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Short communication

Molecular characterization of acute lymphoblastic leukemia in clinical management of pediatric patients from N. Macedonia

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Introduction

Acute lymphoblastic leukemia (ALL) is the most commonly diagnosed type of cancer in the pediatric population (Tran and Hunger, 2020). Continuous improvements in therapeutic protocols and the implementation of novel techniques for monitoring treatment response have resulted in a current five-year survival rate exceeding 92% (Inaba and Pui, 2021). However, survival rates remain significantly lower in patients experiencing disease relapse (20-50%). Increasingly, studies highlight the prognostic significance of minimal residual disease (MRD) and MRD-guided risk stratification, while also demonstrating relapse occurrence in a considerable proportion of patients initially classified as low-risk (Contreras Yametti. et al., 2021). Consequently, the primary objectives of this research are to implement a highly sensitive MRD detection method for the early identification of very low levels of residual disease, evaluate its impact on clinical outcomes, and identify additional genetic features with potential prognostic relevance.

Materials and methods

This study included all patients diagnosed with ALL at the University Clinic for Children's Diseases in Skopje (n=84) over an eight-year period. All patients were uniformly treated according to the ALL-IC-BFM 2002/2022 protocol. Minimal residual disease was evaluated at two time-points following the initiation of chemotherapy (day 33 and day 78) using two molecular methods: initial screening by multiplex PCR followed by capillary electrophoresis (CE) according to the BIOMED-2 protocol (all patients), and next-generation sequencing (NGS) with a sensitivity of 0.01% (50 patients). The most common chromosomal translocations associated with ALL were analyzed using quantitative reverse transcription PCR, while aneuploidies and copy number variations (CNVs) of selected genes were detected through MLPA analysis. The patients who experienced disease relapse (n=11) underwent additional whole-exome sequencing analysis using matched diagnostic and relapse samples.

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Results and discussion

NGS-MRD positivity (MRD>0.01%) was detected in 58% and 18% of patients on days 33 and 78, respectively. This method enabled the detection of MRD in 54% and 15% of patients who were classified as MRD-negative by CE at the respective time-points. Among patients who experienced relapse, only 4 patients (36%) were initially classified as high-risk according to standard clinical and genetic stratification criteria defined by the protocol. Absence of NGS-MRD on day 33, at a sensitivity of 0.01%, was observed in 42% of patients, and was associated with a relapse-free survival of 94%. These findings demonstrate the prognostic significance of negative NGS-MRD status on day 33 for identifying patients with good response to therapy and underlines its value in selecting candidates for treatment de-escalation (Pieters, R. et al., 2016). NGS-MRD positivity was detected in 9 out of 10 and 5 out of 10 relapse cases on days 33 and 78, respectively. However, NGS-MRD positivity on day 33 or at both time-points was also observed in a substantial proportion of relapse-free patients (52% and 12%, respectively), most of whom had a follow-up period exceeding 4 years. These findings indicate the limited prognostic value of NGS-MRD positivity alone and suggest that its presence may be insufficient as an independent criterion for stratifying patients into the high-risk group (Krstevska Bozhinovikjet al., 2025).

The analysis of genetic and chromosomal abnormalities identified several subtypes in B-ALL, the most frequent being high-hyperdiploidy (28%), followed by ETV6::RUNX1 (15%), TCF3::PBX1 (8%), iAMP21 (2,8%), MLL::AFF1 and BCR::ABL1 (1,4% each), and one subtype in T-ALL (STIL::TAL1 in one patient). The most common CNVs detected in the patients with B-ALL were duplication of chromosome 21 (50%) and duplication of PAR1 region of chromosome X (42%). Additionally, deletions in CDKN2A/2B, PAX5, ETV6 and IKZF1 genes were observed in 26%, 18%, 10% and 7% of patients, respectively. In T-ALL, deletions of CDKN2A/2B and IKZF1 were the only CNVs identified, occurring in 50% and 20% of patients, respectively. These findings also enabled the identification of an additional subtype in one patient classified as IKZF1plus.

Disease relapse was observed in one patient each with BCR::ABL1, MLL::AFF1 and iAMP21, and in two patients with hyperdiploidy. Additional CNVs detected in these relapse cases included deletions of TP53, EBF1 and IKZF1, which were uncommon among the remaining patients. Furthermore, these patients harbored additional genetic alterations that may contribute to disease progression, but their clinical significance requires further investigation.

Conclusion

These findings underscore the importance of integrating NGS-MRD assessment together with the evaluation of additional genetic alterations potentially associated with therapy resistance into the final risk stratification process. This approach may improve the identification of patients at increased risk of relapse and contribute to more precise risk-adapted therapeutic decisions.

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