

The 66th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

612.ACUTE LYMPHOBLASTIC LEUKEMIAS: CLINICAL AND EPIDEMIOLOGICAL

Single Center Experience Combining Multiple Tools for Genetic Diagnosis of B-Cell Acute Lymphoblastic Leukemia

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Introduction: Pediatric B-cell acute lymphoblastic leukemia (B-ALL) is characterized by distinct genetic profiles essential for prognosis and treatment. About 70% of B-ALL cases can be classified into well-known genetic subtypes, while 30% remain under the "B-other" category. Accurate genetic diagnosis requires multiple techniques; however, their accessibility varies significantly among laboratories.

This study investigates an integrated approach to classify B-ALL into genetic subtypes, elucidate their frequency and clinical significance, and identify the most effective diagnostic methods.

Methods: This study included 175 pediatric patients with B-ALL treated at Vall d'Hebron University Hospital from 2014 to 2023. Diagnostic techniques included karyotyping, FISH, RT-PCR, MLPA, targeted NGS (t-NGS), and Optical Genome Mapping (OGM).

Results: Utilizing the combined diagnostic approach, we classified 83% (n=145) of B-ALL patients into 17 genetic subtypes. Karyotyping and FISH identified abnormalities in 73% (n=128) of cases, with t-NGS and OGM contributing an additional 10% (n=17) to the classification rate. Among the 145 patients with a defined genetic diagnosis, 71% harbored classical abnormalities: high hyperdiploidy (HeH) (n=46), *ETV6::RUNX1* (n=36), *BCR::ABL1*-like (n=17), *KMT2A* rearrangements (n=8), *TCF3::PBX1* (n=6), *BCR::ABL1* (n=4), *iAMP21* (n=4), and hypodiploidy (n=3). The remaining 29% (n=51) were classified as B-other, with specific genetic subtypes identified 21 cases, including *ZNF384* rearrangements (n=6), *PAX5* alterations (*PAX5alt*) (n=4), *PAX5 P80R* (n=3), *DUX4* rearrangements (n=3), *IGH* rearrangements (n=3), *NUTM1* rearrangements (n=1), and *IKZF1 N159Y* (n=1).

Copy number alterations (CNA) were detected in 60% of patients (median: 1 deletion), with the most frequent deletions observed in *ETV6*, *CDKN2A/B*, *PAX5*, and *IKZF1* genes. CNA profiles were classified as good-risk (CNA-GR; n=69) or poor-risk (CNA-PR; n=51) according to Hamadeh et al.(1). *ETV6::RUNX1* cases showed frequent deletions of *ETV6* and *PAR1* (p<0.01). *BCR::ABL1*-like were associated with a CNA-PR, with significant deletions in *IKZF1* and *PAR1* region (p<0.001). The B-other subgroup displayed a high deletion frequency, particularly in *PAX5*, *CDKN2A/B*, *IKZF1* and *RB1*. Specifically, *PAX5alt* and *ZNF384r* showed a higher number of deletions (median 3 and 2.5, respectively).

Pathogenic mutations were found in 71% of patients (median: 1.5 mutations), with *KRAS*, *NRAS*, and *PAX5* being the genes most commonly affected. *BCR::ABL1*-like patients had a higher mutation frequency, and all *JAK2* mutations occurred in *CRLF2r* patients. The B-other subtype had prevalent *KRAS*, *NRAS*, *PAX5* and *IKZF1* mutations. Concretely, *PAX5alt* group had the highest median number of mutations (3.5), and all *ZNF384r* patients had mutations in *EGFR* and *EZH2*.

A total of 144 B-ALL patients were treated according to SEHOP-PETHEMA 2013 guidelines, which use risk-adapted treatment regimens. The entire B-ALL cohort showed high OS and EFS rates of 88.9% and 78.9%, respectively, with a relapse rate of 17.4%. Genetic subtypes significantly influenced outcomes: HeH and *ETV6::RUNX1* had the best outcomes (5-years 98.5%

OS, 94.9% EFS; $p < 0.001$), while B-other patients showed intermediate-risk profiles (5-years 87.8% OS, 66.8% EFS); specific ZNF384r and PAX5 P80R subtypes exhibited favorable outcomes. BCR::ABL1-like, concretely CRLF2r, had poor outcomes (5-years 52.5% OS, 26.5% EFS), similar to KMT2Ar and hypodiploidy subtypes (5-years 50% OS).

Discussion: An integrated diagnostic approach classified 83% of B-ALL cases into 17 subtypes, the distribution of genetic subtypes aligned with previous studies. Although 58% of B-other cases (17% of all B-ALL) in our study remained unclassified, this is comparable to other large studies like Schwab et al.(2), where even using WGS, 53% were left unclassified.

The study highlights the importance of combining multiple diagnostic tools for accurate genetic classification and risk stratification in pediatric B-ALL. Our study confirms the utility of conventional techniques combined with t-NGS and OGM for comprehensive genetic diagnosis. Karyotyping and FISH remain robust, cost-effective, and widely accessible for rapid chromosomal abnormality assessment, while NGS and OGM significantly improve diagnostic yield, especially in cases with incomplete cytogenetic results.

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