Vita et Sanitas
ISSN 1982-5951

Artigo

LABORATORY DIAGNOSIS OF MYELOID LEUKEMIA: A SYSTEMATIC REVIEW

DIAGNÓSTICO LABORATORIAL DA LEUCEMIA MIELOIDE UMA REVISÃO NARRATIVA

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RESUMO

Objetivo: Revisar a literatura acerca dos diagnósticos laboratoriais das leucemias mieloides, buscando demonstrar a importância de tais sobre o prognóstico e tratamento do paciente. **Metodologia:** Análise de conteúdos teóricos e práticos, já descritos na literatura sobre os diagnósticos laboratoriais da leucemia mieloide, com critério de inclusão referente a data de publicação (15 anos – 2011 a 2025). **Resultados:** O diagnóstico de leucemia mieloide (LM) se baseia no mielograma, que avalia a morfologia e percentual de blastos, e na tríade de técnicas essenciais: citogenética, imunofenotipagem e genética molecular. A imunofenotipagem utiliza citometria de fluxo para classificar as células, enquanto a citogenética analisa alterações cromossômicas com métodos como hibridização fluorescente in situ (FISH). A genética molecular identifica alterações no DNA com técnicas como Reação em Cadeia da Polimerase (PCR) e Sequenciamento de Sanger. Atualmente, novas técnicas de alta resolução, como Next Generation Sequencing (NGS), estão sendo estudadas para aumentar a precisão do diagnóstico, em conjunto aos métodos convencionais, pois oferece maior rapidez e precisão, apesar dos desafios de custo e complexidade. **Conclusão:** O diagnóstico da LM combina métodos tradicionais como o mielograma com técnicas advanced de imunofenotipagem e genética molecular para maior precisão. Embora o Whole Genome Sequencing (WGS) e o sequenciamento de Sanger ofereçam resultados de alta qualidade, seu alto custo e tempo de espera fazem com que os métodos convencionais continuem sendo o padrão-ouro, em conjunto a anamnese do paciente. Este estudo visa unificar esses procedimentos e técnicas para otimizar a eficiência do diagnóstico de LM.

Palavras-chave: Leucemia. Leucemia Mieloide. Diagnósticos Laboratoriais. Hematologia.

ABSTRACT

Objective: To review the literature on laboratory diagnoses of Myeloid Leukemias, seeking to demonstrate their importance for the prognosis and treatment of patients. **Methodology:** Analysis of the theoretical and practical content already described in the literature on laboratory diagnoses of Myeloid Leukemia, with inclusion criteria referring to the publication date (15 years – 2011 to 2025). **Results:** The diagnosis of Myeloid Leukemia (ML) is based on the myelogram, which evaluates the morphology and percentage of blasts, and on the triad of essential techniques: cytogenetics, immunophenotyping, and molecular genetics. Immunophenotyping uses flow cytometry to classify cells, while cytogenetics analyzes chromosomal alterations with methods such as fluorescence in situ hybridization (FISH). Molecular genetics identifies alterations in DNA with techniques such as polymerase reaction (PCR) and Sanger sequencing. Currently, new high-resolution techniques, such as Next-Generation Sequencing (NGS), are being studied to increase diagnostic accuracy, in conjunction with conventional methods, as they offer greater speed and precision, despite the challenges of cost and complexity. **Conclusion:** The diagnosis of LM combines traditional methods, such as myelography, with advanced immunophenotyping and molecular genetics techniques for greater accuracy. Although Whole Genome Sequencing (WGS) and Sanger sequencing offer high-quality results, their high cost and waiting time mean that conventional methods remain the gold standard. This study aims to unify these procedures and techniques to optimize the efficiency of LM diagnosis.

Keywords: Leukemia. Myeloid Leukemia. laboratory diagnostics. hematology.

INTRODUCTION

Myeloid leukemias (ML) are clonal disorders characterized by an increased number of blasts of the myeloid lineage in the bone marrow and peripheral blood. These immature cells, known as myeloblasts, lead to cytopenia of mature cells—such as leukocytes, erythrocytes, and platelets—which, in turn, is associated with an increase in immature leukocytic cells (myeloblasts). This process occurs due to the premature release of myeloblasts, which suppresses the normal maturation of myeloid cells (Nunes, 2022). The origin of this dysfunction lies in a neoplastic hematopoietic cell in the bone marrow, which promotes uncontrolled proliferation and excessive release of the myeloid lineage into the peripheral blood (Silva, 2025).

Due to pathological characteristics—such as nonspecific clinical manifestations including fatigue, weight loss, bone pain, and fever—diagnosis may be delayed. In many cases, the disease is identified incidentally, when patients seek medical care for other conditions (Hamerschlak, 2015). In this context, laboratory diagnosis is crucial for prognosis and treatment. With accurate results, patients can receive specific therapies that lead to significant improvement and potentially to cure (Santos, 2019).

ML is divided into acute myeloid leukemia (AML) and chronic myeloid leukemia (CML), both of which have a major impact on society due to their challenging prognosis and limitations of available treatments. AML predominantly affects individuals over 60 years of age and is characterized by excessive proliferation of myeloblasts in the bone marrow, resulting in neutropenia, anemia, and thrombocytopenia (Braga, 2020). Its origin is associated with genetic and epigenetic alterations—such as point mutations, gene rearrangements, deletions, and amplifications—that affect gene expression. This excessive proliferation of myeloblasts leads to their hyperpopulation in the bone marrow, resulting in reduced synthesis of other differentiated cells (Silva, 2021).

CML affects adults between 40 and 60 years of age and is characterized by a translocation between chromosomes 9 and 22, forming the genetic abnormality known as the Philadelphia chromosome (Ph) (Tunholi, 2023). This chromosome induces the production of the BCR-ABL protein, which stimulates the bone marrow to generate excessive granulocytic progenitors and megakaryocytes, as well as to release immature cells into the bloodstream. This excessive generation of progenitors leads to bone marrow overload, explaining the occurrence of bone pain (Boechat, 2017).

In the case of AML, classification follows the World Health Organization (WHO) system, which guides the definition of subtypes based on cytogenetic examinations—essential for appropriate treatment planning. In Brazil, however, there is a deficit in the application of this approach, resulting in less precise therapeutic decisions (Velloso, 2011). In light of this, the need for systematic reviews becomes evident in order to expand access to updated information on research and treatments.

With the increased use of routine laboratory tests, it has become increasingly common for ML to be discovered incidentally through abnormalities in complete blood count results—such as the presence of myeloblasts in peripheral blood—requested for reasons not directly related to suspicion of the disease (Osman, 2021).

The diagnosis of ML is established through flow cytometry (immunophenotyping), cytogenetic analysis (detection of mutated genes), and bone marrow aspiration (myelogram) with myeloblast counting. For the differential diagnosis between AML and CML, specific tests are used to guide diagnosis, such as alkaline phosphatase for AML and investigation of the molecular marker BCR-ABL and myeloperoxidase for CML (Sossela, 2017).

The objective of this review is to collect and centralize information from previous studies, providing a comprehensive and critical analysis of the available data. In this way, it aims to contribute to the expansion of scientific knowledge in the field and to offer support for the development of future research and investigations.

MATERIAL AND METHODS

The methodology adopted consisted of a systematic review based on the analysis of scientific articles addressing the laboratory diagnosis of myeloid leukemia. Initially, the scope of the study was defined by delimiting the relevant fields of practice, namely oncology and hematology. Subsequently, the research design was established, focusing on the analysis of systematic and narrative review articles addressing the different diagnostic approaches and the pathophysiology of myeloid leukemia (ML).

Next, the information sources were identified, including PubMed, SciELO, Google Scholar, and MEDLINE, which were used to conduct the search for relevant literature. A combination of related terms was employed, using the keywords “Leukemia,” “Myeloid Leukemia,” and “Laboratory Diagnosis.” These descriptors were selected based on controlled vocabularies from BIREME, LILACS, and DeCS, and combined using Boolean operators such as “AND” and “NOT” to refine the search strategy.

Clear inclusion and exclusion criteria were established, considering factors such as language, type of publication, publication period (2010 to 2025) (Figure 1), and relevance of the content to the research topic. The identified studies were then evaluated according to these criteria, and those that best met the proposed objectives were selected. The selected data were analyzed in order to identify patterns, trends, and gaps in the existing literature.

After the stepwise application of these parameters—including descriptors, Boolean operators, and inclusion and exclusion criteria—it was observed that the use of the descriptor “Leukemia” initially yielded 13,352 articles. After adding the descriptor “Myeloid Leukemia,” the number of results was reduced to 3,489 articles. Following the application of the inclusion and exclusion criteria, 80 articles remained, as illustrated in Figure 1.

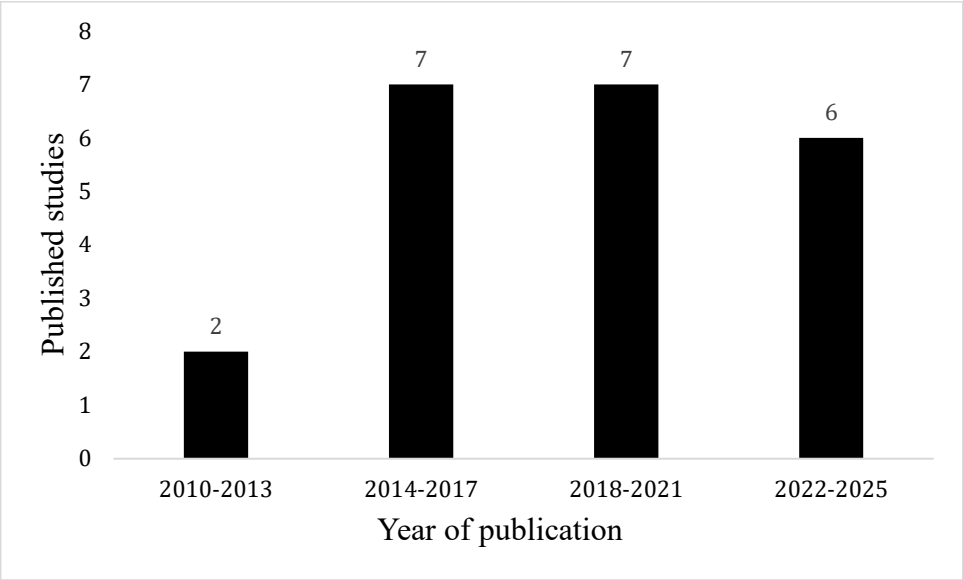

Figure 1. Studies included according to the review period (2010–2025).

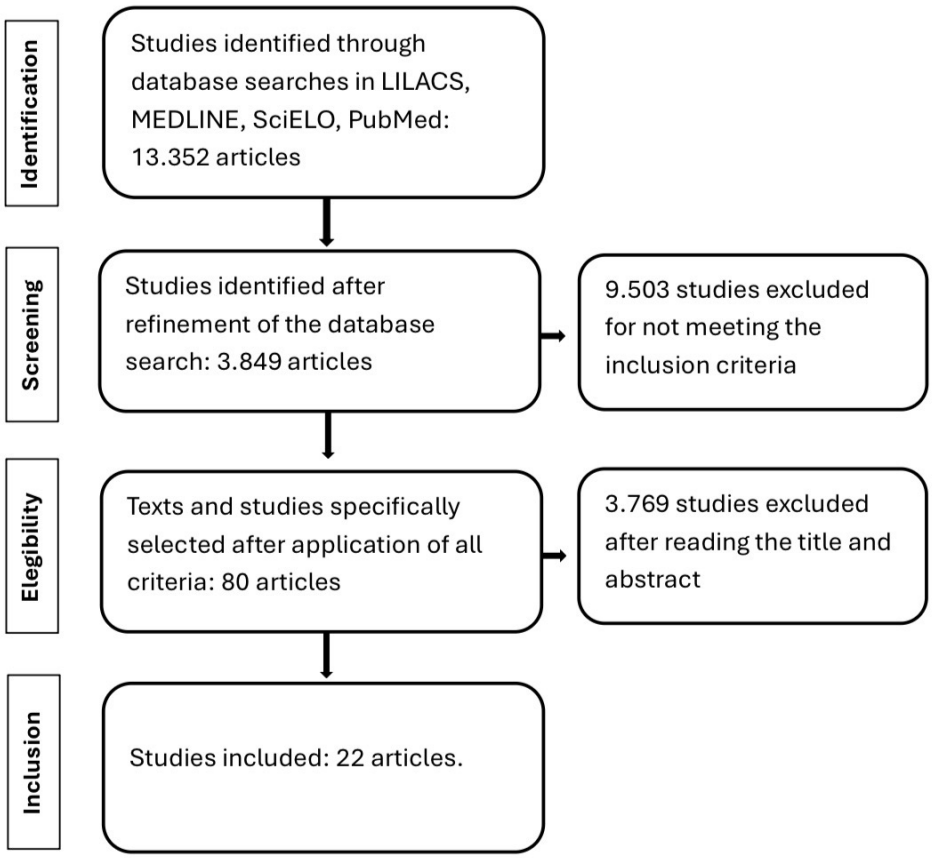

Figure 2. Flow diagram illustrating the identification, screening, eligibility, and inclusion of studies in the review according to PRISMA guidelines.

RESULTS

To synthesize the main findings and methodologies reported in the literature, Table 1 below provides a summary of the articles reviewed in this study. The table organizes the information according to author, year, and main focus, highlighting the specific diagnostic techniques and the contribution of each reference to the understanding of myeloid leukemia (ML) diagnosis.

Table 1. **Methodological characteristics and main findings of the evaluated studies.**

Authors/Year	**Study Type**	**Objective**	**Conclusion**
Nunes et al., 2022	Recommendation report	To evaluate the effectiveness of alpha-epoetin in adults with low-risk myelodysplastic syndrome.	Alpha-epoetin is effective in low-risk myelodysplastic syndrome, increasing hemoglobin levels and reducing transfusion requirements.
Silva, L.V.B., 2025	Specialization thesis	To evaluate the phenotype of HLA-DR–negative acute myeloid leukemias.	Although rare, the absence of HLA-DR in AML has diagnostic and prognostic relevance, and immunophenotypic characterization is essential for risk stratification and treatment definition.
Hamerschlak et al., 2015	Cross-sectional study	To assess Brazilian CML patients' perceptions of diagnosis and treatment.	Limited patient understanding highlights the need for improved physician–patient communication and health education.
Santos et al., 2019	Narrative review	To address diagnostic approaches and treatments for myeloid leukemia.	Early and accurate diagnosis is crucial for selecting appropriate therapy, including targeted therapies and bone marrow transplantation.
Braga, 2020	Literature review	To review clinical and therapeutic aspects of AML.	AML shows significant clinical and genetic heterogeneity, reinforcing the importance of molecular diagnosis to guide therapy.
Silva & Conceição, 2021	Systematic review	To evaluate advances in molecular diagnosis of AML.	Molecular biology has revolutionized clinical practice by improving classification, prognosis, and identification of therapeutic targets in AML.

Boechat et al., 2017	Pharmaceutical chemistry article	To describe synthesis and properties of BCR-ABL tyrosine kinase inhibitors.	Tyrosine kinase inhibitors had a major impact on CML treatment, enabling effective disease control.
Tunholi, 2023	Undergraduate thesis	To investigate mechanisms of progression and resistance to TKIs in CML.	Resistance to TKIs occurs due to mutations in the BCR-ABL gene and alternative signaling pathways, highlighting the need for second- and third-generation therapies.
Velloso et al., 2011	Review and case description	To report cytogenetic and molecular alterations in AML.	Cytogenetic and molecular alterations are decisive for AML diagnosis, prognosis, and treatment selection.
Osman & Deininger, 2021	International review	To review modern therapies and challenges in CML.	Despite the success of TKIs, challenges such as resistance, relapse, and patient adherence remain; future strategies focus on personalized therapies and functional cure.
Sossela et al., 2017	Review	To present clinical, diagnostic, and hemogram aspects of CML.	The complete blood count is essential for suspecting CML; molecular tests are required for confirmation and monitoring.
Oliveira et al., 2021	Review	To describe the pathophysiology, diagnosis, and treatment of CML.	Early molecular understanding and diagnosis enabled targeted therapies such as TKIs, significantly improving survival and quality of life.
Chen & Cherian, 2000	Review article	To describe the role of immunophenotyping in AML.	Immunophenotyping is essential for diagnosis, subtype identification, and prognostic stratification of AML.
Borim & Amilcar, 2017	Academic study	To emphasize differential diagnosis between leukemias and reactive conditions.	Careful evaluation is vital to avoid misdiagnosis and inappropriate therapies.

Bonfim et al., 2022	Review	To discuss the role of cytogenetics and molecular biology in CML.	Molecular tests are crucial for early diagnosis, therapeutic monitoring, and prognostic definition in CML.
Lago & Petroni, 2017	Systematic review	To address the pathophysiology and diagnosis of CML.	Molecular understanding and early diagnosis enabled targeted therapies such as TKIs, improving survival and quality of life.
Osman; Deininger, 2021	International review	To review modern therapies and challenges in CML.	TKIs transformed CML treatment; however, major challenges related to resistance, adherence, and definitive cure persist.
Duncavage et al., 2022	Translational study	To assess genomic profiling in myeloid neoplasms and AML.	Genomic profiling supports clinical decision-making in AML, enabling personalized therapies.
Reis, Visentainer & Macedo, 2017	Integrative review	To discuss molecular techniques in myeloproliferative neoplasms.	Molecular techniques increase diagnostic and prognostic accuracy in myeloproliferative neoplasms.
Haider et al., 2020	Clinical-laboratory study	To evaluate neutrophil scatter data as a pre-microscopic AML flag.	Neutrophil scatter data are promising for rapid screening of suspected AML, optimizing laboratory workflows and reducing diagnostic time.
Gajendra et al., 2023	Observational study	To analyze CD34−/HLA-DR− AML cases and their association with mutations.	The CD34−/HLA-DR− AML subtype is strongly associated with mutations such as NPM1 and FLT3-ITD, impacting prognosis and therapeutic decisions.
Bollmann & Del Giglio, 2011	Historical review	To review the evolution of CML treatment.	TKIs revolutionized CML treatment, transforming a fatal disease into a manageable chronic condition.

In myeloid leukemia (ML), diagnosis is primarily based on the bone marrow aspirate (myelogram), an examination that evaluates cell morphology through blood or bone marrow smears, aiming to identify the presence of myeloblasts. In addition to the myelogram, diagnosis relies on an essential diagnostic triad composed of cytogenetics, immunophenotyping, and molecular genetics, which allows differentiation and identification of the involved cell lineage. Other complementary tests include fluorescence in situ hybridization (FISH) and polymerase chain reaction (PCR), which are more sensitive and therefore important for diagnosis, therapeutic monitoring, and differentiation from other myeloproliferative disorders (Oliveira, 2021).

Immunophenotyping is a procedure derived from flow cytometry, which consists of suspending cells in a fluid stream and analyzing them using a laser beam. This technique provides qualitative and quantitative data on the cells and enables their differentiation. Immunophenotyping is essential in myeloid leukemias, as it allows the classification of cellular abnormalities related to the myeloid lineage (Chen & Cherian, 2017).

Among cytochemical assays, alkaline phosphatase staining is relevant because it indicates the presence of granules in mature neutrophils, helping to exclude a possible diagnosis of myeloid leukemia. Another important cytochemical test is myeloperoxidase staining, since this enzyme is present in myeloid precursors and its detection supports the diagnosis of myeloid leukemia (Borim, 2017).

Regarding cytogenetic techniques, cells at the maximum stage of mitotic condensation are obtained from bone marrow samples, allowing chromosome extraction and analysis of translocations, mutations, and deletions. Among these techniques, fluorescence in situ hybridization (FISH) uses fluorescently labeled probes to identify the chromosomal location of genetic alterations (Bonfim, 2022).

Through molecular genetic analyses, it is possible to identify alterations in specific DNA sequences at defined chromosomal loci. The main techniques include real-time PCR, in situ hybridization, and Sanger sequencing (Lago & Petroni, 2017).

Sanger sequencing enables the detection of small genetic variations in DNA using fragments amplified by PCR. In this method, individual DNA bases are detected by electrophoresis due to the random incorporation of fluorescently labeled dideoxynucleotide chain terminators by DNA polymerase during in vitro DNA replication. This technique is generally used to detect genetic mutations in single exons (Duncavage, 2022).

Currently, additional diagnostic techniques are being discussed for potential implementation, as they may replace conventional cytogenetic methods used in leukemia diagnosis due to their high resolution, speed, and accuracy. However, challenges remain for their routine adoption, including high costs and the complexity of result interpretation (Silva, 2021).

Among these emerging techniques, next-generation sequencing (NGS)—also known as massively parallel or high-throughput sequencing—uses millions to billions of parallel sequencing reactions to identify genomic abnormalities. Whole genome sequencing (WGS) is a derivative of NGS and involves comprehensive genomic sequencing assays that enable the detection of alterations across the entire genome. WGS functions as a broad genomic panel in which sequencing adapters are incorporated into sample DNA, allowing binding and detection of genomic alterations throughout the genome (Duncavage, 2022).

DISCUSSION

The laboratory diagnosis of myeloid leukemia is a complex process that requires the integration of multiple techniques, ranging from screening tests, such as the complete blood count (CBC) and cytochemical assays, to highly specific methods, including cytogenetics and molecular genetics (Reis, 2017).

Although often regarded as a screening test, the complete blood count plays a fundamental role in the initial suspicion of myeloid leukemia. Quantitative and morphological abnormalities in blood cells—such as the presence of immature leukocytes and blasts—can guide the diagnostic investigation, making the CBC an accessible and clinically relevant tool. Despite advances in techniques such as flow cytometry and molecular analyses, the initial hematological examination remains essential for indicating the need for confirmatory and more complex diagnostic methods (Haider, 2020).

The integration of diagnostic tests is indispensable, as each approach provides complementary information regarding cell morphology, genetic alterations, and the expression of specific markers, contributing to more accurate disease classification and treatment definition (Osman; Deininger, 2021).

Analysis of the data included in this review demonstrates that, as reported by several authors, the diagnostic triad composed of bone marrow examination (myelogram), immunophenotyping, and cytogenetics continues to represent the gold standard for confirming

myeloid leukemia, allowing differentiation between acute myeloid leukemia (AML) and chronic myeloid leukemia (CML) (Santos, 2019).

In AML, immunophenotyping has proven particularly relevant for subtype classification, as the expression of specific surface markers may be associated with distinct prognostic profiles (Gajendra, 2023). The use of flow cytometry, through qualitative and quantitative cellular analysis, enables precise identification of immunophenotypic abnormalities, confirms myeloid lineage involvement, and supports therapeutic decision-making.

In CML, detection of the Philadelphia chromosome by fluorescence in situ hybridization (FISH) or real-time PCR remains a determining criterion for diagnosis and disease monitoring. By identifying the BCR-ABL fusion gene, these methods allow not only diagnostic confirmation but also assessment of therapeutic response and potential resistance to tyrosine kinase inhibitors (TKIs) (Bollmann, 2011).

Cytochemical tests, such as alkaline phosphatase and myeloperoxidase staining, maintain clinical relevance, particularly due to their practicality and low cost, functioning as initial tools to suggest or exclude diagnostic hypotheses (Borim, 2017). However, although useful for screening, these methods present lower sensitivity and specificity, reinforcing the importance of confirmatory diagnostic tests.

Cytogenetic techniques continue to play a central role by enabling the analysis of translocations, deletions, and other chromosomal abnormalities from metaphase cells obtained from bone marrow samples. The use of methods such as FISH, which employs fluorescent probes to localize mutations, and conventional karyotyping remains widely recommended due to their ability to characterize both structural and numerical chromosomal abnormalities (Lago & Petroni, 2017). In parallel, molecular genetics expands this approach through techniques such as real-time PCR, in situ hybridization, and Sanger sequencing, allowing the identification of point mutations and specific alterations relevant to prognosis.

More recently, emerging technologies such as next-generation sequencing (NGS) and whole genome sequencing (WGS) have gained prominence due to their ability to detect genomic alterations with high resolution and broad coverage (Duncavage, 2022). These methods enable the simultaneous analysis of multiple genes and genomic regions, facilitating the identification of rare mutations and genomic patterns associated with therapeutic resistance. Despite their potential,

routine diagnostic implementation remains challenging due to high costs, the need for specialized infrastructure, and the complexity of result interpretation.

CONCLUSION

This literature review highlights that the laboratory diagnosis of myeloid leukemia (ML) is a complex yet essential process for early clinical definition and selection of the most appropriate treatment. Conventional methods, such as the bone marrow examination, remain important initial tests for identifying the presence of blasts. However, advances in immunophenotyping, cytogenetics, and molecular genetics have significantly increased diagnostic sensitivity and specificity, enabling not only differentiation of myeloid leukemias from other hematological disorders but also the identification of subtypes and clinically relevant genomic alterations.

This study also emphasizes the role of additional diagnostic procedures, such as whole genome sequencing (WGS) and Sanger sequencing, which, despite providing highly specific and qualified diagnostic information, are associated with high costs and longer turnaround times. Consequently, established diagnostic methods continue to represent the gold standard in routine clinical practice.

Overall, this review contributes to improving the understanding of myeloid leukemia diagnosis by consolidating standard procedures and emerging technologies into a single article, thereby supporting more efficient diagnostic and prognostic strategies and reinforcing the perspective of personalized management of myeloid leukemias.

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