REVIEW

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MINIMAL RESIDUAL DISEASE ASSESSMENT IN ACUTE LYMPHOBLASTIC LEUKEMIA: METHODS FOR DETECTION AND QUANTIFICATION

Recently, the identification of minimal residual disease (MRD), also known as measurable residual disease, has seen significant advances driven by the evolving technological landscape in this field. Initially based on raw morphology, the field has since evolved to incorporate karyotyping, cytogenetics, flow cytometry, and other sensitive methods. This updated review discusses key technical considerations for successful MRD detection. We explore the correlation between the results obtained by flow cytometry and molecular genetic techniques.

Keywords: MRD, flow cytometry, molecular technique.

Acute lymphoblastic leukemia (ALL) is a clonal disease of hematopoietic stem cells of either B- or T-cell lineage. It manifests as lymphoblastoid cells with blocked differentiation. Despite being rare, ALL is the most common type of leukemia in children, affecting 4—5 children per 100,000 annually [1]. Precursor B-cell ALL accounting for about 85% to 90% of ALL cases in children is more common than T-cell precursor ALL. T-cell precursor leukemia accounts for 10%—15% of cases in children and 25% in adults, with T-lymphoblastic lymphoma (T-LBL) more prevalent than B-LBL [1, 2]. The therapeutic strategy involves three stages, including induction, consolidation, and maintenance [3]. In children with B-ALL, the five-year disease-free survival rate exceeds 85%, with a cure rate of 90% [4, 5]. Similarly, in children with T-ALL, the ffive-year event-free survival (EFS) rate has increased from 75% to > 90% [6]. However, a proportion of patients exhibit more risk factors, higher relapse probabilities, and, consequently, an unfavorable overall condition. In 1993, the National Cancer Center identified risk factors for childhood B-ALL for prognostic purposes. These factors include age, white blood cell count, central nervous system involvement, cytogenetic alterations, aneuploidy, and early response to therapy. These factors reliably predict patient outcomes, and risk stratification was developed based on EFS. In contrast, risk factors for

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T-ALL are less well-defined [7]. Previous research by the Pediatric Oncology Group (POG) indicates that, unlike in B-ALL, advanced age and a high white blood cell count are not reliable indicators in T-ALL [8]. However, POG has observed a correlation between T-cell maturation and patient outcomes. This discovery has been the focus of multiple studies, including the ALL 2000 study at St. Jude Children's Research Hospital, which provided deeper insights into this association [9]. A pivotal question in the management of ALL in children is identifying those who require more intensive therapy to mitigate the risk of relapse. Clinical and biological factors, such as white blood cell count, age, immunophenotype, ploidy, structural chromosomal abnormalities, and genetic rearrangements, can be used to stratify treatment, although these factors are not ideal. While some patients with promising indicators experience recurrence, others at a higher risk of relapse receive more intensive treatment than necessary.

Several studies focused on minimal residual disease (MRD) to obtain more reliable estimates of lymphoblast counts during the clinical remission phase. MRD serves as an indicator of treatment sensitivity and intensity, enabling the adoption of tailored treatment protocols. The reliable detection of MRD aims to identify children at high risk of relapse as early as possible. Additionally, it is used to assess bone marrow and peripheral blood for the suitability of autologous hematopoietic stem cell transplantation. Moreover, MRD plays a crucial role in evaluating the clearance process [10]. Finally, MRD allows for comparing the effectiveness of different chemotherapy regimens. This paper aims to provide a comprehensive overview of MRD identification techniques. Indeed, one of the major challenges in childhood ALL is identifying those who require more intensive treatment to prevent the risk of relapse. During the clinical remission phase, MRD assessment contributes significantly to improving the quantification of the total burden of leukemia cells. This is crucial as it indicates disease aggressiveness and treatment sensitivity, thus facilitating the selection of the most appropriate therapeutic strategy.

MRD detection and quantification methods

An effective platform for identifying MRD must possess three key advantages encompassing optimal sensitivity, specificity, and reproducibility [11]. Despite advances in immunohistochemistry testing methods, morphological assessment remains insufficient in terms of sensitivity and specificity. Similarly, traditional methods such as karyotyping and fluorescence in situ hybridization lack the required sensitivity to detect submicroscopic leukemia cells, often achieving detection rates as low as 1%—5% [12]. The primary techniques used to assess MRD include flow cytometry, which relies on identifying cells with abnormal immunophenotypes, and molecular genetic methods such as polymerase chain reaction (PCR) for identifying tumor-specific antigen receptor sequences. Today, next-generation sequencing (NGS) has emerged as another valuable tool for molecularly identifying these genetic abnormalities [13]. The accuracy and scope of MRD tests are undergoing significant changes, driven by the development of improved antibodies, upgrades to flow cytometers, and advancements in computing [14]. Flow cytometry has revealed significant correlations between MRD findings, treatment efficacy, and clinical features, thereby enhancing the credibility of this approach [15—18]. Flow cytometry, with its accessibility and straightforward concept, stands out as the most readily available technique for identifying MRD [19]. It directly measures MRD, simplifying quantification and enabling more precise measurements [20]. Moreover, flow cytometry can also detect dying cells and cellular fragments. This is particularly useful for analyzing fresh samples (e.g., those collected within several hours) because it allows researchers to identify cell death caused by a lack of survival factors before spontaneous cell death occurs. Nevertheless, the application of flow cytometry may have several limitations. To identify a single leukemia cell among 10^5 or more normal cells is challenging with flow cytometry but achievable with PCR. This level of sensitivity is particularly valuable in MRD studies involving patients with irregular disease distribution, as well as in the context of cell collection for autograft procedures. It is also important to note that the immunophenotype of leukemia cells evolves as the disease progresses. However, if these changes affect the markers used for MRD surveillance, there is a risk of obtaining false-negative results [14, 21—23]. This risk is inversely proportional to the number of marker combinations applicable to each patient. In other words, if cells

exhibit multiple suitable phenotypes, the loss of one phenotypic pattern can be compensated by the persistence of other abnormal patterns. Another limitation of flow cytometry techniques is that their results rely on the analysis of immunophenotypic characteristics of leukemia, while immunophenotype may change substantially between diagnosis and relapse. These frequent changes complicate the use of the original diagnostic markers for targeted MRD detection by flow cytometry [23].

Immature T cells are homologs of normal T-cell lineage ALL cells. Unlike leukemic T-cell lymphoblasts, which circulate freely throughout the body, these cells are limited to the thymus. To detect MRD in patients with T-cell lineage ALL, the focus is on identifying cells with the immature T-cell phenotype in bone marrow or peripheral blood. Effective immunophenotypes for this purpose involve the simultaneous expression of T-cell markers such as CD3, CD5, and either TdT or CD34 [24]. Some studies also suggest that certain combinations of antibodies, such as CD7 and CD3 with CD2 or CD5, may exhibit abnormal expression in a subset of T-ALL patients [25, 26]. B cell progenitors, which are the normal counterparts of B-cell lineage ALL cells, are primarily found in the bone marrow and in smaller quantities in peripheral blood [25]. Therefore, MRD studies in the B-cell ALL lineage require distinguishing between leukemia cells and their normal counterparts, which can be achieved by examining abnormal levels of expression of various molecules in leukemia cells [25—27]. T-ALL MRD backbone panels are set up to evaluate both the aberrant expression of mature T/NK-cell antigens (i.e., surface/cytoplasmic CD3, CD5, CD7, CD2, CD4, CD8, CD45, CD16, and CD56) and immature markers (CD34, CD1a, TdT, and CD99) These immature markers are frequently absent or very dim, and CD45 is commonly quite bright in the residual leukemic cells after treatment. It is hard to say whether the immature nature of residual T-ALL and the "different-from-normal" strategies are available to identify a T-cell population different from normal T cells [28]. In most cases, the second strategy includes looking for abnormal CD4/8 double-negative (DN), CD4/8 double-positive (DP), and/or $sCD3^{-}cCD3^{+}$ cells.

In the context of B-ALL, markers such as CD13, CD15, CD33, and CD65 myeloid markers, along with the CD21 marker, associated with mature B cells, have the potential to be expressed by $CD19^{+}CD34^{+}$ cells. Conversely, normal $CD19^{+}CD34^{+}$ B cell progenitors do not express these markers or express them only weakly [25]. When comparing B-ALL to their normal counterparts, the expression levels of CD19, CD10, TdT, and CD34 vary considerably, either being higher or lower [29]. Leukemia cells often exhibit underexpression of CD38 and CD45. The EuroFlow consortium is currently developing advanced protocols for flow cytometry, known as Next Generation Flow [30]. These protocols aim to establish standards for MRD detection [31]. The first step involves a mass lysis procedure to remove erythrocytes and concentrate leukocytes, thereby increasing the number of cells available for labeling. While traditional four-color flow cytometry has a sensitivity of 0.01%, this new technique achieves a sensitivity of 0.001% due to its ability to label more than 4×10^6 cells [32]. The Children's Oncology Group (COG) has a standardized 3-tube 6-color panel for B-ALL MRD detection, which is used for patients in COG studies. This panel includes CD34, CD19, CD10, CD20, CD38, CD45, CD9, CD58, CD13/33, CD71, CD3, and Syto16. Although it has limitations such as not being able to detect CD19-negative MRD due to its gating strategy and consuming more samples, it is highly standardized with a good sensitivity (10^{-4}) in most cases. A highly sensitive standardized multiparameter flow cytometry (MFC) B-ALL MRD assay has also been designed by the EuroFlow group, and it demonstrates the application of a fully standardized bulk lysis protocol and two stepwise-designed 8-color tubes (including the backbone panel plus CD81 and either CD66c/CD123 or CD73/CD304) [33]. The inclusion of B-cell markers other than CD19 has been emphasized in recent years due to the increasing clinical use of immunotherapies targeting CD19 (i.e., blinatumomab and CAR-T19). CD22 and CD24 are expressed in B lymphoblasts and can be essential for tracking B-ALL leukemic cells with downregulation of CD19 as well as for identification of $CD22^{+}$ cases eligible for inotuzumab ozogamicin therapy [34].

In summary, flow cytometry is used in more than 95% of patients and provides valuable information on the immunophenotypic heterogeneity of leukemia and the cellular state of the bone marrow microenvironment — aspects that are insufficiently considered by other MRD techniques [32]. While flow

cytometry has lower costs and faster turnaround time (1 day) compared to the alternative approaches mentioned below (4 days to weeks), it involves rapid manipulation due to its execution on living cells. Additionally, achieving sensitivity levels above 10^{-5} requires a significant quantity of cells [30, 35].

Real-time quantitative PCR (RQ-PCR)

The use of RQ-PCR is an accurate method to identify MRD, with sensitivity potentially reaching levels as low as 10^{-6}. When using PCR to identify MRD, the genetic marker typically employed to differentiate ALL from healthy cells is the rearranged sequence of the Ig/TCR gene or fusion gene.

Rearrangements of immunoglobulin and T cell receptor genes can be used to identify a small number of ALL cells among a large population of normal lymphocytes expressing different gene rearrangements. In B-cell precursor ALL, incomplete and complete *IGH, IGK*, and *IGL* rearrangements are more commonly detected and used as MRD targets. However, sometimes leukemia cells of the B- and T-lineage display lineage rearrangements, identifiable by multipoint PCR and usable for the determination of MRD [36, 37]. Sequencing of PCR products is performed to determine junctional regions and acquire allele-specific primers, with successful cloning confirmation through homo-heteroduplex analysis [38, 39]. This qPCR technique, using fluorescent marker-labelled probes, is extremely accurate, capable of identifying one leukemia cell among 100,000 healthy lymphoid cells under ideal circumstances [40]. The EuroMRD consortium, a dedicated group of expert laboratories, has played an important role in establishing the most widely accepted MRD approach. This consortium offers training, standardization, quality control, and guidelines for the interpretation of RQ-PCR data [41]. MRD experiments conducted in EuroMRD laboratories can be reliably replicated, allowing for the comparison of MRD datasets from different clinical protocols. However, due to its complexity, the identification of a small leukemia population using this MRD approach requires specialized knowledge and expertise [42]. Additionally, technical issues hinder the identification of MRD targets in 5%–10% of cases using RQ-PCR-based MRD. This method has a limitation related to the possibility of false-negative MRD results due to clonal evolution of Ig/TCR rearrangements during the disease and relapse. The study by Szczpansky et al. [43] demonstrated that 22% of leukemia patients lost most or all their Ig/TCR targets assessed at diagnosis after the onset of hematological relapse. The amount of diagnostic DNA available for analysis is another limitation for this type of MRD assessment. Since it quantifies tumor burden at diagnosis, the initial DNA sample obtained at the first diagnosis is required for each MRD experiment and the identification of Ig/TCR clonal rearrangements [44].

Identification of the fusion gene. Precise identification of leukemia subtypes containing fusion genes is achieved using RQ-PCR. However, this method is reliable for only 40% of patients with ALL distinct characteristics and molecular abnormalities. Among pediatric cases of ALL, four prevalent types of fusion genes should be distinguished:

- The *TEL/AML1* fusion gene (also known as *ETV6-RUNX1*) from the t(12;21)(p13;q22) translocation. It occurs in 22% of children and 2% of adults with ALL [45]. This translocation is the most common in childhood B-ALL. Moreover, it is associated with an excellent prognosis with intensive chemotherapy, including asparaginase therapy.
- The *TCF3/PBX1* fusion gene from the t(1;19)(q23;p13) translocation represents 5% of childhood ALL and 3% of adult ALL and is frequently associated with the pre-B immunophenotype, in about 25% of cases [45, 46].
- *BCR/ABL* from the t(9;22)(q34;q11) translocation is found in approximately 3% of children and 30% of adults, and is associated with an unfavorable prognosis [46].
- *AF4/MLL* is derived from the t(4;11)(q21;q23) translocation [45,46]. Chromosomal rearrangements of the human *MLL* gene are the most common genetic abnormality in the first year of life, but it occurs in only 8% of children and 10% of adults with ALL [46].

The first two fusion genes have higher incidence rates in children with B-ALL. In contrast, the *BCR/ABL* fusion gene is more frequently found in chronic myeloid leukemia and remains rare in pediatric ALL. In more than 2/3 of childhood leukemia cases, the *AF4/MLL* fusion gene is present. According to Ajuba et al. [47], this fusion gene is associated with poor prognosis and is predominant in B-ALL of infants.

Variations in the detection rates of different fusion genes in different regions are observed. This is probably a result of genetic diversity among breeds. Although RQ-PCR has some advantages, such as simple and fast operation, high specificity, and sensitivity in the detection of fusion genes, it is limited to specific subtypes of leukemia. The variations in the expression of fusion genes between individuals and the potential for false-positive results due to PCR contamination are additional challenges. Furthermore, RQ-PCR faces difficulties in achieving accurate ratios between the number of leukemia cells and PCR products, making it inadequate for precise cellular-level MRD assessment.

Next Generation Sequencing (NGS)

In the late 1990s, new DNA sequencing techniques emerged. These methods were further developed into high-throughput sequencing (HTS), known as NGS, since 2000. This has rapidly gained adoption by researchers in various fields. The advantages of NGS are twofold: it can sequence large amounts of DNA quickly and has a sensitivity to detect and quantify very small clones, such as one cell in a population of 10^{-6} cells, as well as very low-frequency cells. Researchers have been particularly interested in exploring the use of NGS for MRD detection. Initially, they used this method to detect MRD in T-ALL and then in B-ALL [48, 49].

NGS in the context of T-ALL. Wu et al. [48] used HTS to study MRD in patients diagnosed with T-ALL and compared these results with those obtained by flow cytometry. HTS showed better sensitivity in detecting MRD. Interestingly, among cases without TCR clonal beta rearrangements at diagnosis, some had substantial clones identifiable by flow cytometry on day 29. Almost half of these cases had an immunophenotype of early T-cell precursors (ETP), while the other half had a similar phenotype, except for the presence of CD5, which is referred to as 'quasi-ETP'. Conversely, none of the cases with detectable TCR clonal beta rearrangements had an ETP phenotype.

NGS in the context of B-ALL. In line with previous research on T-ALL, NGS was used for MRD detection in patients with B-ALL [49]. NGS was extended to all B-ALL patients, regardless of their known IGH rearrangements [50]. The authors discovered identifiable clonal rearrangements in 93% of patients in the pretreatment sample [49, 50]. Among MRD-positive cases, the clone most frequently identified in the pretreatment sample corresponds to the equally dominant clone on day 29. Like in T-ALL, a subset of MRD patients was identifiable by NGS but not by flow cytometry. Patients without clonal rearrangements of the Immunoglobulin Superfamily had poorer outcomes than patients with clonal IGH. Maybe these cases represent more primitive clones [51]. NGS offers several advantages over flow cytometry and conventional PCR when examining MRD. It involves less subjectivity in interpretation compared to flow cytometry. Once the protocol is established, the technical requirements are easier than those of other molecular genetic methods. By using primers to simultaneously amplify any possible combination of rearranged IGH or TCR loci, NGS bypasses the need for patient-specific primers. This explains the use of HTS because of its speed in real-time therapeutic decision-making, although it is slower than flow cytometry in practical applications. However, NGS can only identify cases with complete rearrangements, leaving behind a limited number of cases without rearrangements or with incomplete rearrangements. In addition, this method involves matching pre- and post-processing samples. Several studies demonstrated that NGS can accurately predict the rate of recurrence and survival by detecting MRD as compared to flow cytometry and conventional PCR [50] and can potentially replace these methods in the future [52, 53].

New technologies

Despite significant progress in standardizing RQ-PCR for MRD detection and ensuring reproducibility within and between laboratories, there are still two main concerns that need to be addressed. One is related to the significant amount of DNA required for accurate diagnosis. The other pertains to the risk of non-specific amplification in RQ-PCR technology, especially in the presence of low levels of MRD. Indeed, a substantial amount of diagnostic DNA is essential for the detection of clonal rearrangements in MRD genes by IG/TR. This is because, in addition to early detection of clonal rearrangements, a standard dilution curve must be con-

structed for each qPCR MRD experiment, posing limitations in monitoring patients, especially when assessing multiple time points. Third-generation PCR, known as droplet digital PCR (ddPCR), makes it possible to overcome these limitations of conventional RQ-PCR. ddPCR technology compartmentalizes the sample into thousands of individual droplets, each representing an independent PCR reaction. This allows for endpoint amplification and utilizes Poisson statistics [54, 55]. Several studies on medical diagnosis focused on the use of ddPCR techniques in oncology, including hematological malignancies [56, 57]. The recent studies compared ddPCR and qPCR in adult patients with mature lymphoid malignancies and adolescent and young adult patients with ALL, respectively [58], and reported that ddPCR exhibits sensitivity, accuracy, and reproducibility comparable to qPCR. Moreover, ddPCR technology made it possible to determine the absolute amount of specific DNA molecules in a given sample without the need to compare to a standard curve. While the final clinical validations of ddPCR are still pending, the first guidelines for interpreting ddPCR data in a clinical setting have been published [59].

ddPCR can certainly serve as a viable alternative to qPCR and as an instrument to preserve DNA samples. Owing to the extensive partitioning of the reaction mixture into a large number of individual compartments, ddPCR can achieve a very high level of analytical precision. Furthermore, ddPCR is particularly well suited for the detection of low-abundance targets in the presence of a high background of non-target DNA. Within partitions that contain the target molecule, the relative proportion of target to non-target DNA becomes significantly higher than in the original sample, thereby facilitating detection.

Detection methods used to monitor MRD in ALL

Method	Advantages	Disadvantages
FCM (Flow cytometry)	Fast Sensitivity 10^{-4} Relatively inexpensive Ability to quantify Aberrant antigen expression	Variable sensitivity due to similarities between normal (regenerating) cells and malignant cells Limited standardization, no QA (quantification) results
FCM-DfN (Flow cytometry different from normal)	Sensitivity 10^{-3} to 10^{-5} No diagnostic sample required Phenotypic shifts will not interfere with results Applicable to > 90% of patients Rapid turnaround time	Significant operator experience required Limited standardization
RT-PCR of fusion genes	Sensitivity 10^{-4} to 10^{-5} Simple (uses some primers as used for diagnosis)	Limited QA rounds Limited applicability in ALL (absence of targets in 50% of cases) Risk of contamination
PCR of Ig/TCR genes	Sensitivity 10^{-4} to 10^{-5} Applicable to most patients Standardized guidelines in Europe	Time-consuming Expensive Relies on pre-treatment sample Requires extensive experience and labor
Digital PCR	Highly sensitive (10^{-4} to 10^{-5}) Rapid and widely applicable	Limited standardization, available only in a few labs, no approved guidelines for data analysis, relatively expensive
Next Generation Sequencing	Very sensitive (10^{-6}) Applicable to almost all patients Clone–unbiased (can track multiple clones and evolution) Only US FDA-approved assay (ClonoSEQ) Data for MRD use in peripheral blood	Expensive Longer turnaround time than FCM Requires diagnostic pre-treatment sample

Finally, dPCR amplification — and consequently, the resulting quantification — is generally less affected by partial PCR inhibition compared to conventional PCR. Several digital PCR (dPCR) platforms are currently available on the market, reflecting the growing importance of this relatively recent technology in quantitative molecular biology applications. Existing dPCR instruments can be broadly categorized according to their partitioning formats.

Amplification of non-specific, false Ig/TCR rearrangements cannot be distinguished from cases of very low-level positive results that cannot be quantified. These non-quantifiable positive cases present a risk of detecting false positives or false negatives in the analysis of MRD, creating an additional challenge. In this context, the EuroMRD recommendations are aimed at the management of non-specific amplification, considering different clinical objectives. These guidelines focus on the prevention of false negatives or false positives [60]. In any case, it is important to evaluate the results carefully, especially for samples collected after treatment has been completed or after HSCT. Indeed, the identification of MRD in these situations can be complicated by the reconstitution of B cells. In such circumstances, a specific study of the size or profile of the RQ-PCR is necessary before making a medical decision [61]. HTS based on PCR of Ig/TCR gene rearrangements represents an interesting advance in this context. HTS identifies clone-specific IG/TR indexing sequences. The same procedure is used for each follow-up copy, allowing the determination and quantification of clones detected during diagnosis [62]. Several recent studies highlighted the greater specificity of HTS in identifying recurrence in ALL patients following HSCT [63] or induction [64] compared to RQ-PCR. HTS for MRD detection also has the advantage of allowing early identification of clonal evolution, which is a relatively common phenomenon in recurrent ALL [65]. With the use of universal primers, this technique allows simultaneous monitoring of all Ig/TCR gene rearrangements, providing a global view of MRD as well as the normal immune repertoire. However, to date, standardization of HTS MRD is very limited, and guidelines for interpreting the information are not yet available. The working group of the Euroclonality-NGS consortium is working to fill this gap. At the same time, the practicability of HTS MRD is dependent on cost and complexity, although this tends to diminish [66].

The methods in current use for monitoring MRD in ALL are summarized in the Table.

To sum up, in clinical practice, incorporating MRD follow-up as a component of the initial treatment regimen is routine for nearly all cases of ALL in children and a substantial portion of adult cases as well. This monitoring procedure serves as a valuable tool, especially in identifying the necessity for HSCT or intensified chemotherapy in patients who test positive for MRD later in the induction phase, particularly those categorized as high-risk individuals.

REFERENCES

1. Swerdlow SH, Campo E, Pileri SA, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. *Blood.* 2016;127(20):2375-2390. https://doi.org/10.1182/blood-2016-01-643569
2. Coustan-Smith E, Mullighan CG, Onciu M, et al. Early T-cell precursor leukaemia: a subtype of very high-risk acute lymphoblastic leukaemia. *Lancet Oncol.* 2009;10(2):147-156. https://doi.org/10.1016/S1470-2045(08)70314-0
3. Gaynon PS, Trigg ME, Heerema NA, et al. Children's Cancer Group trials in childhood acute lymphoblastic leukemia: 1983–1995. *Leukemia.* 2000;14(12):2223-2233. https://doi.org/10.1038/sj.leu.2401939
4. Schultz KR, Pullen DJ, Sather HN, et al. Risk- and response-based classification of childhood B-precursor acute lymphoblastic leukemia: a combined analysis of prognostic markers from the Pediatric Oncology Group (POG) and Children's Cancer Group (CCG). *Blood.* 2006;109(3):926-935. https://doi.org/10.1182/blood-2006-01-024729
5. Hunger SP, Loh ML, Whitlock JA, et al. COG Acute Lymphoblastic Leukemia Committee. Children's Oncology Group's 2013 blueprint for research: Acute lymphoblastic leukemia. *Pediatr Blood Cancer*. 2013;60:957-963. https://doi.org/10.1002/pbc.24420
6. Goldberg JM, Silverman LB, Levy DE, et al. Childhood T-cell acute lymphoblastic leukemia: the Dana-Farber Cancer Institute acute lymphoblastic leukemia consortium experience. *J Clin Oncol.* 2003;21(19):3616-3622. https://doi.org/10.1200/JCO.2003.10.116
7. Wood BL, Winter SS, Dunsmore KP, et al. T-lymphoblastic Leukemia (T-ALL) shows excellent outcome, lack of significance of the early thymic precursor (ETP) immunophenotype, and validation of the prognostic value of end-induction minimal residual disease (MRD) in Children's Oncology Group (COG) study AALL0434. *Blood.* 2014;124(21):1. https://doi.org/10.1182/blood.V124.21.1.1

8. Smith M, Arthur D, Camitta B, et al. Uniform approach to risk classification and treatment assignment for children with acute lymphoblastic leukemia. *J Clin Oncol.* 1996;14(1):18-24. https://doi.org/10.1200/JCO.1996.14.1.18
9. Pullen J, Shuster JJ, Link M, et al. Significance of commonly used prognostic factors differs for children with T cell acute lymphocytic leukemia (ALL), as compared to those with B-precursor ALL. A Pediatric Oncology Group (POG) study. *Leukemia.* 1999;13(11):1696-1707. https://doi.org/10.1038/sj.leu.2401555
10. Rambaldi A, Borleri G, Dotti G, et al. Innovative two-step negative selection of granulocyte colony-stimulating factor–mobilized circulating progenitor cells: Adequacy for autologous and allogeneic transplantation. *Blood.* 1998;91(6):2189-2196. https://doi.org/10.1182/blood.V91.6.2189
11. Gaipa G, Basso G, Biondi A, et al. Detection of minimal residual disease in pediatric acute lymphoblastic leukemia. *Cytometry B Clin Cytom.* 2013;84(6):359-369. https://doi.org/10.1002/cyto.b.21101
12. Mancini M, Cedrone M, Diverio D, et al. Use of dual-color interphase FISH for the detection of inv(16) in acute myeloid leukemia at diagnosis, relapse and during follow-up: a study of 23 patients. *Leukemia.* 2000;14(3):364-368. https://doi.org/10.1038/sj.leu.2401678
13. Bielorai B, Golan H, Trakhtenbrot L, et al. Combined analysis of morphology and fluorescence in situ hybridization in follow-up of minimal residual disease in a child with Philadelphia-positive acute lymphoblastic leukemia. *Cancer Genet Cytogenet.* 2002;138(1):64-68. https://doi.org/10.1016/S0165-4608(02)00578-2
14. Coustan-Smith E, Behm FG, Sanchez J, et al. Immunological detection of minimal residual disease in children with acute lymphoblastic leukaemia. *Lancet.* 1998;351(9102):550-554. https://doi.org/10.1016/S0140-6736(97)10295-1
15. Farahat N, Morilla A, Owusu-Ankomah K, et al. Detection of minimal residual disease in B-lineage acute lymphoblastic leukaemia by quantitative flow cytometry. *Br J Haematol.* 1998;101(1):158-164. https://doi.org/10.1046/j.1365-2141.1998.00675.x
16. Coustan-Smith E, Sancho J, Hancock ML, et al. Clinical importance of minimal residual disease in childhood acute lymphoblastic leukemia. *Blood.* 2000;96(8):2691-2696. https://doi.org/10.1182/blood.V96.8.2691
17. San Miguel JF, Vidriales MB, López-Berges C, et al. Early immunophenotypical evaluation of minimal residual disease in acute myeloid leukemia identifies different patient risk groups and may contribute to postinduction treatment stratification. *Blood.* 2001;98(6):1746-1751. https://doi.org/10.1182/blood.V98.6.1746.
18. Dworzak MN, Fröschl G, Printz D, et al.; Austrian Berlin-Frankfurt-Münster Study Group. Prognostic significance and modalities of flow cytometric minimal residual disease detection in childhood acute lymphoblastic leukemia. *Blood.* 2002;99(6):1952-1958. https://doi.org/10.1182/blood.V99.6.1952
19. Coustan-Smith E, Sancho J, Behm FG, et al. Prognostic importance of measuring early clearance of leukemic cells by flow cytometry in childhood acute lymphoblastic leukemia. *Blood.* 2002;100(1):52-58. https://do.org/10.1182/blood-2002-01-0006
20. Coustan-Smith E, Sancho J, Hancock ML, et al. Use of peripheral blood instead of bone marrow to monitor residual disease in children with acute lymphoblastic leukemia. *Blood.* 2002;100(7):2399-2402. https://doi.org/10.1182/blood-2002-04-1130
21. Neale GA, Coustan-Smith E, Pan Q, et al. Tandem application of flow cytometry and polymerase chain reaction for comprehensive detection of minimal residual disease in childhood acute lymphoblastic leukemia. *Leukemia.* 1999;13(8):1221-1226. https://doi.org/10.1038/sj.leu.2401459
22. Oelschlägel U, Nowak R, Schaub A, et al. Shift of aberrant antigen expression at relapse or at treatment failure in acute leukemia. *Cytometry.* 2000;42(4):247-253. https://doi.org/10.1002/1097-0320(20000815)42:4<247::AID-CYTO5>3.0.CO;2-V
23. Baer MR, Stewart CC, Dodge RK, et al. High frequency of immunophenotype changes in acute myeloid leukemia at relapse: implications for residual disease detection (Cancer and Leukemia Group B Study 8361). *Blood.* 2001;97(11):3574-3580. https://doi.org/10.1182/blood.V97.11.3574
24. Porwit-MacDonald A, Björklund E, Lucio P, et al. BIOMED-1 concerted action report: Flow cytometric characterization of CD7+ cell subsets in normal bone marrow as a basis for the diagnosis and follow-up of T cell acute lymphoblastic leukemia (T-ALL). *Leukemia.* 2000;14(5):816-825. https://doi.org/10.1038/sj.leu.2401741
25. Campana D, Coustan-Smith E. Advances in the immunological monitoring of childhood acute lymphoblastic leukaemia. *Best Pract Res Clin Haematol.* 2002;15(1):1-19. https://doi.org/10.1053/beha.2002.0182
26. Dworzak MN, Fritsch G, Fleischer C, et al. Comparative phenotype mapping of normal vs. malignant pediatric B-lymphopoiesis unveils leukemia-associated aberrations. *Exp Hematol.* 1998;26(4):305-313. PMID: 9546313
27. Ciudad J, San Miguel JF, López-Berges MC, et al. Detection of abnormalities in B-cell differentiation pattern is a useful tool to predict relapse in precursor-B-ALL. *Br J Haematol.* 1999;104(4):695-705. https://doi.org/10.1046/j.1365-2141.1999.01236.x
28. Fuda F, Chen W. Minimal/measurable residual disease detection in acute leukemias by multiparameter flow cytometry. *Curr Hematol Malig Rep*. 2018;13(6):455-466. https://doi.org/10.1007/s11899-018-0479-1
29. Lavabre-Bertrand T, Janossy G, Ivory K, et al. Leukemia-associated changes identified by quantitative flow cytometry: I. CD10 expression. *Cytometry.* 1994;18(4):209-217. https://doi.org/10.1002/cyto.990180404
30. Flores-Montero J, Sanoja-Flores L, Paiva B, et al. Next Generation Flow for highly sensitive and standardized detection of minimal residual disease in multiple myeloma. *Leukemia.* 2017;31(10):2094-2103. https://doi.org/10.1038/leu.2017.29

31. Theunissen P, Mejstrikova E, Sedek L, et al. Standardized flow cytometry for highly sensitive MRD measurements in B-cell acute lymphoblastic leukemia. *Blood.* 2017;129(3):347-357. https://doi.org/10.1182/blood-2016-07-726307
32. Della Starza I, Chiaretti S, De Propris MS, et al. Minimal residual disease in acute lymphoblastic leukemia: Technical and clinical advances. *Front Oncol.* 2019;9:726. https://doi.org/10.3389/fonc.2019.00726
33. Theunissen P, Mejstrikova E, Sedek L, et al. Standardized flow cytometry for highly sensitive MRD measurements in B-cell acute lymphoblastic leukemia. *Blood.* 2017;129(3):347-357. https://doi.org/10.1182/blood-2016-07-726307
34. Liu Z, Li Y, Shi C. Monitoring minimal/measurable residual disease in B-cell acute lymphoblastic leukemia by flow cytometry during targeted therapy. *Int J Hematol.* 2021;113(3):337-343. https://doi.org/10.1007/s12185-021-03085-y
35. Kruse A, Abdel-Azim N, Kim HN, et al. Minimal residual disease detection in acute lymphoblastic leukemia. *Int J Mol Sci.* 2020;21(3):1054. https://doi.org/10.3390/ijms21031054
36. Szczepański T, Beishuizen A, Pongers-Willemse MJ, et al. Cross-lineage T cell receptor gene rearrangements occur in more than ninety percent of childhood precursor-B acute lymphoblastic leukemias: Alternative PCR targets for detection of minimal residual disease. *Leukemia.* 1999;13(2):196-205. https://doi.org/10.1038/sj.leu.2401277
37. Langerak AW, Groenen PJTA, Brüggemann M, et al. EuroClonality/BIOMED-2 guidelines for interpretation and reporting of Ig/TCR clonality testing in suspected lymphoproliferations. *Leukemia.* 2012;26(10):2159-2171. https://doi.org/10.1038/leu.2012.246
38. Langerak AW, Szczepański T, van der Burg M, et al. Heteroduplex PCR analysis of rearranged T cell receptor genes for clonality assessment in suspect T cell proliferations. *Leukemia.* 1997;11(12):2192-2199. https://doi.org/10.1038/sj.leu.2400887
39. Germano G, del Giudice L, Palatron S, et al. Clonality profile in relapsed precursor-B-ALL children by GeneScan and sequencing analyses. Consequences on minimal residual disease monitoring. *Leukemia.* 2003;17(8):1573-1582. https://doi.org/10.1038/sj.leu.2403008
40. Verhagen O, Willemse MJ, Breunis WB, et al. Application of germline IGH probes in real-time quantitative PCR for the detection of minimal residual disease in acute lymphoblastic leukemia. *Leukemia.* 2000;14(8):1426-1435. https://doi.org/10.1038/sj.leu.2401801
41. van der Velden VH, Cazzaniga G, Schrauder A, et al.; European Study Group on MRD detection in ALL (ESG-MRD-ALL). Analysis of minimal residual disease by Ig/TCR gene rearrangements: guidelines for interpretation of real-time quantitative PCR data. *Leukemia.* 2007;21(4):604-611. https://doi.org/10.1038/sj.leu.2404586
42. van der Velden VH, Noordijk R, Brussee M, et al. Minimal residual disease diagnostics in acute lymphoblastic leukaemia: Impact of primer characteristics and size of junctional regions. *Br J Haematol.* 2014;164(3):451-453. https://doi.org/10.1111/bjh.12621
43. Szczepański T, Velden VHJ van der, Raff T, et al. Comparative analysis of T-cell receptor gene rearrangements at diagnosis and relapse of T-cell acute lymphoblastic leukemia (T-ALL) shows high stability of clonal markers for monitoring of minimal residual disease and reveals the occurrence of second T-ALL. *Leukemia.* 2003;17(11):2149-2156. https://doi.org/10.1038/sj.leu.2403081
44. Szczepanski T, Willemse MJ, Brinkhof B, et al. Comparative analysis of Ig and TCR gene rearrangements at diagnosis and at relapse of childhood precursor-B–ALL provides improved strategies for selection of stable PCR targets for monitoring of minimal residual disease. *Blood.* 2002;99(7):2315-2323. https://doi.org/10.1182/blood.v99.7.2315
45. Pui CH, Relling MV, Downing JR. Acute lymphoblastic leukemia. *N Engl J Med.* 2004;350(15):1535-1548. https://doi.org/10.1056/NEJMra023001
46. Downing JR, Shannon KM. Acute leukemia: A pediatric perspective. *Cancer Cell.* 2002;2(6):437-445. https://doi.org/10.1016/S1535-6108(02)00211-8
47. Ajuba IC, Madu AJ, Okocha C, et al. Frequency and clinical impact of ETV6/RUNX1, AF4-MLL, and BCR/ABL fusion genes on features of acute lymphoblastic leukemia at presentation. *Niger J Clin Pract.* 2016;19(2):237-241. https://doi.org/10.4103/1119-3077.164351
48. Wu D, Sherwood A, Fromm JR, et al. High-throughput sequencing detects minimal residual disease in acute T lymphoblastic leukemia. *Sci Transl Med.* 2012;4(134):134ra63. https://doi.org/10.1126/scitranslmed.3003656
49. Wu D, Emerson RO, Sherwood A, et al. Detection of minimal residual disease in B lymphoblastic leukemia by high-throughput sequencing of IGH. *Clin Cancer Res.* 2014;20(17):4540-4548. https://doi.org/10.1158/1078-0432.CCR-13-3231
50. Wood B, Wu D, Crossley B, et al. Measurable residual disease detection by high-throughput sequencing improves risk stratification for pediatric B-ALL. *Blood.* 2018;131(12):1350-1359. https://doi.org/10.1182/blood-2017-09-806521
51. Wood BL, Wu D, Kirsch IM, et al. Residual disease monitoring by high throughput sequencing provides risk stratification in childhood B-ALL and identifies a novel subset of patients having poor outcome. *Blood.* 2016;128(22):1086. https://doi.org/10.1182/blood.V128.22.1086.1086
52. Pulsipher MA, Carlson C, Langholz B, et al. IgH-V(D)J NGS-MRD measurement pre-and early post-allotransplant defines very low-and very high-risk ALL patients. *Blood.* 2015;125(22):3501-3508. https://doi.org/10.1182/blood-2014-12-615757

53. Kotrova M, Muzikova K, Mejstrikova E, et al. The predictive strength of next-generation sequencing MRD detection for relapse compared with current methods in childhood ALL. *Blood.* 2015;126(8):1045-1047. https://doi.org/10.1182/blood-2015-07-655159
54. Huggett JF, Whale A. Digital PCR as a novel technology and its potential implications for molecular diagnostics. *Clin Chem.* 2013;59(12):1691-1693. https://doi.org/10.1373/clinchem.2013.214742
55. Huggett JF, Cowen S, Foy CA. Considerations for digital PCR as an accurate molecular diagnostic tool. *Clin Chem.* 2015;61(1):79-88. https://doi.org/10.1373/clinchem.2013.214742
56. Reinert T, Schøler LV, Thomsen R, et al. Analysis of circulating tumour DNA to monitor disease burden following colorectal cancer surgery. *Gut.* 2016;65(4):625-634. https://doi.org/ 10.1136/gutjnl-2014-308859
57. Waterhouse M, Follo M, Pfeifer D, et al. Sensitive and accurate quantification of JAK2 V617F mutation in chronic myeloproliferative neoplasms by droplet digital PCR. *Ann Hematol.* 2016;95(5):739-744. https://doi.org/10.1007/s00277-016-2623-0
58. Della Starza I, De Novi LA, Elia L, et al. Optimizing molecular minimal residual disease analysis in adult acute lymphoblastic leukemia. *Cancers.* 2023;15(2):374. https://doi.org/10.3390/cancers15020374
59. Pecoraro S, Berben G, Burns M, et al. *Overview and recommendations for the application of digital PCR*. Luxembourg: Publications Office of the European Union, ISBN 978-92-76-00180-5, Article 115736. https://doi.org/10.2760/192883
60. van der Velden VH, Cazzaniga G, Schrauder A, et al.; European Study Group on MRD detection in ALL (ESG-MRD-ALL). Analysis of minimal residual disease by Ig/TCR gene rearrangements: guidelines for interpretation of real-time quantitative PCR data. *Leukemia.* 2007;21:604-611. https://doi.org/10.1038/sj.leu.2404586
61. Fronkova E, Muzikova K, Mejstrikova E, et al. B-cell reconstitution after allogeneic SCT impairs minimal residual disease monitoring in children with ALL. *Bone Marrow Transplant.* 2008;42(3):187-196. https://doi.org/10.1038/bmt.2008
62. van Dongen JJ, van der Velden VH, Brüggemann M, et al. Minimal residual disease diagnostics in acute lymphoblastic leukemia: need for sensitive, fast, and standardized technologies. *Blood.* 2015;125(26):3996-4009. https://doi.org/10.1182/blood-2015-03-580027
63. Kotrova M, van der Velden VHJ, van Dongen JJM, et al. Next-generation sequencing indicates false-positive MRD results and better predicts prognosis after SCT in patients with childhood ALL. *Bone Marrow Transplant.* 2017;52(7):962-968. https://doi.org/10.1038/bmt.2017.16
64. Kotrova M, Muzikova K, Mejstrikova E, et al. The predictive strength of next-generation sequencing MRD detection for relapse compared with current methods in childhood ALL. *Blood.* 2015;126(8):1045-1047. https://doi.org/10.1182/blood-2015-07-655159
65. Eckert C, Flohr T, Köhler R, et al. Very early/early relapses of acute lymphoblastic leukemia show unexpected changes of clonal markers and high heterogeneity in response to initial and relapse treatment. *Leukemia.* 2011;25(8):1305-1313. https://doi.org/10.1038/leu.2011.89
66. Ladetto M, Brüggemann M, Monitillo L, et al. Next-generation sequencing and real-time quantitative PCR for minimal residual disease detection in B-cell disorders. *Leukemia.* 2014;28(6):1299-1307. https://doi.org/10.1038/leu.2013.375

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МІНІМАЛЬНА ЗАЛИШКОВА ХВОРОБА ПРИ ГОСТРІЙ ЛІМФОБЛАСТНІЙ ЛЕЙКЕМІЇ: МЕТОДИ ВИЯВЛЕННЯ ТА КІЛЬКІСНОГО ВИЗНАЧЕННЯ

Ідентифікація мінімальної залишкової хвороби (МЗХ) зазнала значного прогресу завдяки розвитку технологій, застосованих у цій галузі. Якщо спочатку вона базувалася виключно на морфології, згодом методологія визначення МЗХ зазнала докорінних змін та включила каріотипування, цитогенетику, проточну цитометрію та інші чутливі методи. В огляді обговорено ключові технічні аспекти успішного виявлення МЗХ і проаналізовано кореляцію між результатами, отриманими за допомогою проточної цитометрії та молекулярно-генетичних методів.

Ключові слова: МЗХ, проточна цитометрія, молекулярно-генетичні методи.