Editorial

Role of cytochemical staining in differentiate types of acute leukemia

Natsaruth Songthawee

Division of Hematology and Oncology, Department of Pediatrics, Faculty of Medicine, Prince of Songkla University, Hat Yai

Acute leukemia is the most common malignancy in children and can be classified into acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML). ALL can be further subclassified into B-cell and T-cell ALL, which is important for designing therapeutic plans and predicting the prognosis. Several methods are available to differentiate the subtypes of acute leukemia, including morphology, cytochemical staining, immunophenotyping, cytogenetics and molecular genetics. Immunophenotyping, using either immunohistochemical staining and/or flow cytometry, is accurate and widely used for lineage classification–i.e., differentiating between AML and ALL, as well as B-cell and T-cell ALL. Additionally, this technique can further subclassify ALL according to the maturation stage, such as precursor, common, pre-B-cell ALL, or early T-cell precursor ALL. With the advancement of molecular diagnostics, the classification of acute leukemia has been refined based on the fifth edition of the World Health Classification of Tumors of the Hematopoietic and Lymphoid Tissue, which is essential for risk stratification and treatment planning.[1,2]

Microscopic assessment of blast cell morphology can aid in distinguishing between ALL and AML. However, in some cases, blast cells are difficult to classify based on morphology alone. Cytochemical staining, a simple, rapid and low-cost technique, can be used to distinguish between these subtypes in such cases. The most commonly used cytochemical stains include myeloperoxidase (MPO), Sudan Black B (SBB), combined esterase (CEA), non-specific esterase (NSE), Periodic Acid-Schiff (PAS) and acid phosphatase (AP).

MPO is a lysosomal enzyme found in the azurophilic granules of neutrophils and their precursors, as well as in eosinophils and monocytes. The main purpose of MPO staining is to differentiate between AML and ALL, as MPO positivity is highly specific for AML and acute promyelocytic leukemia (APL). SBB stains phospholipids and neutral fats contained in neutrophilic granules. Since lymphoblasts are SBB-negative, this stain is used as an adjunct to MPO and CAE staining.

CEA and NSE are used to distinguish AML subtypes, such as granulocytic, monocytic or megakaryocytic origin. CAE is present in myeloid cell granules and shows negativity in most myeloblasts, erythroblasts and megakaryocytes, while showing positivity in APL and some AML cases. Its staining pattern is similar to that of MPO and SBB. NSE is positive in monocytic and megakaryocytic lineages, and is particularly useful in diagnosing acute monoblastic/monocytic leukemia (AML-M5) and acute megakaryoblastic leukemia (AMKL).

PAS stains polysaccharides such as glycogen, glycoproteins, glycolipids and mucins. It shows positivity in mature neutrophils and cells of the megakaryocytic lineage. PAS is typically positive in ALL, APL, AMKL and acute erythroleukemia, although staining patterns vary depending on the differentiation stage of blasts in AML.

AP is used as a supporting stain for T-cell ALL. Although it is present in all hematopoietic cells and may show positivity in AML, APL, AML-M5 and AMKL, it is generally used as an adjunctive test to support diagnosis.[3]

Correspondence to: Natsaruth Songthawee, Division of Hematology and Oncology, Department of Pediatrics, Faculty of Medicine, Prince of Songkla University, Hat Yai

The correlation between morphology and cytochemical staining and immunophenotype is variable. The concordance rate ranges from 66.7% to 87.5% in ALL and 91.6% to 100% in AML patients.[4-8] Despite advances in diagnostic technology, cytochemical staining remains valuable as a rapid, low-cost diagnostic tool-especially useful for guiding urgent management, such as cytoreduction therapy, before definitive treatment is initiated. Moreover, it continues to play an essential role in differentiating leukemia subtypes in settings where advanced diagnostic techniques are not readily available.

References

1. *Loghavi S, Kanagal-Shamanna R, Khoury JD, Medeiros LJ, Naresh KN, Nejati R, et al. Fifth edition of the World Health Classification of Tumors of the Hematopoietic and Lymphoid Tissue: myeloid neoplasms. Mod Pathol. 2024;37:100397.*
2. *Alaggio R, Amador C, Anagnostopoulos I, Attygalle AD, Araujo IBO, Berti E, et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: lymphoid neoplasms. Leukemia. 2022;36:1720-48.*
3. *Paessler ME, Helfrich M, Wertheim GBW. Cytochemical staining. Methods Mol Biol. 2017;1633:19-32.*
4. *Resende GA, Gileno MD, Moraes-Souza H, Carlos AM, Leal AS, Martins PR. The role of cytochemistry in the diagnosis of acute leukemias. IJHSR. 2017;7:290-5.*
5. *Belurkar S, Mantravadi H, Manohar C, Kurien A. Correlation of morphologic and cytochemical diagnosis with flowcytometric analysis in acute leukemia. J Cancer Res Ther. 2013;9:71-9.*
6. *Ahmad N, Kumari N, Tirkey D, Mahto SK, Jawaid M. Evaluation of acute leukaemias by flow cytometry and its correlation with diagnosis using morphological and special staining techniques. Cureus. 2024;16:e54126.*
7. *Mhawech P, Buffone GJ, Khan SP, Gresik MV. Cytochemical staining and flow cytometry methods applied to the diagnosis of acute leukemia in the pediatric population: an assessment of relative usefulness. J Pediatr Hematol Oncol. 2001;23:89-92.*
8. *Pandey G, Nichlani R, Jalaly F, Durrani N, Ali MA. Correlation of cytomorphology with flow cytometric immunophenotyping in patients of acute leukemia in tertiary care hospital. J Pathol Nep. 2022;12:1917-21.*