

Unique pathologic features and gene expression signatures distinguish blastoid high-grade B-cell lymphoma from B-acute lymphoblastic leukemia/lymphoma

High-grade B-cell lymphoma (HGBL) encompasses two categories based on genetic features: HGBL with *MYC*, *BCL2*, with/without *BCL6* rearrangements (HGBL-DH), and HGBL not otherwise specified (HGBL-NOS).¹ A small subset of cases of both categories show a blastoid morphology (designated as blastoid HGBL here), which is rare and not well characterized.² The immature morphology often overlaps with other blastoid B-cell neoplasms, like B-acute lymphoblastic leukemia/lymphoma (B-ALL) or blastoid mantle cell lymphoma. The distinction between blastoid HGBL and CD34 negative B-ALL can be very challenging, especially when blastoid HGBL expresses immature markers such as TdT and/or lacks surface light chains.³⁻⁶ On the other hand, B-ALL cases may show unusual fea-

tures such as lack of CD34 and/or TdT, bright CD38 and decreased or absence of CD10.^{7,8} We previously studied 31 cases of blastoid HGBL initially presented in bone marrow and developed a 6-point scoring system and a diagnostic algorithm to aid in the differential diagnosis of blastoid B-cell neoplasms.⁴ However, this 6-point scoring system was largely focused on flow cytometric parameters, which may not be available in every practice setting or every case. In addition, the underlying gene expression signature of blastoid HGBL *versus* B-ALL remains unexplored.

Here we collected 64 cases of blastoid HGBL with complete immunohistochemistry (IHC) studies. These cases involved nodal and/or extranodal sites and included 17 cases from a previous study.⁴ This cohort included 39 men

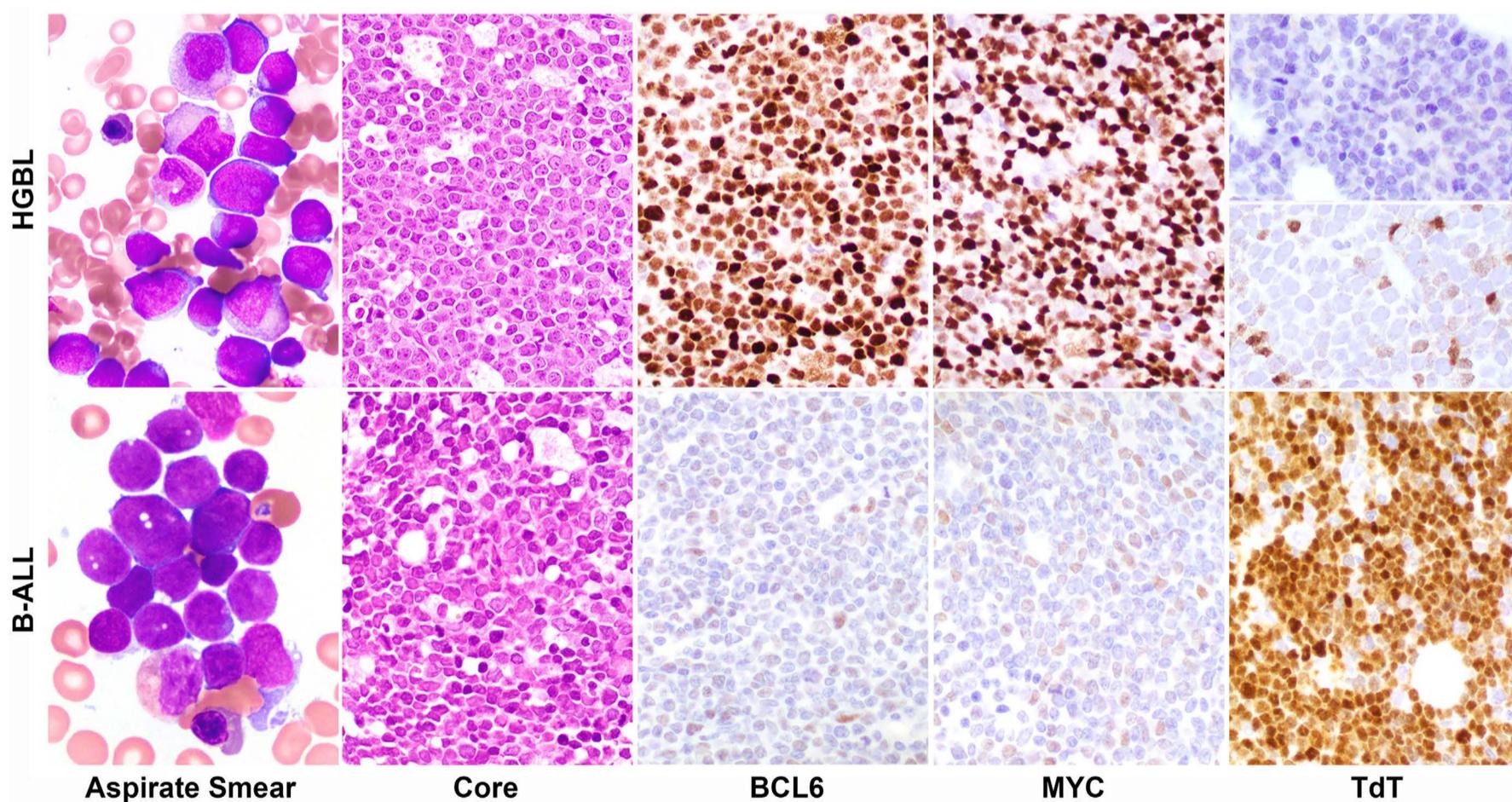


Figure 1. Comparison of morphologic and immunohistochemical features between blastoid high-grade B-cell lymphoma and B-acute lymphoblastic leukemia/lymphoma. Top row of panels: a representative case of blastoid high-grade B-cell lymphoma (HGBL) with marrow entirely replaced by blastoid lymphoma cells with a starry sky pattern and finely dispersed blastoid chromatin. The lymphoma cells were strongly and diffusely positive for BCL6 and MYC but negative for TdT (upper). Occasional cases may be variably positive for TdT (lower) in a small subset of lymphoma cells. Lower row of panels: in contrast, the lymphoblasts of a representative B-acute lymphoblastic leukemia/lymphoma (B-ALL) case showed similar blastoid morphology but had an opposite immunophenotype: negative for BCL6 and MYC, and positive for TdT. (Magnifications: column 1, Wright Giemsa stains, 100X; column 2, hematoxylin and eosin stains, 50X; the rest: immunohistochemical stains, 50X).

and 25 women with a median age of 60 years (range, 23–87 years), with 40% (25/64) being HGBL-NOS and 60% HGBL-DH. Thirty percent of the patients had a history of follicular lymphoma or other type of non-Hodgkin lymphoma. Eighty percent of patients showed extra-nodal site involvement at presentation, with bone marrow involvement in 75% (40/53) biopsied. A B-ALL comparison group (n=37) which were disproportionally enriched with CD34-negative cases (51%) were included to serve the purpose of the study. The B-ALL group included 21 men and 16 women with a younger median age of 44 years (range, 18–83 years). All B-ALL patients presented with bone marrow disease, although extramedullary involvement was seen in 50% of patients. None of the B-ALL patients had a history of B-cell lymphoma.

By comparison with B-ALL, blastoid HGBL demonstrated distinctive immunophenotypic and molecular cytogenetic features. By immunohistochemistry, blastoid HGBL showed more frequent MYC and BCL6 expression than B-ALL cases (86% vs. 27% and 67% vs. 16%, respectively), but less frequent TdT expression defined by $\geq 5\%$ positivity (12/64, 19% vs. 18/21, 86%), with median of 20% (range, 5–60%) versus 70% (range, 10–100%) (all $P < 0.0001$). TdT expression was dim partial in blastoid HGBL in contrast to strong diffuse in B-ALL (Figure 1). Fluorescence *in situ* hybridization (FISH) analysis detected MYC-R in 75% cases of blastoid HGBL while none of the B-ALL cases had MYC-R. Blastoid HGBL more frequently had a complex karyotype than B-ALL cases (93% vs. 45%, $P = 0.0001$). However, B-ALL-related translocations were exclusively seen in B-ALL cases, including *BCR::ABL1* in seven CD34-positive B-

ALL cases and *MLL-R* and translocations involving *TCF3 (E2A)* (n=10 each) in CD34-negative B-ALL cases. Targeted next-generation sequencing demonstrated *TP53* was the most frequently mutated gene in blastoid HGBL, relatively more frequent than in B-ALL (35% vs. 15%, $P = 0.17$). Mutations in *NRAS* and *KRAS* were seen exclusively in B-ALL patients (24% and 18%, respectively) ($P < 0.05$).

Detailed flow cytometric study may not be available for every blastoid B-cell neoplasm or in every practice setting. Therefore, we developed a 3-point scoring system using three immunohistochemical markers, MYC, BCL6 and TdT. Expression of MYC, BCL6 and lack of TdT expression were assigned a score of 1 each. In a blastoid B-cell neoplasm, a total score of ≥ 2 would support a diagnosis of blastoid HGBL and < 2 favor a diagnosis of B-ALL (Figure 2). Similar to the previously reported 6-point scoring system, MYC expression serves as a surrogate for *MYC-R* and presence of either one was sufficient for 1 point. Since the concordance rate of TdT between flow cytometry and IHC was 95% in this cohort, TdT by either method can be used for this scoring system. Of the 64 blastoid HGBL cases, 59 (92%) cases had a score of ≥ 2 and five (8%) had a score of 1. The performance is similar between those with (44/48, 92%) and without *MYC-R* (15/16, 94%). In the 37 B-ALL cases, 34 had a score of < 2 while three had a score of 2.

In order to validate the above-mentioned scoring system and also to understand the underlying gene expression signatures associated with blastoid HGBL, RNA-sequencing-based whole transcriptome profiling was performed on archived formalin-fixed paraffin-embedded tissue sec-

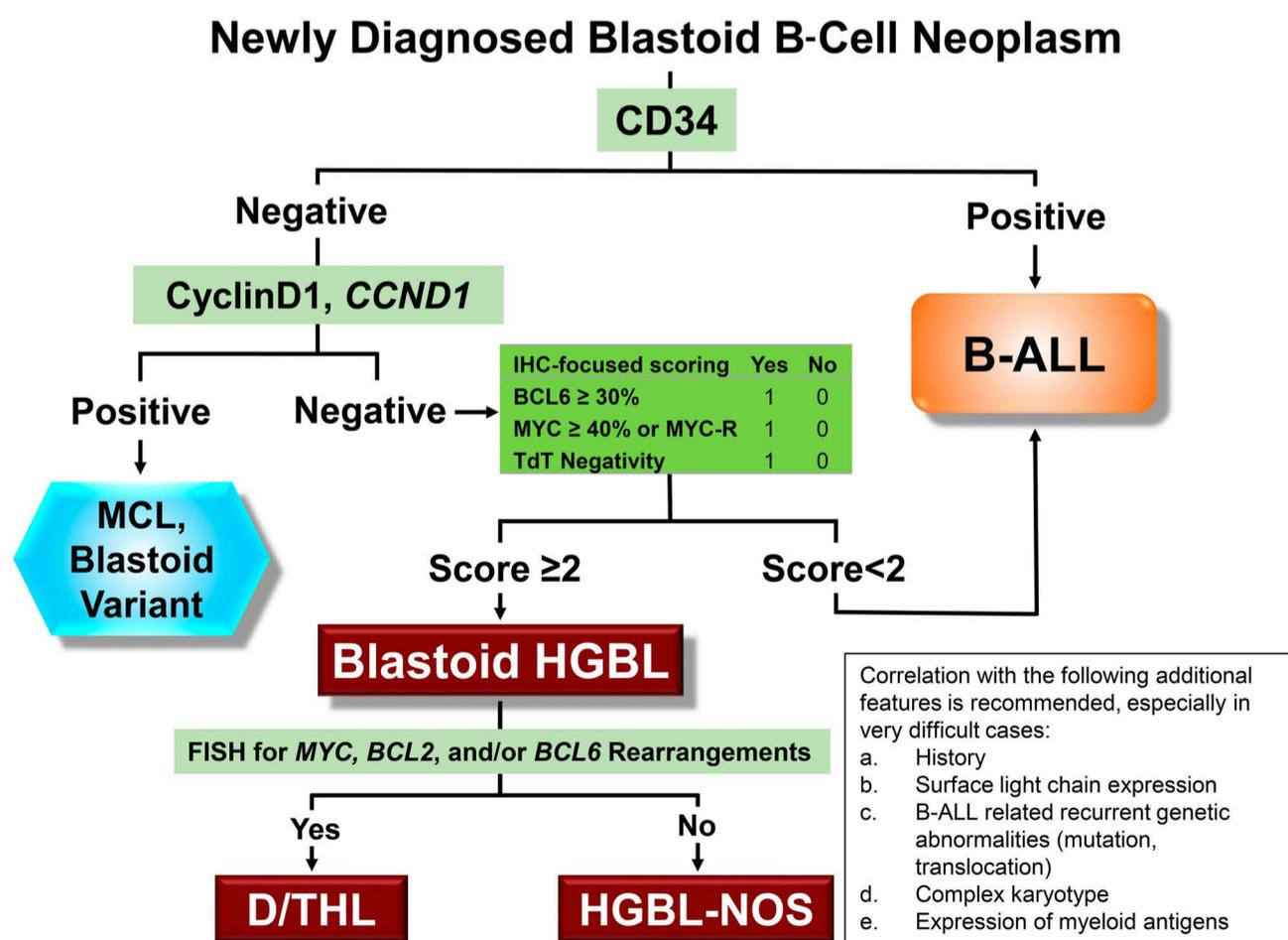


Figure 2. Recommended algorithm for the differential diagnosis of blastoid B-cell neoplasms. HGBL: high-grade B-cell lymphoma; B-ALL: B-acute lymphoblastic leukemia/lymphoma; MCL: mantle cell lymphoma; D/THL: HGBL with MYC, BCL2, and/or BCL6 rearrangements, or double/triple hit lymphoma. FISH: fluorescence *in situ* hybridization

tions on a subset of cases utilizing extraction-free HTG EdgeSeq technology (HTG Molecular Diagnostics, Inc, Tucson, AZ). Differential expression analysis (DESeq2 package) revealed 4,678 significantly differentially expressed genes between blastoid HGBL (n=52) and B-ALL (n=26) cases, of which, 2,199 (47%) and 2,479 (53%) genes were upregulated and downregulated, respectively (Figure 3A), and clustered in distinct regions by principal component analysis (Figure 3B). In blastoid HGBL, there was enrichment of genes in association with multiple signaling pathways when compared with B-ALL (Figure 3C), supporting distinct gene expression signatures in the two disease entities. These pathways and differentially expressed molecules are mostly responsible for the differential regulation of cell cycle progression in association with cell growth, differentiation, homeostasis and immune response, with predicted activation of the *p53* and *PI3K/AKT* signaling pathways and inhibition of the *ERK/MAPK* signaling pathway. Blastoid HGBL cases showed upregulation and enrichment in genes relevant for germinal center B-

cell maturation and differentiation, such as *MYC* (1.85-fold), *BCL6* (3.32-fold), *MS4A1* (CD20) (2.89-fold), *PTPRC* (*CD45*) (2.20-fold) ($P < 0.05$ for all, vs. B-ALL), consistent with their relatively increased protein expression levels. In contrast, B-ALL cases maintained high abundance of genes responsible for self-renewal of immature B cells at a precursor level. Six cases with unexpected scores by the 3-point scoring systems had HTG gene expression study performed, and their diagnosis were confirmed by the gene expression signatures for the three blastoid HGBL cases with a score of 1 and three B-ALL cases with a score of 2 (Figure 3D, arrows).

As such, this 3-point IHC-focused scoring system demonstrated a comparable high-performance as the previous 6-point flow-focused scoring system, and showed a sensitivity, specificity, positive and negative predictive values of 92%, 92%, 95% and 87% respectively for establishing a diagnosis of blastoid HGBL versus B-ALL. We therefore propose a diagnostic algorithm for the differential diagnosis of blastoid B-cell neoplasms by incorporating this

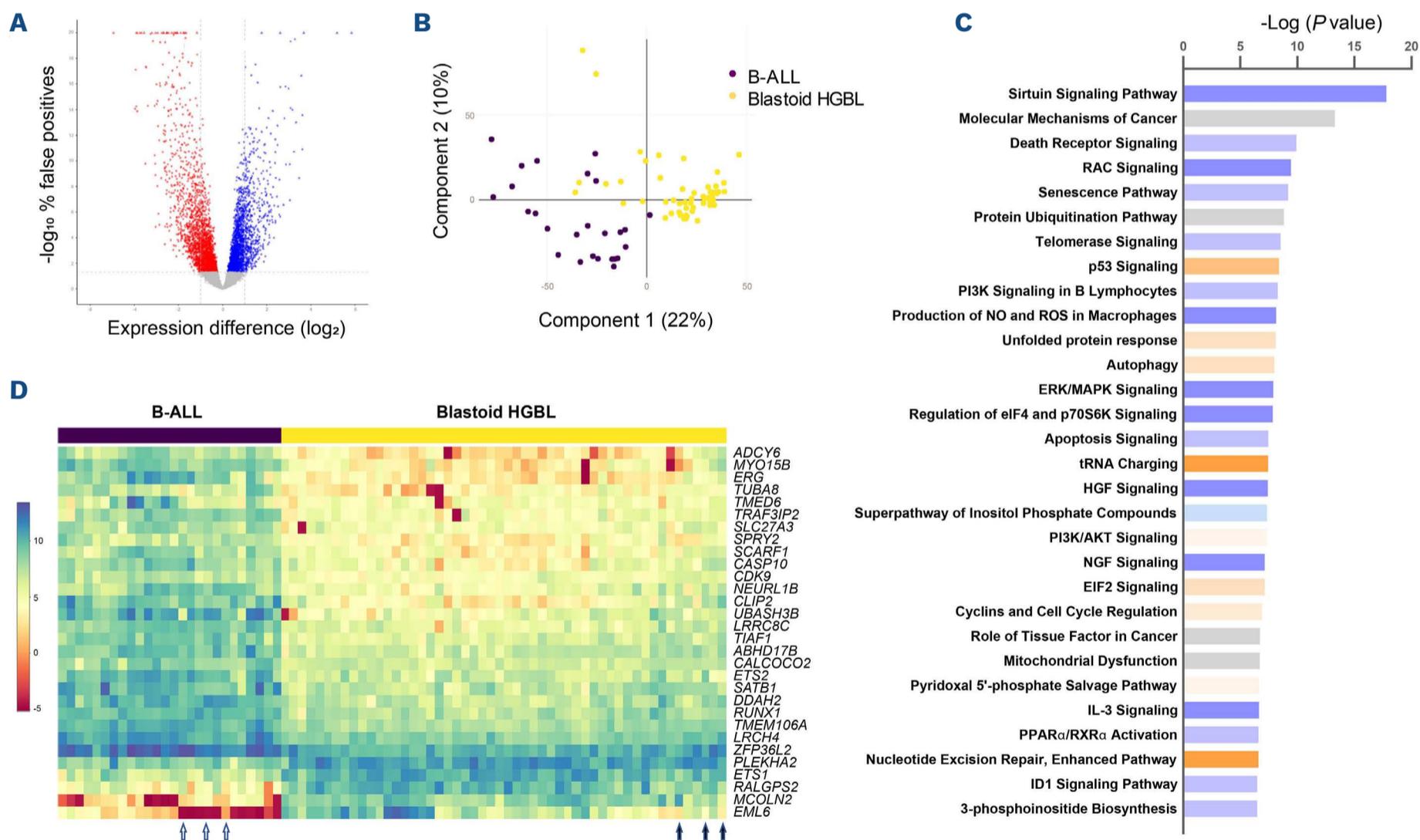


Figure 3. Transcriptome profiling in blastoid high-grade B-cell lymphoma in comparison with B-acute lymphoblastic leukemia/lymphoma. (A) Volcano plot of 4,678 significantly differentially expressed (DE) genes with upregulated genes in blue and downregulated genes in red. (B) Principal component analysis revealed distinct clusters formed by differentially expressed genes in blastoid high-grade B-cell lymphoma (HGBL) vs. B-acute lymphoblastic leukemia/lymphoma (B-ALL) cases. (C) Top 30 enriched signaling pathways of DE genes. The bar color corresponds to their respective Z-scores by Ingenuity Pathway Analysis (Invitrogen), with orange colors indicating positive scores for predicted activation, blue colors for negative scores with predicted inhibition, and grey colors for no activity pattern available. NO: nitric oxide; ROS: reactive oxygen species. (D) Heatmap of the top 30 DE genes. Solid arrow - blastoid HGBL cases with 3-point score of 1; open arrow - B-ALL cases with 3-point score of 2.

3-point IHC-focused scoring system (Figure 2). For rare extremely challenging cases with equivocal scores, correlation with all available clinicopathologic features is helpful.

This study aimed to improve our understanding of blastoid HGBL with a focus on the differential diagnosis of blastoid HGBL, either HGBL-DH or NOS, *versus* B-ALL. In general, lymphoma is a nodal-based disease that often occurs in adults, whereas B-ALL is a bone marrow-based disease and frequently affects children. However, blastoid HGBL often presents in or involves bone marrow, whereas up to 25% of B-ALL cases occur in adults, with extramedullary presentations in a subset of cases that often cause diagnostic challenges.⁹⁻¹¹ In this study, bone marrow presentation was present in one third of blastoid HGBL cases, mimicking the clinical manifestations of B-ALL patients. Although most B-ALL cases are CD34-positive and easy to recognize, up to a third of B-ALL may lack CD34 expression, making the differential diagnosis with blastoid HGBL difficult.^{7,8} Distinguishing blastoid HGBL from B-ALL is important, since their treatment and prognosis are substantially different from each other. The diagnostic challenge not only lies in the similar blastoid morphology, but also arises in the overlapping immunophenotypic features between blastoid HGBL and B-ALL. A subset of blastoid HGBL cases may show an immature immunophenotype, such as TdT expression, decreased CD45, and absence of surface light chains, as shown in this study. On the other hand, B-ALL cases may show unusual features such as lack of CD34, CD10, TdT and bright CD38, particularly in B-ALL patients harboring translocations involving *MLL* and *TCF3*. Therefore, no single marker can distinguish blastoid HGBL from B-ALL with the exception of CD34. A scoring system simultaneously evaluating multiple markers is helpful for guiding the work-up and establishing a correct diagnosis. The 3-point scoring system is a simplified scoring system by IHC, which usually can be completed within 1-2 days of the biopsy and is widely available in general practice settings. The IHC-focused 3-point scoring system has an accuracy of 92% and is practically relevant and useful. The score systems were further validated by RNA-sequencing-based transcriptome profiling, which revealed more than 4,000 differentially expressed genes underlying the unique molecular signature of blastoid HGBL as compared with B-ALL. The 5th edition of the World Health Organization classification of hematolymphoid neoplasms¹² does not recommend to differentiate morphologic subtypes anymore due to the poor interpersonal reproducibility¹³ while the ICC classification keeps the morphology subtypes.¹⁴ So, this score system may also be useful for all HGBL, especially in bone marrow presentation. In addition, a double/triple hit is extremely rare in B-ALL,¹⁵ therefore the presence of such would favor a diagnosis of blastoid HGBL.

In summary, blastoid HGBL cases have distinctive immunophenotypic, molecular and cytogenetic characteristics as compared with B-ALL. We proposed an IHC-focused 3-point scoring system for the differential diagnosis of blastoid HGBL *versus* B-ALL, which were further validated by RNA-sequencing-based gene expression profiling. We also reported the transcriptome profiling of blastoid HGBL cases as compared with B-ALL cases, revealing unique molecular signatures underlying the distinct biological processes involved in blastoid HGBL.

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Disclosures

No conflicts of interest to disclose.

Contributions

LQ collected data, analyzed the data, and wrote the manuscript. JX, PL, ENC, GT, SAW, MK, WW, JDK, SK, CCY, JLJ and FV contributed data and edited the manuscript. LJM contributed data and wrote the manuscript. SL designed the study, collected and analyzed the data, supervised the study and wrote the manuscript. All authors reviewed and approved the manuscript.

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Data-sharing statement

Data are available for sharing upon request to the corresponding author.

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