and flow cytometry analysis. Importantly, the experience about differential diagnosis and our appreciation among the T-LBL, ETP-ALL and MPAL was discussed to enlighten readers.

Diagnoses: The patient was diagnosed with mixed phenotype acute leukemia (T+My)-NOS.

Interventions: The patient received 1 cycle of VDCLP scheme treatment firstly. The effect of chemotherapy is satisfactory, and then he received continuous treatment and was currently in good condition.

Outcomes: This patient is alive at present. The follow-up period has been 1 year.

Lessons: For the diagnosis of T-LBL, the molecular markers of the myeloid and lymphoid tissues need to be included, such as CD117, CD33, Lys and MPO. The bone marrow puncture also needs to be conducted to distinguish T-LBL and T-ALL. Secondly, to identify ETP-ALL and MPAL, bone marrow cell morphology, cytochemical staining as well as flow cytometric analysis were needed to make a clear diagnosis. It is recommended that at least CD8, CD1a, Lys and MPO should be included in the panel to identify ETP-ALL.

Abbreviations: AML = acute myeloid leukemia, ETP-ALL = early T-cell precursor acute lymphoblastic leukemia, IHC = immunohistochemical, MPAL = mixed phenotype acute leukemia, MPO = myeloperoxidase, T-LBL = T-lymphoblastic lymphoma.

Keywords: early T-cell precursor acute lymphoblastic leukemia, laboratory analysis, mixed phenotype acute leukemia, T-lymphoblastic lymphoma

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1. Introduction

Precursor T-lymphoblastic lymphoma (T-LBL), which is biologically similar to precursor T-acute lymphoblastic leukemia (T-ALL), is derived from immature lymphoid cells of T-cell lineage.^[1] The initial clinical manifestation of T-LBL usually takes the form of a mediastinal mass or lymphadenopathy, whereas T-ALL patients present with predominant manifestation of bone marrow and peripheral blood disease. T-LBL is distinguished from T-ALL primarily based on the degree of bone marrow involvement by T-lymphoblasts.^[2] Patients with <25% bone marrow involvement are classified as T-LBL while patients with 25% or more bone marrow blasts are diagnosed with T-ALL.^[1,3] Early T-cell precursor ALL/LBL (ETP-ALL/LBL) is a recently described, rare, high risk subtype of T-cell ALL/LBL (T-ALL/LBL) by the WHO in 2016.^[4,5] Additionally, the WHO classification has established strict criteria for the diagnosis of mixed phenotype acute leukemia (MPAL), the differentiation between MPAL (T/myeloid) and ETP-ALL mainly is the morphologic presence of Auer rods and myeloperoxidase

(MPO) staining, although both exhibit a similar flow cytometric phenotype.^[6] Here we reported 1 patient with diagnosis changing from T-LBL to MPAL, and the discrimination of T-LBL, ETP-ALL, and MPAL (T+My)-NOS was proposed, which was unique for present case.

2. Case presentation

2.1. Clinical history

On September 12, 2017, a 51-year-old man presented at our department of hematology and the main symptom was an increase in superficial lymph nodes. According to the medical history, the patient was admitted to the department of otolaryngology due to cervical lymph node mass on March 20, 2017. The largest tumor mass was $6.5 \times 5 \times 2.8$ cm by ultrasonography. Lymph node biopsies and immunohistochemical (IHC) staining displayed as $CD3^{++}$ $CD7^{+++}$ $CD56^{++}$ $CD5^{par+}$ TDT^{++} $BCL2^{+++}$ $Ki67$ (80%+) and $CD1a^{-}$ $CD10^{-}$ CK^{-} $CD30^{-}$ $Granzyme^{-}$ $Perforin^{-}$ $CyclinD1^{-}$ $EBER^{-}$ $BCL6^{-}$ TIA^{-} , while few scattered cells were positive for CD4, CD8, CD2, Pax5, CD20, CD23, CD21, and CD79a. Diagnosis was T-lymphoblastoid non-Hodgkin lymphoma (T-LBL). However, this patient

himself only accepted the treatment of traditional Chinese medicine which turned out to have poor efficacy. As the disease progressed, manifesting as generalized lymphadenopathy, the patient went to a local hospital for bone marrow biopsy, and the result of bone marrow cytology suggested acute myeloid leukemia (AML). Although this conclusion was contradictory with former T-LBL diagnosis, no further laboratory tests such as flow cytometry were performed. For further diagnosis and treatment, he visited our department, one of the most outstanding hospitals in southwest China.

Informed written consent was obtained from the patient for publication of this case details and accompanying images. This study was also approved by the ethics committee of the Second Affiliated Hospital of the Army Medical University.

Routine peripheral blood examination showed white blood cell count $1.99 \times 10^9/L$, red blood cell count $3.81 \times 10^{12}/L$, hemoglobin 129 g/L, and platelet $134 \times 10^9/L$. Magnetic resonance imaging revealed multiple swollen lymph nodes in the body, including bilateral lymph nodes in bilateral axillary, tonsil, neck, submandibular, subclavian, axillary, anterior superior mediastinal, hilar, abdominal aortic, periaortic, intraperitoneal, and pelvic bilateral inguinal regions.

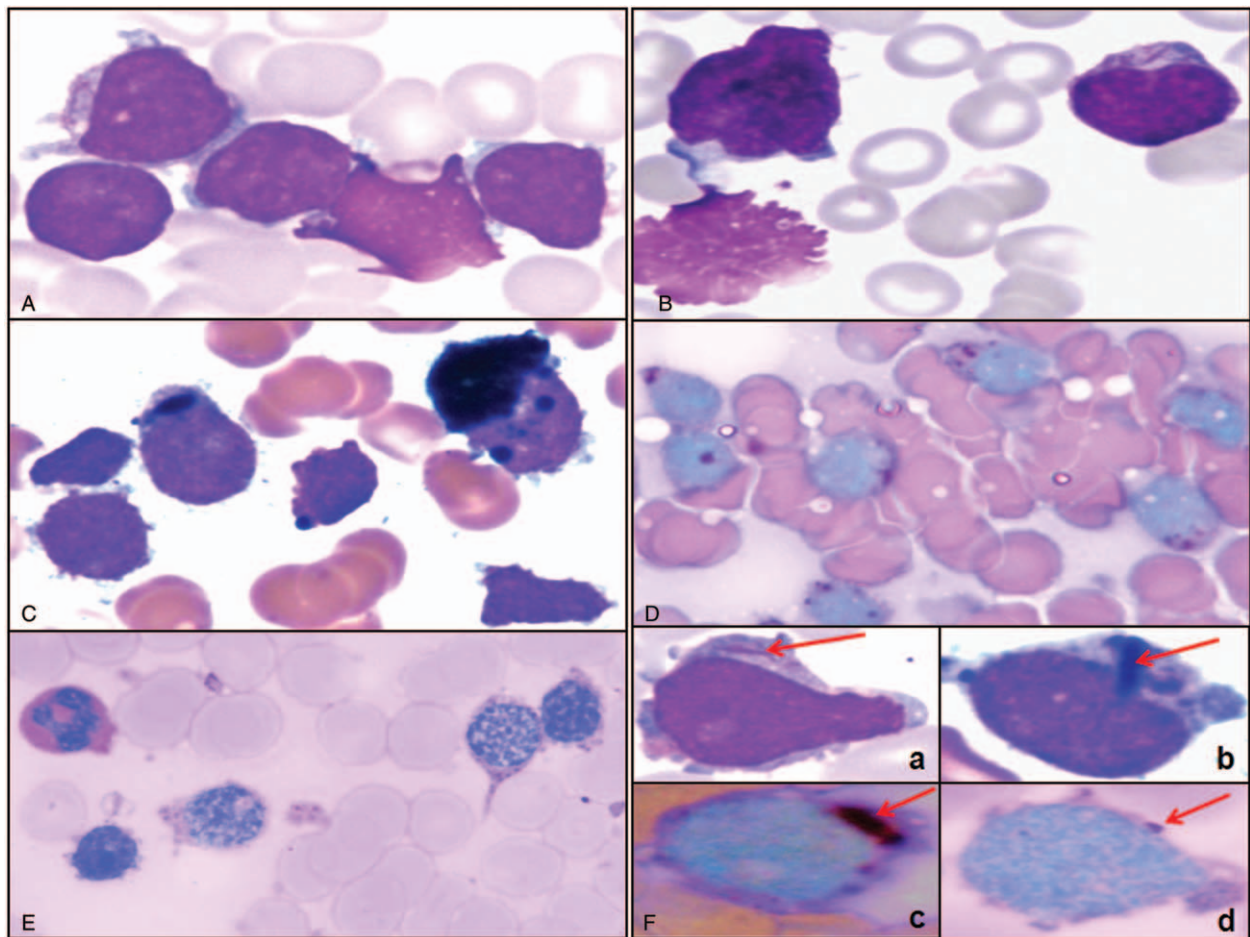


Figure 1. Bone marrow cell morphology and cytochemical analysis. About 64% of cells in bone marrow biopsy were primary tumor cells. The original cell has a cell volume of about 15 to 20 μL . The aberrant cell nuclei were large, irregular, folded, and twisted. The cytoplasm was less and irregular in shape with trailing and burr-like changes. Some contained small amounts of azurophilic granule, some do not (A and B). About 11% of the tumor cells were positive for myeloperoxidase (MPO) staining, and Auer body also could be seen in those blast cells (C). The NAE staining of cytoplasm was scattered or massive positive (D), and the PAS staining of cytoplasm showed spotty or bead-like positive (E). By observing individual primitive cells, a slender Auer body could be seen in the cytoplasm (F-a), and MPO staining of Auer body showed positive signal (F-b). NAE staining (F-c) and PAS staining (F-d) were positive in cytoplasm.

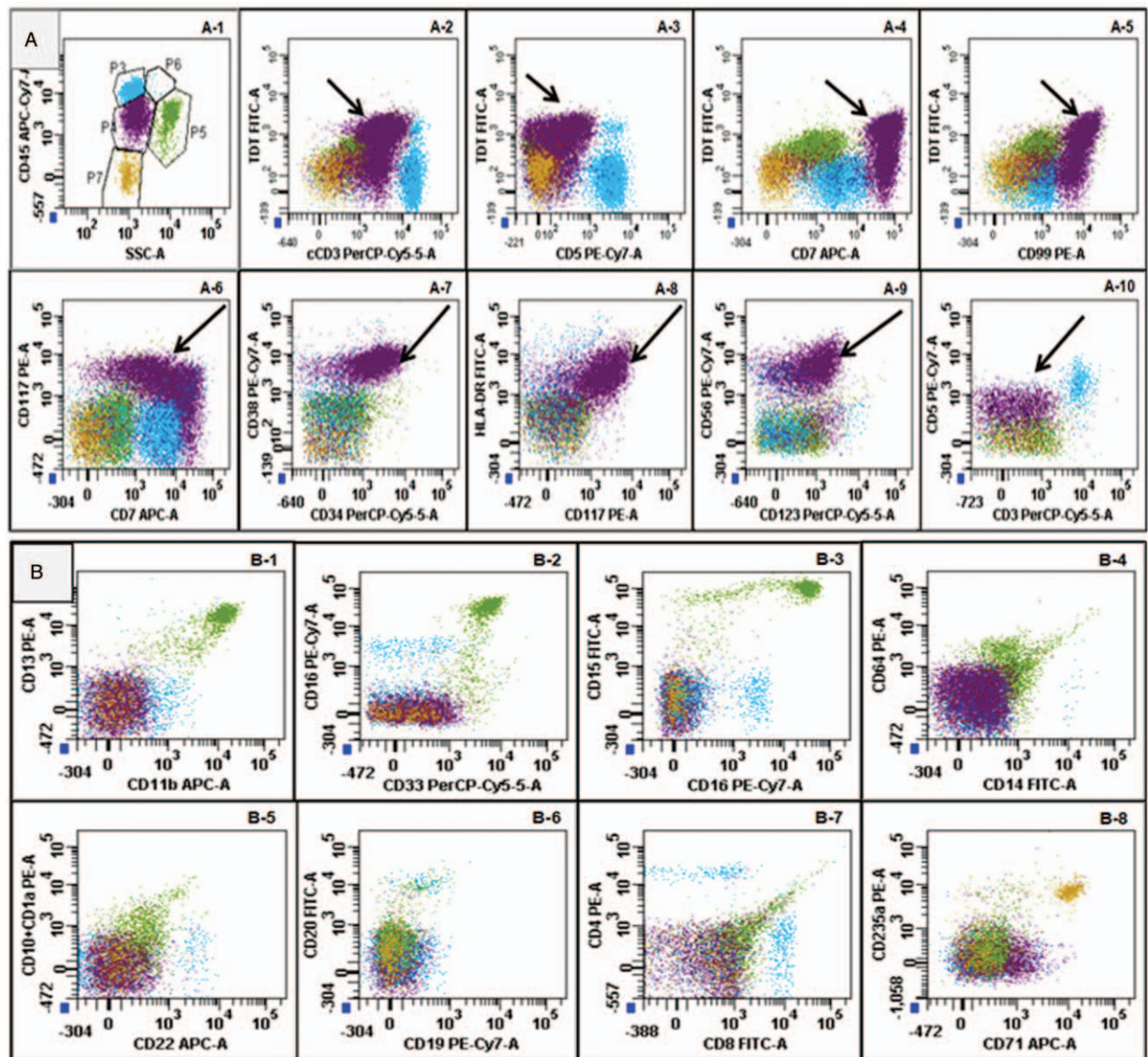


Figure 2. Flow cytometry analysis related to early T-cell precursor acute lymphoblastic leukemia (ETP-ALL)-like immunophenotyping. Through a wide monoclonal antibody panel by 6-color flow cytometry analysis, blast cells were gated, and accounting for 48.5% of nuclear cells (P4). The immunophenotype of blasts was CD45^{dim} CD7^{bri+} CD117⁺ CD34⁺ CD38⁺ HLA-DR⁺ CD123^{dim} CD5^{dim} CD71^{dim} CD99⁺ TDT⁺ cCD3⁺ (A1 to A10), and CD1a⁻ CD8⁻ CD4⁻ CD13⁻ CD33⁻ CD14⁻ CD64⁻ CD11c⁻ CD19⁻ CD20⁻ CD22⁻ CD79a⁻ (B1 to B8). The T-lymphoblastic lymphoma/ALL clone was detected, and the phenotype was consistent with ETP-ALL according to WHO 2016 criteria.

2.2. Pathologic findings

Bone marrow biopsy was rechecked in our department and displaying approximately 64% ALL blast cells, interestingly, approximately 11% of primitive tumor cells were peroxidase staining positive, and Auer bodies were visible in a few cells (Fig. 1). The diagnosis was still unclear: was it an ETP-ALL or MPAL was the question.

Flow cytometry analysis suggested primitive tumor cells in P4 gate occupy 48.5% of nuclear cells, CD45^{dim} CD7^{bri+} CD117⁺ CD34⁺ CD38⁺ HLA-DR⁺ CD123^{dim} CD5^{dim} CD71^{dim} CD99⁺ TDT⁺ cCD3⁺, and CD1a⁻ CD8⁻ CD4⁻ CD13⁻ CD33⁻ CD14⁻ CD64⁻ CD11c⁻ CD19⁻ CD20⁻ CD22⁻ CD79a⁻ (Fig. 2). T-cell leukemia clones were found and in accordance with the ETP-ALL phenotype according to WHO 2016 criteria.

However, additional leukemia clones appeared. CD7^{low/-} CD3⁺ MPO⁺ CD117^{bri+} cells accounted for 11.1% of CD3⁺ cells. This

cannot be explained only by ETP (Fig. 3). According to the EGIL criteria, biphenotypic leukemia is diagnosed when scores are >2 for the myeloid and one of the lymphoid lineages.^[7] Therefore, this case was ultimately diagnosed as MPAL (T+My)-NOS by combining morphology and flow immunophenotype. In this case, chromosome karyotype is 46XY, normal. No abnormalities were found by polymerase chain reaction and fluorescent in situ hybridization, such as PML/RARa, BCR/ABL, AML1/ETO, CBFβ/MYH11, MLL fusion gene.

The patient received 1 cycle of VDCLP scheme treatment. The effect of chemotherapy is satisfactory, and most of enlarged lymph nodes shrank, some even disappeared. The proportion of bone marrow blasts was significantly decreased. This patient received continuous treatment and was currently in good condition. So far, the follow-up period has been 1 year.

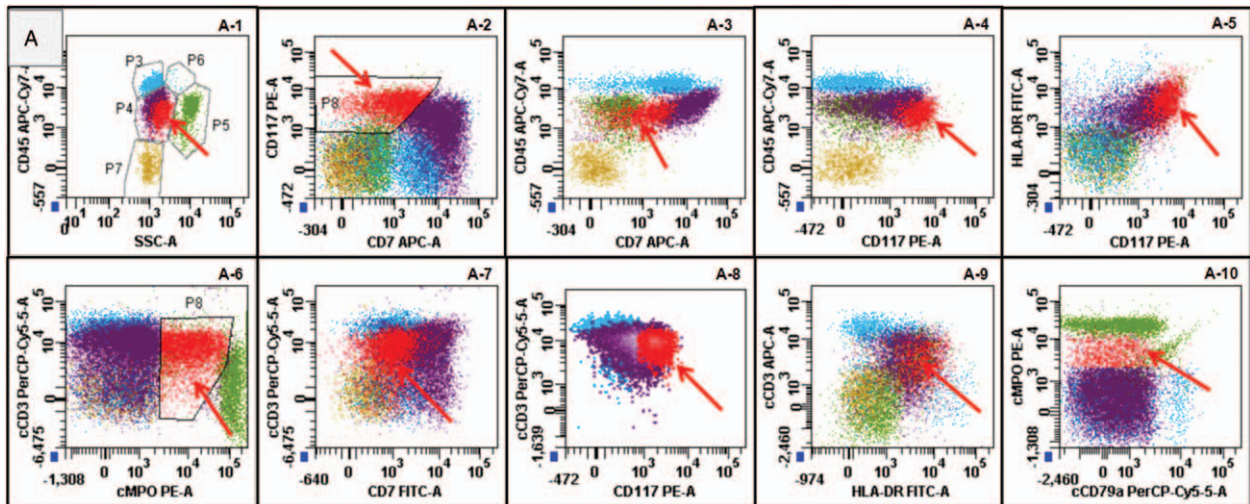


Figure 3. Flow cytometry analysis related to mixed phenotype acute leukemia (MPAL) immunophenotyping. Apart from early T-cell precursor acute lymphoblastic leukemia (ETP-ALL)-like immunophenotyping, an additional leukemic clone appeared, with immunophenotyping CD117⁺ CD7^{low/-}. This population cells were further positive for HLA-DR⁺ cCD3⁺ cMPO⁺. Cells with MPO⁺ cCD3⁺ CD117⁺ accounted for 11.1% of CD3⁺ cells. Combining the results of Figures 2 and 3, this case was finally diagnosed as MPAL(T+My)-NOS.

3. Discussion

Here we reported a case with a tortuous experience of leukemia patient, at first, regional cervical lymph node biopsy showed T-LBL, then a bone marrow puncture examination suggested AML, finally, diagnosed as MPAL(T+My)-NOS (not otherwise specified) in our department. Since the initial inspection was not perfect as myeloid marker expression and bone marrow puncture were not implemented, it could be extramedullary invasion of MPAL at the beginning. There was another possibility that this case could be ETP at that time but misdiagnosed as T-LBL, gradually the myeloid clone of ETP amplified and eventually evolved into MPAL under the influence of the microenvironment. This is in agreement with former conclusion that adult T-cell progenitors retain myeloid potential.^[8]

The ETP-ALL/LBL is a recently described, rare, high-risk subtype of T-ALL/LBL by the WHO in 2016,^[5] which is characterized by the aberrant expression of myeloid and hematopoietic stem-cell markers (e.g., CD13, CD33, CD34, and CD117), weak or absent expression of CD5, lack of expression of the T-lineage cell surface markers CD1a and CD8, and a gene expression profile reminiscent of the murine early T-cell precursor,^[9] but MPO⁻.

It is assumed that ETP has the potential to differentiate into the myeloid lineage and may develop into mixed cell leukemia,^[10] which is highly malignant and has a poor prognosis. Present case supports this viewpoint. ETPs originate from both bone marrow and thymic precursor cells, and expresses some markers of myeloid cells. Different microenvironment renders ETPs with different directional differentiation capabilities. Thymus and lymph nodes are more suitable for T-lymphocyte proliferation, and the bone marrow environment for myeloid differentiation. So, flow cytometry analysis of bone marrow cells indicates 2 populations of clonally propagated tumor cells, that is, the mixed T-cells and myeloid cells^[11]; meanwhile, the IHC staining of lymph nodes needs extra-antibodies against myeloid marker. This kind of double-lineage detection is more helpful.

The ETP-ALL is a kind of “T/myeloid” leukemia. It is worth discussing how to distinguish the T/myeloid MPAL and ETP-ALL. Both of them are T- and myeloid-cell markers positive; however, MPO staining is the principle difference, ETP-ALL is MPO⁻ (Table 1), but MPAL is MPO⁺ simultaneously with the scores >2 for the myeloid according to the EGIL criteria.^[7] Therefore, our case was diagnosed as MPAL for MPO⁺ cells and Auer bodies in cytoplasm.

Table 1

How to distinguish T-LBL, ETP-ALL, and MPAL(T+My) by flow cytometry.

Diagnosis	Markers
MPAL(My+T)	
Myeloid	Myeloperoxidase or monocytic differentiation: at least 2 of the following markers: Lys, CD11c, CD14, CD64, NSE
T-cell	Cytoplasmic CD3 or surface CD3
ETP-ALL	Positive: CD7, CD2, cCD3, at least 1 or more of the myeloid/stem-cell markers: CD34, CD117, HLADR, CD13, CD33, CD11b, CD65. CD5 ^{weak+/-} Negative: MPO, CD1a, CD8, Lys, and NSE on IHC
T-LBL/ALL	Positive: TdT, CD7, CD2, CD5, cytoplasmic and/or surface CD3, CD1a, CD43 When TdT: CD3, CD34, CD1a, CD10, CD4, CD8, CD99, Ki-67 need evaluation. Importantly, myeloid markers CD117, CD33, Lys, and MPO, need to be included

ALL = acute lymphoblastic leukemia, ETP = early T-cell precursor, IHC = immunohistochemistry, Lys = lysozyme, MPAL = mixed phenotype acute leukemia, NSE = nonspecific esterase, T-LBL = T-lymphoblastic lymphoma.

The T-LBL accounts for approximately 85% to 90% of all lymphoblastic lymphomas and has similar clinical manifestation as ALL. T-LBL occurs most frequently in underage males and a small proportion of adults. T-LBL usually invades the mediastinum and bone marrow. In addition, the skin, tonsils, lymph nodes, liver, spleen, central nervous system, and male testes may also be invaded by tumor cells.^[12] This case at beginning was diagnosed as T-LBL, but ended as MPAL. Honestly, there are some limitations about this case, for instance, bone marrow puncture was not performed in the beginning, so we have no idea whether the bone marrow was invaded or not? Secondly, because there is no standard methods about histopathologic staining for T-LBL. IHC studies from lymph node biopsy did not contain the myeloid markers. Therefore, we emphasize that the diagnosis of T-LBL should not be based only on histopathologic examination of lymph node, especially when swollen lymph nodes are the first symptoms for the case. The expression of myeloid markers in lymph nodes, such as CD117, CD33, lysozyme (Lys), and MPO should be screened in case of extramedullary extensions of ETP-ALL or MPAL. In addition, the bone marrow puncture and flow cytometry are necessary for diagnosis.

4. Conclusion

For the diagnosis of T-LBL, we recommend that the molecular markers of both myeloid and lymphoid tissues should be included in IHC staining of lymph node biopsy, such as CD117, CD33, Lys, and MPO. Similarly, bone marrow puncture also needs to be conducted to distinguish T-LBL and T-ALL.

The ETP-ALL and MPAL are difficult to identify, which needs combination of bone marrow cell morphology, cytochemical staining as well as flow cytometric analysis to make a diagnosis. We recommend that at least CD8, CD1a, Lys, and MPO should be included in the panel to identify ETP-ALL.

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