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Evaluation of the Cytomorphology, Immunophenotype, and Molecular Genetics of Lymphoblastic Lymphoma/Leukemia in Serous Effusion

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Keywords
Serous effusion · Lymphoblastic lymphoma/leukemia · Cytology · Molecular genetics · Immunocytochemistry

Abstract
Introduction: This study aimed to elucidate the spectrum of clinical manifestations, cytomorphology, immunophenotype, and the molecular genetic features of lymphoblastic lymphoma/acute lymphoblastic leukemia (LBL/ALL) in the context of serous effusions (SE). ***Methods:*** A retrospective analysis evaluated the cytomorphological features, immunophenotype, and the cyto-histological correlations of twenty-one LBL/ALL associated with SE. Concurrently, bone marrow (BM) aspiration samples were analyzed using an integrated approach, including flow cytometry, reverse transcription PCR (RT-PCR), next-generation sequencing (NGS), or whole transcriptome sequencing (WTS). ***Results:*** Of the 21 cases of SE LBL/ALL, 16 cases were T-LBL/ALL and 5 cases were B-LBL/ALL. The cases included 17 pleural, 2 peritoneal, and 2 pericardial fluid samples. Both T-LBL/ALL and B-LBL/ALL in SE exhibit a blast-like morphology, characterized by small to medium size, irregular nuclear membranes, and inconspicuous nucleoli, alongside frequent nuclear fragmentation and apoptotic bodies. LBL/ALL express immaturity markers such as terminal deoxynucleotidyl transferase (7/17, 41.2%), CD10 (6/12, 50%), CD43 (8/8, 100%), and CD99 (6/6, 100%). T-LBL/ALL and B-LBL/ALL specifically express T-cell markers (CD2 [3/6, 50%], CD3 [10/12, 83.3%], CD5 [2/11, 18.2%], CD7 [10/10, 100%]) or B-cell markers (CD20 [3/5, 60%], CD79a [4/4, 100%], PAX5 [5/5, 100%]), respectively. A high proportion of primitive and immature lymphocytes exceeding 25% in BM was observed in T-LBL/ALL (5/7) and in one case of B-LBL/ALL. No *BCR/ABL* gene rearrangements were detected in any cases. Furthermore, fusion gene *MLL*::*ENL* and *PLCALM*::*MLLT10*, as well as mutations in genes including *WT1*, *NOTCH1*, *PAX5*, *IKZF*, *ARID1A*, *BCOR*, *SETD2*, *ARID2*, *TET2*, *JAK3*, *NF1*, and *CEBPA*, were identified in LBL/ALL through RT-PCR, NGS, or WTS analyses. ***Conclusion:*** The integration of clinical manifestations, cytological evaluation, and gene

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expression profiles is instrumental in achieving accurate diagnosis, subclassification, and prognosis of LBL/ALL within the context of SE.

Introduction

Lymphoblastic lymphoma/leukemia (lymphoblastic lymphoma/acute lymphoblastic leukemia [LBL/ALL]), particularly the T-LBL/ALL, frequently involves serous effusions (SEs) [1]. Despite the literature suggesting that SE cytology can serve as a reliable and precise diagnostic tool for hematopoietic malignancies [2–4], the cytological diagnosis of LBL/ALL in SE remains challenging. This study presents a retrospective analysis of 21 cases of LBL/ALL involving the serous cavity. Detailed and comprehensive descriptions of clinical manifestations, cytomorphology, immunophenotype, and cyto-histological correlations, with a particular emphasis on genetic abnormalities or gene expression profiles, are provided. The study evaluates the efficacy of SE specimens in diagnosing LBL/ALL, with a specific focus on their distinct immunophenotype. Furthermore, LBL/ALL subtypes were explored in conjunction with cytogenetic characteristics derived from bone marrow (BM) aspiration samples.

Materials and Methods

Study Cases

SE cases of ALL/LBL diagnosed at Xijing Hospital between January 2016 and May 2025 were systematically reviewed. Clinical data and follow-up information were extracted from the patients' medical records. Hematoxylin and eosin staining was conducted on alcohol-fixed smears, while May-Grünwald-Giemsa staining was applied to air-dried direct smears. Additionally, the remaining specimens underwent centrifugation to precipitate the sediment, which was subsequently embedded in paraffin for cell block preparation. All slides were reviewed by two senior cytopathologists, and diagnoses were made in accordance with the 5th edition of the World Health Organization Classification of Tumors of Hematopoietic and Lymphoid Tissues. This study utilized archival samples and received approval from the Ethics Committee of Xijing Hospital (approval number: KY20253201-1).

Immunophenotype and Gene Rearrangement

Immunocytochemistry was performed on the cell block sections using the EnVision method for ICC or immunohistochemistry staining, with diaminobenzidine serving as the substrate. The antibodies employed included anti-CD45, CD20, PAX5, CD79a, CD2, CD3, CD5, CD7, CD10, terminal deoxynucleotidyl transferase (TdT), CD34, CD43, CD99, CD1a, CD56, CD117, Bcl-2, Bcl-6, c-MYC, cyclin D1, MPO, Lys, CD38, CD138, ALK, CD30, MUM1, and Ki-67 (from Fuzhou Maixin Biotechnology Development Co., LTD.). In addition, EBER in situ hybridization was conducted following the provided protocol. Genomic DNA was extracted from cell blocks or formalin-fixed paraffin-embedded tissues. Polymerase chain reaction amplification assays were performed to detect immunoglobulin (Ig) monoclonal gene rearrangements and T-cell receptor rearrangements.

Laboratory Tests Related to BM Aspiration Samples

The BM aspiration samples underwent morphological analysis (smears stained with May-Grünwald-Giemsa), immunophenotyping via flow cytometry (FCM), fluorescent in situ hybridization (FISH) assay, real-time fluorescence reverse transcription PCR (RT-PCR), targeted next-generation sequencing (NGS), or whole transcriptome sequencing (WTS) in succession. The diagnosis of ALL was established based on the presence of more than 25% lymphoblast cells in BM aspiration samples. FISH was employed for the identification of *BCR/ABL* gene rearrangements. Total RNA from nucleated cells in BM aspiration samples was extracted and analyzed using the Agilent Technologies Stratagene Mx3000P fluorescence quantitative PCR instrument with the TaqMan probe method. Additionally, the total DNA from nucleated cells in the BM aspiration samples was extracted and subsequently analyzed using Illumina MiSeq second-generation sequencer. RT-PCR was useful for detecting fusion transcripts. Specific fusions and mutations were identified through NGS or WTS.

Results

Out of the 14,156 SE specimens received for cytological evaluation, only 21 cases (0.15%) were diagnosed as LBL/ALL (12 males, 9 females, age range 1–72 years), comprising 16 cases of T-LBL/ALL and 5 cases of B-LBL/ALL. There were 17 (81.0%) pleural fluids, 2 (9.5%) peritoneal fluids, and 2 (9.5%) pericardial fluids. In 19 patients, SE was the initial clinical presentation. Patients with pleural and pericardial effusions frequently exhibited symptoms such as chest tightness and shortness of breath, alongside chest pain, coughing, or expectoration. Conversely, patients with ascites typically experienced abdominal distension and pain. Notably,

Table 1. Clinical manifestations of 21 cases of LBL/ALL in SE

Patient characteristics	Patients, *n* (%)
Gender	
Male	12 (57.1)
Female	9 (42.9)
Age at diagnosis, years (mean)	1–72 (24.0)
Previous history of ALL/LBL	
First occurrence	19 (90.5)
Recurrence	2 (9.5)
Location of effusions	
Pleural	17 (81.0)
Peritoneal	2 (9.5)
Pericardial	2 (9.5)
Diagnosis	
T-LBL/ALL	16 (76.2)
B-LBL/ALL	5 (23.8)
BM involved	
Yes	7 (33.3)
No	3 (14.3)
NA	11 (52.4)
Mediastinal mass	
Yes	10 (47.6)
No	11 (52.4)
Therapeutic schemes	
Allo-HSCT or ASCT	4 (19.0)
Chemotherapy[a]	14 (66.7)
Radiotherapy	1 (4.8)
CAR-T	2 (9.5)
NA	6 (28.6)
Follow-up data [clinical follow-up intervals, months]	
Alive	11 (52.4) [1–91]
Death	7 (33.3) [3–57]
NA	3 (14.3)

LBL/ALL, lymphoblastic lymphoma/leukemia; SE, serous effusion; BM, bone marrow; NA, not available; Allo-HSCT, allogeneic hematopoietic stem cell transplantation; ASCT, autologous peripheral blood stem cell transplantation; CAR-T, chimeric antigen receptor T-cell immunotherapy; hyper-CVAD, cyclophosphamide, vincristine, adriamycin, dexamethasone; VIDCP, vincristine, idarubicin hydrochloride, dexamethasone, cyclophosphamide, pirarubicin; VDLP, vincristine, daunorubicin, L-asparaginase, prednisone; VM, vincristine, methotrexate; VMCLP, vincristine, methotrexate, cyclophosphamide, L-asparaginase, prednisone. [a]The specific chemotherapy regimen involves hyper-CVAD, VIDCP, VDLP, VM, and VMCLP.

patients with T-LBL/ALL often presented with a mediastinal mass (9/16, 56.3%) and BM involvement (6/8, 75.0%). The patients received various treatments, including chemotherapy regimens, allogeneic hematopoietic stem cell transplantation, autologous peripheral blood stem cell transplantation, or chimeric antigen receptor T-cell immunotherapy. Follow-up data were available for 18 out of the 21 patients. The follow-up period ranged from 1 to 91 months, with a median duration of 28 months. During this period, 7 patients succumbed to the progression of LBL/ALL. Comprehensive clinical data are provided in Table 1.

Microscopically, smears of pleural, pericardial, or peritoneal effusions from both B-LBL/ALL and T-LBL/ALL patients revealed numerous atypical lymphoid cells interspersed with variable mesothelial cells and histiocytes. The atypical lymphocytes were observed to be singly dispersed and diffusely distributed, exhibiting a lack of adhesion. The cytomorphological characteristics of B-LBL/ALL and T-LBL/ALL were similar, characterized by a monotonous appearance, small to medium cell size, irregular nuclear membranes, sparse or absent cytoplasm, fine granular chromatin, inconspicuous nucleoli, and a blastoid morphology. Mitoses were prominent, accompanied by nuclear fragmentation and apoptotic bodies. Figure 1a–d (case 9) and c, d (case 11) illustrate the cytomorphological features of T-LBL/ALL, while Figure 1e, f (case 17) and g, h (case 18) depict those of B-LBL/ALL involving pleural effusion. Detailed cytomorphological characteristics are outlined in Table 2.

Online supplementary Table S1 (for all online suppl. material, see https://doi.org/10.1159/000548726) provides an in-depth summary of the immunophenotype and cyto-histological correlation of the 21 cases of LBL/ALL examined. Through cytological immunophenotypic analysis, 16 cases of T-LBL/ALL and 5 cases of B-LBL were identified. ICC staining of cell block sections from SE samples revealed that LBL/ALL expressed markers indicative of immaturity, including TdT (7/17, 41.2%), CD43 (8/8, 100%), CD10 (6/12, 50%), and CD99 (6/6, 100%). T-LBL/ALL always exhibited positivity for T-lymphoid markers, such as CD3 (10/12, 83.3%), CD2 (3/6, 50%), CD5 (2/11, 18.2%), and CD7 (10/10, 100%) (Fig. 2a–i, case 13). In contrast, B-LBL/ALL expressed B-lymphoid markers, including CD20 (3/5, 60%), PAX5 (5/5, 100%), CD79a (4/4, 100%), CD38 (3/3, 100%), and CD138 (0/2, 0%) (Fig. 3a–i, case 17). Among 15 cases of LBL/ALL with Ki-67 immunostaining, 2 cases exhibited a Ki-67 proliferation index below 60%, 8 cases had a Ki-67 proliferation index between 60% and 90%, and 5 cases demonstrated a Ki-67 proliferation index exceeding 90%. Additionally, EBER in situ hybridization results were negative in all cases (12/12). Histopathological specimens were available for comparison in nine cases, and the cyto-histological correlation demonstrated a high

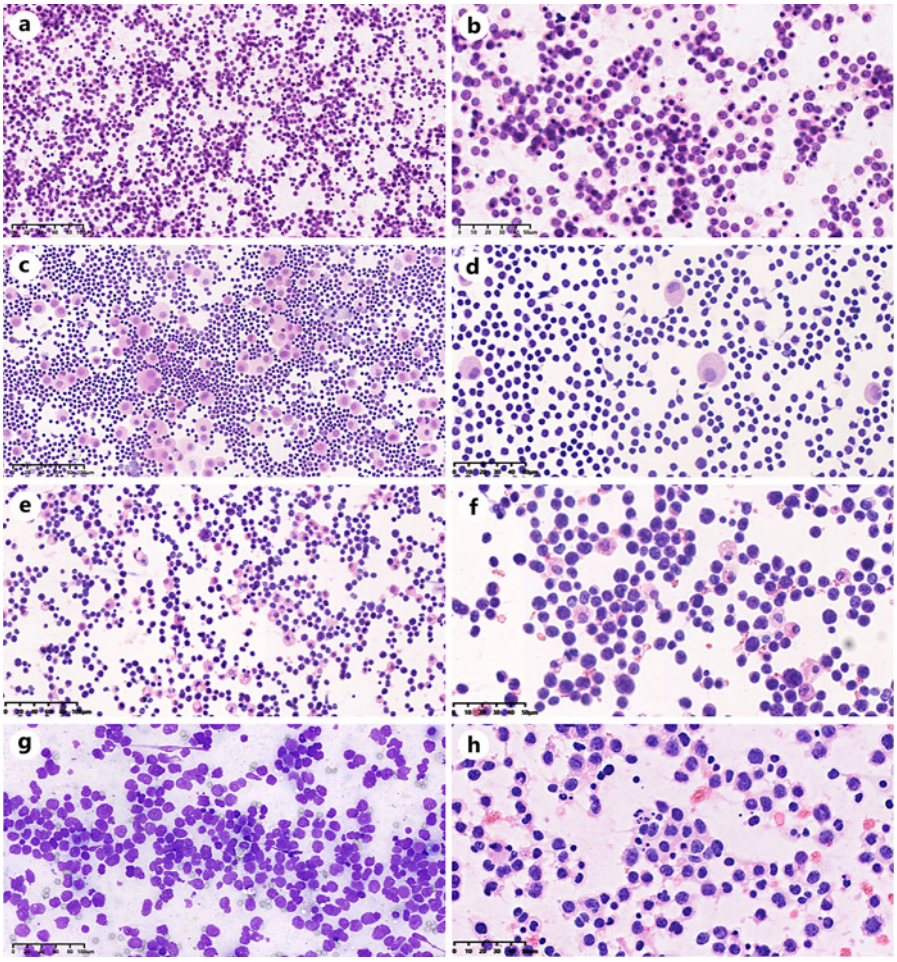

Fig. 1. Cytomorphological features of LBL/ALL in SE. T-LBL/ALL involving pleural effusion in case 9 (**a**, **b**) and case 11 (**c**, **d**). Pleural fluid smears reveal a dispersed population of atypical lymphoid cells (**a**, ×200) characterized by scant cytoplasm, a high N/C ratio, fine chromatin, inconspicuous nucleoli, and apoptotic bodies (**b**, ×400). Atypical lymphocytes are intermixed with numerous reactive mesothelial cells and histiocytes (**c**, ×200). High-magnification microscopy shows monomorphic small atypical lymphocytes (**d**, ×400). B-LBL/ALL involving pleural effusion in case 17 (**e**, **f**) and case 18 (**g**, **h**). The smear showed a substantial presence of dispersed atypical lymphocytes intermingled with reactive histiocytes (**e**, ×200). Small- to medium-sized atypical lymphocytes with irregular nuclear membranes and fine chromatin (**f**, ×400). MGG staining showed amount of medium-sized atypical lymphocytes exhibiting irregular nuclear shapes (**g**, ×400). Lymphocytes with significant atypia are accompanied by nuclear fragmentation and apoptotic bodies (**h**, ×400). MGG, May-Grünwald-Giemsa.

concordance rate (9/9, 100%) in the diagnosis of LBL/ALL. Furthermore, T-cell receptor rearrangement was detected in 1 case of T-LBL/ALL (case 6). Ig monoclonal gene rearrangements were identified in two cases of B-LBL/ALL (case 19 and case 21).

As detailed in online supplementary Table S2, a comprehensive analysis involving cytological morphology of BM smears, FCM, FISH, RT-PCR, NGS, or WTS was conducted on BM aspiration samples from ten cases. Among these, 5 out of 7 cases of T-LBL/ALL and 1 case of B-LBL/ALL exhibited a high proportion of primitive and immature lymphocytes, exceeding 25%. FCM analysis was performed on four cases of T-LBL/ALL (cases 6, 11, 12, and 14) and one case of B-LBL/ALL (case 18), revealing positive expression of TdT (3/5), CD34 (4/5), CD58 (5/5), and CD10 (0/5). FISH analysis was employed to identify *BCR/ABL* gene rearrangements; however, no such rearrangements were detected in any of the cases (T-LBL/ALL: 0/4; B-LBL/AL: 0/2). Figure 4a illustrates the integrated genetic abnormalities or gene expression profiles of ALL as determined by RT-PCR, targeted NGS, or WTS data. A 27-gene panel associated with acute leukemia was analyzed using RT-PCR in 6 cases (cases 4, 6, 7, 11, 12, and 14) of T-LBL/ALL and 2 cases (cases 17 and 18) of B-LBL/ALL. Genetic alterations were not detected in three cases of T-LBL/ALL (cases 4, 6, and 11). *WT1* mutations were identified in cases 12 and 14, and *MLL::ENL* fusion gene was detected in case 7. In two cases of B-LBL/ALL, case 17 exhibited *WT1* mutation, while case 18 demonstrated *WT1* mutation and *PLCALM::MLLT10* fusion gene. Furthermore, NGS of a 41-gene panel was performed on five cases (case 5, 6, 7, 12, and 14) of T-LBL/ALL to identify hotspot genes associated with T-cell lymphoma, whereas WTS was conducted on one case (case 18) of B-LBL/ALL. NGS identified *NOTCH1* and *PAX5* mutations in case 5, *PAX5* mutation in case 6, *IKZF* mutation in case 7, and mutations in *ARID1A*, *BCOR*, and *NOTCH1* in case 12, as well as mutations of *SETD2*, *ARID2*, and *TET2* in case 14. WTS identified mutations in *JAK3*, *NF1*, *CEBPA*,

Table 2. Cytomorphological features of 21 cases of LBL/ALL in SE

Case No.	Diagnosis	Cell quantity	Cytomorphological features of neoplastic lymphocytes						Background cell components, %
			cell morphology	cell size	nucleolus	chromatin	apoptotic debris, %	mitosis[a]	
1	T-LBL/ ALL	Abundant	Uniform, irregular nuclear membrane, nuclear invaginations	Medium	Not obvious	Fine granular	<10	5	Few mesothelial cells (<5)
2	T-LBL/ ALL	Abundant	Uniform, irregular nuclear membrane, nuclear invaginations	Small to medium	Not obvious	Fine granular	<50	3	Few mesothelial cells (<5)
3	T-LBL/ ALL	Abundant	Uniform, irregular nuclear membrane, nuclear invaginations	Small to medium	Not obvious	Fine granular	<10	2	Few mesothelial cells (<5)
4	T-LBL/ ALL	Abundant	Uniform, irregular nuclear membrane, nuclear invaginations	Medium	Not obvious	Fine granular	<50	7	Few mesothelial cells (<5)
5	T-LBL/ ALL	Abundant	Uniform, irregular nuclear membrane, nuclear invaginations	Small to medium	Not obvious	Fine granular	<1	1	Few mesothelial cells (<5)
6	T-LBL/ ALL	Abundant	Uniform, irregular nuclear membrane, nuclear invaginations	Small to medium	Not obvious	Fine granular	<10	2	Few mesothelial cells (<5)
7	T-LBL/ ALL	Abundant	Uniform, irregular nuclear membrane, nuclear invaginations	Medium	Not obvious	Fine granular	<50	4	Few mesothelial cells (<5)
8	T-LBL/ ALL	Abundant	Uniform, irregular nuclear membrane, nuclear invaginations	Small to medium	Not obvious	Fine granular	<10	1	Histiocyte and mesothelial cells (15)
9	T-LBL/ ALL	Abundant	Uniform, irregular nuclear membrane, nuclear invaginations	Medium	Not obvious	Fine granular	<50	1	Few mesothelial cells (<5)
10	T-LBL/ ALL	Abundant	Uniform, irregular nuclear membrane, nuclear invaginations	Medium	Not obvious	Fine granular	<10	2	Few mesothelial cells (<5)
11	T-LBL/ ALL	Abundant	Uniform, irregular nuclear membrane, nuclear invaginations	Small to medium	Not obvious	Fine granular	<1	1	Histiocyte and mesothelial cells (20)

Table 2 (continued)

Case No.	Diagnosis	Cell quantity	Cytomorphological features of neoplastic lymphocytes						Background cell components, %
			cell morphology	cell size	nucleolus	chromatin	apoptotic debris, %	mitosis[a]	
12	T-LBL/ ALL	Abundant	Uniform, irregular nuclear membrane, nuclear invaginations	Small	Not obvious	Fine granular	<1	1	Few mesothelial cells (<5)
13	T-LBL/ ALL	Abundant	Uniform, irregular nuclear membrane, nuclear invaginations	Small	Not obvious	Fine granular	<10	3	Few mesothelial cells (<5)
14	T-LBL/ ALL	Abundant	Uniform, irregular nuclear membrane, nuclear invaginations	Small to medium	Not obvious	Fine granular	<50	2	Few mature small lymphocytes
15	T-LBL/ ALL	Moderate	Uniform, irregular nuclear membrane, nuclear invaginations	Small to medium	Not obvious	Fine granular	<10	2	Few mesothelial cells (<5)
16	T-LBL/ ALL	Scant	Uniform, irregular nuclear membrane, nuclear invaginations	Medium	Not obvious	Fine granular	<10	1	Abundant blood cells
17	B-LBL/ ALL	Abundant	Polymorphism, irregular nuclear membrane, nuclear invaginations	Medium	Not obvious	Fine granular	<50	10	Histiocyte (20)
18	B-LBL/ ALL	Abundant	Polymorphism, irregular nuclear membrane, nuclear invaginations	Medium	Not obvious	Fine granular	<50	5	Few mesothelial cells (<5)
19	B-LBL/ ALL	Abundant	Polymorphism, irregular nuclear membrane, nuclear invaginations	Medium	Not obvious	Coarse granular	≥50	NA	Few mesothelial cells (<5)
20	B-LBL/ ALL	Abundant	Polymorphism, irregular nuclear membrane, nuclear invaginations	Medium	Not obvious	Coarse granular	≥50	NA	Few mesothelial cells (<5)
21	B-LBL/ ALL	Moderate	Uniform, irregular nuclear membrane, nuclear invaginations	Small to medium	Not obvious	Fine granular	<10	2	Abundant blood cells

[a]Evaluation of mitosis in cell block sections, per 40 high-power fields (HPFs); NA, not available (nuclear fragmentation and apoptotic bodies were too abundant to permit accurate mitotic assessment.); SE, serous effusion.

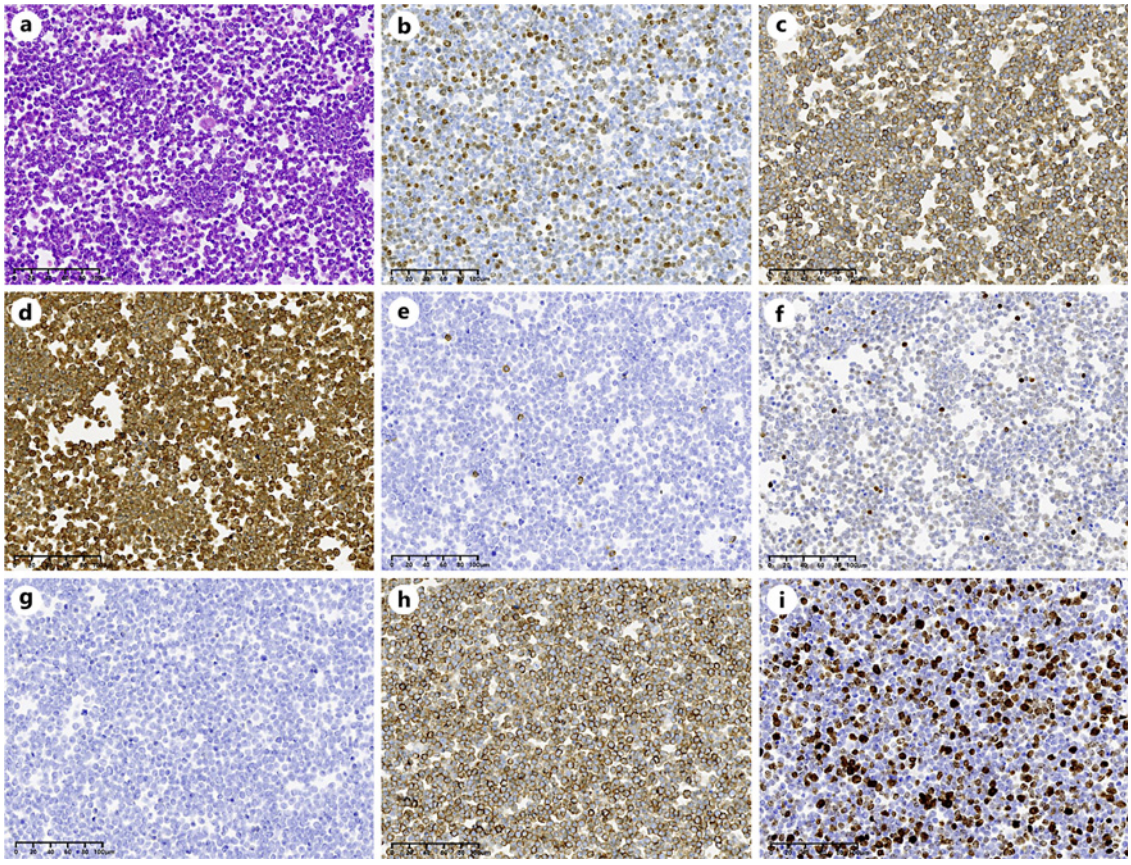

Fig. 2. a Cytological examination of a cell block derived from pleural centrifuge sediment (case 13) indicating T-LBL/ALL. H&E staining revealed amount tumor cells with uniformly medium-sized nuclei and irregular nuclear membranes. ICC staining demonstrated positive expression for TdT (**b**, patchy, ×200), CD43 (**c**, diffusely, ×200), CD99 (**d**, diffusely, ×200), negative expression for CD20 (**e**, ×200), PAX5 (**f**, ×200), and CD3 (**g**, ×200), diffusely positive expression for CD7 (**h**, ×200), and a high Ki-67 proliferation index (**i**, ×200).

GSKIP, and *SIN3A* in case 18. Based on molecular typing, this case was classified as the *KMT2A*-like B-LBL/ALL high-risk subtype, due to its gene expression profile similarity to *KMT2A* rearrangements and associated poor prognosis (Fig. 4b).

Discussion

LBL/ALL is a highly aggressive neoplasm derived from lymphoblasts of either B-cell or T-cell origin (T-LBL/ALL; B-LBL/ALL). LBL is traditionally distinguished from ALL by the presence of less than 20–25% BM infiltrating blast cells [5]. T-LBL/ALL is the most common hematopoietic malignancy involving the effusion fluid [3, 6]. In this study, 21 cases of SE LBL/ALL were evaluated, comprising 16 cases of T-LBL/ALL and 5 cases of B-LBL/ALL. Among the cases studied, 19 cases (90.5%) initially presented with SE as their first symptom. The clinical manifestations of B-LBL/ALL are nonspecific and include symptoms such as fever, loss of appetite, weight loss, and localized lymphadenopathy. In contrast, T-LBL/ALL typically presents with a mediastinal mass (9/16, 56.3%), accompanied by symptoms such as dyspnea, chest pain, and abdominal distension. The first step in the cytological diagnosis of ALL/LBL involves evaluating the cell quantity, composition, and distribution pattern within SE. Cytological smears of ALL/LBL in SE typically reveal an abundance of atypical lymphoid cells that are scattered without adhesion and exhibit few or no mesothelial cells, particularly in peritoneal cavity effusions. The cytomorphological characteristics of ALL/LBL in smears are monomorphic, with cells being small to medium in size, possessing scant cytoplasm, irregular nuclei, fine granular chromatin, and inconspicuous

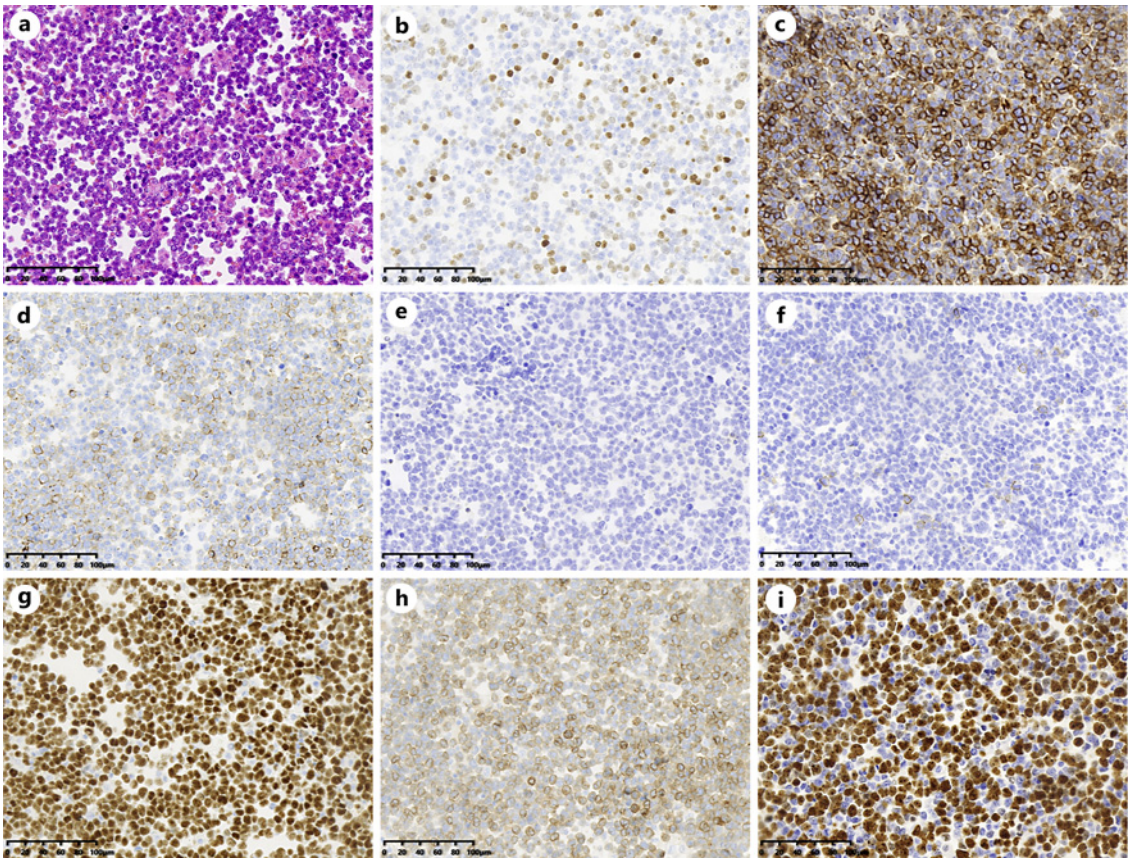

Fig. 3. a Cytological examination of a cell block derived from pleural centrifuge sediment (case 17) indicating B-LBL/ALL. H&E staining showed atypical lymphoid cells characterized by irregular nuclear membranes. ICC staining revealed positive expression for TdT (**b**, patchy, ×200), CD10 (**c**, diffusely, ×200), CD43 (**d**, diffusely, ×200), negative expression for CD3 (**e**, ×200) and CD20 (**f**, ×200), diffusely positive expression for PAX5 (**g**, ×200) and CD79a (**h**, ×200), and a high Ki-67 proliferation index (**i**, ×200).

nucleoli. The presence of mitoses, nuclear fragmentation, and apoptotic bodies strongly suggests a diagnosis of lymphoma. Notably, compared to B-LBL/ALL, T-LBL/ALL more frequently mimics reactive lymphocytosis due to the presence of monomorphic small- and medium-sized lymphocytes. In light of significant morphological atypia, the diagnosis of malignant lymphoma was more strongly indicated in the case of B-LBL/ALL. Li et al. [2] have proposed that the presence of mitotic figures, hand mirror-shaped blasts, nuclear fragmentation, and apoptotic bodies may serve as diagnostic indicators for LBL/ALL.

Typically, B-LBL/ALL is always positive for at least two B-cell markers among CD19, CD79a, and CD22. Additionally, markers such as CD10, CD24, PAX5, and TdT are commonly expressed, whereas the expression of CD20 and the stem cell antigen CD34 is variable, and CD45 may be absent. According to the beta version of the World Health Organization Classification of Tumors of Hematopoietic and Lymphoid Tissue, B-LBL/ALL subtypes are delineated based on specific genetic abnormalities or gene expression profiles, which can be identified through karyotyping, FISH, whole transcriptome or targeted RNA or DNA sequencing, or alternative assays such as low-density arrays [7]. Cases of B-LBL/ALL lacking defining genetic abnormalities are categorized as not otherwise specified, while those without a comprehensive workup should be classified as B-LBL/ALL, not further classified [8]. T-LBL/ALL are neoplasms comprising lymphoblasts committed to the T-cell lineage. The defining features of T-LBL/ALL include the expression of CD3 (surface and/or cytoplasmic) along with markers indicative of immaturity, such as TdT, CD1a, CD34, CD117, and/or CD99. The diagnosis can be further supported by identifying clonal rearrangements of TRB and/or TRG, which are

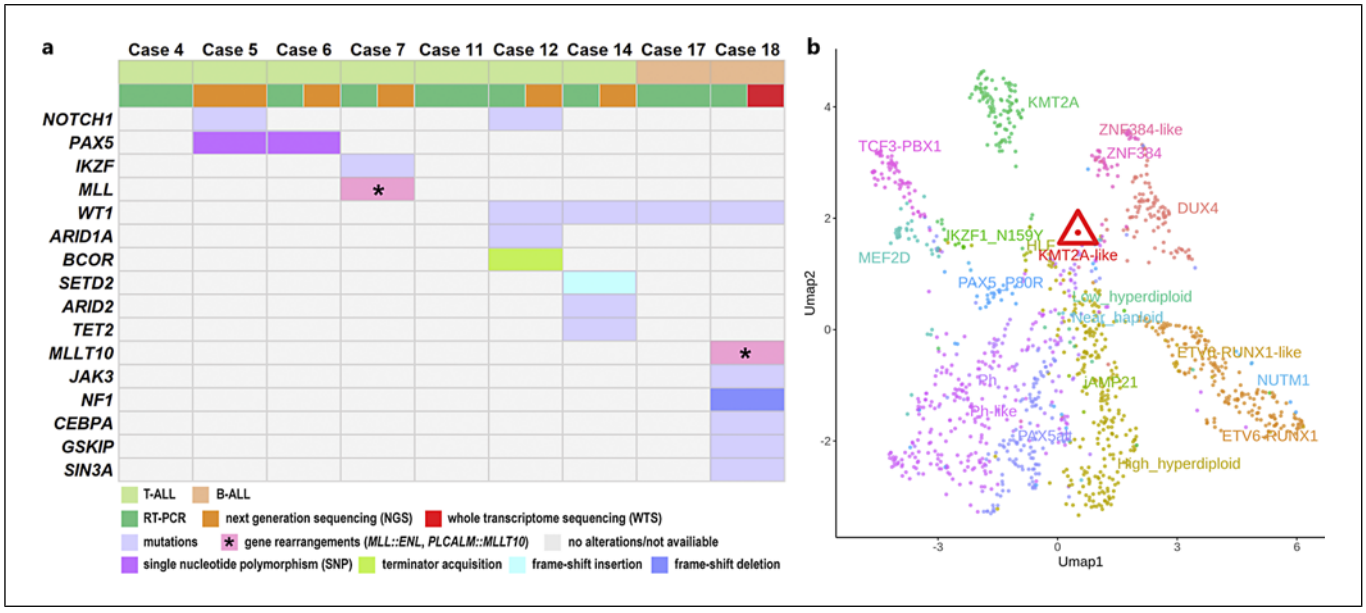

Fig. 4. a Integrated genetic abnormality landscape of 9 LBL/ALL cases based on the BM aspiration samples. Genetic abnormalities or gene expression profiles of LBL/ALL were analyzed using an integrated approach, including real-time fluorescence RT-PCR, targeted NGS or WTS. The red triangular area in the two-dimensional plot map highlights a subgroup of *KMT2A*-like B-LBL/ALL, as determined by WTS data in case 18. **b** Groups recognized as "like" cluster together with samples carrying the associated translocations, attributed to a gene expression profile similar to that of *KMT2A* rearrangement B-ALL.

present in over 90% of cases. It should be noted that about 20% of T-LBL/ALL cases also have Ig gene clonal rearrangement [9].

The assessment of contaminating lymphoid cells in SE can facilitate a rapid diagnosis, while the immunophenotypic analysis of cell blocks allows for precise determination of cell lineage and subtyping. In our study, B-LBL/ALL demonstrated positivity for CD20 (3/5, 60%), CD79a (4/4, 100%), and PAX5 (5/5, 100%), whereas T-LBL/ALL showed positivity for CD3 (10/12, 83.3%), CD2 (3/6, 50%), CD5 (2/11, 18.2%), and CD7 (10/10, 100%). Notably, CD79a positivity was observed in 1 case of T-LBL/ALL, and CD7 positivity was detected in B-LBL/ALL (2/4, 50%). Heterogeneous expression of CD79a, which is often ambiguous, is observed in at least 10% of T-LBL/ALL cases, and CD19 expression is occasionally noted; thus, neither marker alone defines B lineage differentiation. Of note, we found that the Ki-67 proliferation index was lower in cell block sections compared to histological samples, with only 5 cases (35.7%) exhibiting a Ki-67 proliferation index exceeding 90%. Among the five paired SE and histological specimens, two cases demonstrated discordant TdT results, with all histological specimens testing positive, whereas all SE specimens were negative for TdT. The positivity rate of TdT in SE was merely 41.2% (7/17). These false negatives could be attributed to factors such as cell degeneration, dehydration, alcohol fixation, or other technical issues, which may lead to protein leakage [10]. Consequently, the potential for false-negative staining in cytological specimens must be highlighted to prevent missed diagnoses and misdiagnoses of LBL/ALL.

Various subtypes of B-LBL/ALL, including those with high hyperdiploidy, *BCR::ABL1* fusion, *BCR::ABL1*-like features, *KMT2A* rearrangement, *ETV6::RUNX1* fusion, *ETV6::RUNX1*-like features, *TCF3::PBX1* fusion, *TCF3::HLF* fusion, *IGH::IL3* fusion, and other defined genetic alterations can be detected by karyotype analysis, FISH, and PCR. A comprehensive analysis of genetic alterations and gene expression profiles led to dividing T-ALL/LBL into eight new interim entity subgroups: *TAL1*, *TAL2*, *TLX1*, *TLX3*, *HOXA*, *LMO1/LMO2*, *LMO2/LYL1*, and *NKX2-1* [11]. Other genetic abnormalities, including del(9p)/*CDKN2A* and the deletion or inactivation of *BCL11B*, *LEF1*, *WT1*, as well as mutations in *NF1*, *NOTCH1*, and/or *FBXW7*, have been linked to better clinical outcomes. Conversely, loss of heterozygosity at 6q and mutations in *PTEN* and *KMT2D* are linked to adverse prognosis

[12]. Genetic sequencing should be conducted as much as possible for risk stratification and determination of therapeutic targets [13]. In our study, a significant proportion of primitive and immature lymphocytes, exceeding 25%, was observed in T-LBL/ALL (5/7) and one case of B-LBL/ALL. None of the cases exhibited *BCR/ABL* gene rearrangements. Moreover, fusion genes *MLL::ENL* and *PLCALM::MLLT10*, along with mutations in *WT1*, *NOTCH1*, *PAX5*, *IKZF*, *ARID1A*, *BCOR*, *SETD2*, *ARID2*, *TET2, JAK3*, *NF1*, and *CEBPA*, were identified in LBL/ALL through RT-PCR, NGS, or WTS. Notably, one case was classified as a *KMT2A*-like B-LBL/ALL high-risk subtype, attributed to its gene expression profile resembling that of *KMT2A* rearrangement and associated poor prognosis. In a cohort of pediatric T-ALL, whole exome sequencing data revealed frequent mutations in *NOTCH1* (35%), followed by *WT1* (23%), *FBXW7* (12%), *KRAS* (12%), *PHF6* (12%), and *JAK3* (12%) [14]. The *MLLT10* gene has been reported to undergo translocation with partners such as *PLCALM*, *DDX3X*, and *KMT2A* in ALL/LBL and acute myeloblastic leukemia. Regardless of the fusion partner, patients harboring *MLLT10* fusions exhibit very high-risk features [15, 16]. It has been documented that partner genes such as *MLL::AF4*, *MLL::PTD*, and *MLL::ENL* are more common in MLL rearrangement-positive ALL. Patients with *MLL* rearrangement-positive ALL demonstrate moderate remission rates but are susceptible to relapse, resulting in low overall survival [17].

Lymphocyte-rich effusions often present diagnostic challenges in clinical cytology. In our practice, T-LBL/ALL can be morphologically indistinguishable from reactive hyperplasia of activated lymphocytes caused by conditions such as tuberculosis and pulmonary embolism due to its monomorphic small and medium-sized lymphocytes. Bhaker et al. [18] proposed that nuclear cleavage and nuclear notching are the most prominent features of LBL/ALL, which can be utilized to differentiate between reactive and neoplastic lesions. It is essential to differentiate LBL/ALL from other hematopoietic malignancies involving SE, such as Burkitt lymphoma, diffuse large B-cell lymphoma, anaplastic large cell lymphoma, and extranodal NK/T-cell lymphoma, nasal type. Specific cytomorphological features, along with immunophenotyping and molecular genetic alterations, can be identified through cytological analysis. Involvement of SE in LBL/ALL can occur at any stage of the disease, including initial diagnosis, advanced stages, relapse, and even post-stem cell transplantation [19]. Although the impact of SE involvement on the treatment and prognosis of LBL/ALL remains uncertain, aggressive treatment is recommended. In our practice, SE cytology has proven to be a reliable and effective method for diagnosing of LBL/ALL. The integration of clinical manifestations, cytomorphological characteristics, immunophenotyping, and gene expression profiles significantly aids in diagnosing LBL/ALL in SE, particularly when SE involvement presents as the initial symptom. Additionally, cytopathology plays a crucial role in monitoring tumor recurrence and assessing prognosis in recurrent cases.

Statement of Ethics

The study was approved by the Ethics Committee of Xijing Hospital and was exempted from requiring written informed consent (Approval No. KY20253201-1). The study was based on archive samples and was conducted according to the 1964 Helsinki Declaration, its later amendments, or comparable ethical standers.

Conflict of Interest Statement

The authors declare no conflicts of interest.

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Author Contributions

W.C.: clinical information collection, review of cases and ancillary study results, writing – original draft. X.D. and J.L.: study conduction and progression, data analysis, and curation. P.H.: molecular biology experiment and financial support. S.M.: study assistance. C.Y.: study conduction and progression and writing – original draft. H.X.: study conceptualization, study design, study supervision, writing – review and editing, and project administration. All authors reviewed and approved the manuscript for submission.

Data Availability Statement

All data generated or analyzed during this study are included in this article and its online supplementary material files. Further inquiries can be directed to the corresponding author (H.X.).

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