VOLUME - 13, ISSUE - 07, JULY - 2024 • PRINT ISSN No. 2277 - 8160 • DOI : 10.36106/gjra

Original Research Paper

Pathology

CHALLENGING MORPHOLOGICAL DIAGNOSIS IN ACUTE LEUKAEMIA - A STUDY OF TWO CASES

Shaily Goyal Senior Resident Department of Pathology, Vardhman Mahavir Medical College & Safdarjung Hospital, New Delhi, India.

Chintamani Pathak* Professor, Department of Pathology, Vardhman Mahavir Medical College & Safdarjung Hospital, New Delhi, India. *Corresponding Author

Sunil Ranga Professor & Head, Department of Pathology, Vardhman Mahavir Medical College & Safdarjung Hospital, New Delhi, India.

ABSTRACT Acute leukemias are characterized by the unrestricted proliferation of lymphoid or myeloid blasts, typically defined as 20% or more in peripheral blood or bone marrow. A peripheral smear test and a complete blood count are the first steps in the diagnosis of acute leukemia. These cases often show anemia, leukocytosis/leucopenia, and thrombocytopenia. Cell morphology is crucial for diagnosis and helps with further assessment, but it can also be misleading in some situations. Morphological identification of blasts as lymphoblasts or myeloblasts is the initial point to classify acute leukemia, however diagnosis requires integrating clinical history, morphology, immunophenotype, and molecular analysis. This article illustrates the significance of flow cytometry and cytogenetics in leukemia cases by presenting two examples with deceptive morphology. Relying solely on morphology alone may result in a mistaken diagnosis.

KEYWORDS : Acute leukemia, morphology, flow cytometry, cytogenetics

INTRODUCTION

Acute leukemias are characterized by the unrestricted proliferation of either lymphoid or myeloid blasts, or both, and are classically defined as 20% or more in either peripheral blood or bone marrow [1,2]. In most of these cases complete hemogram and peripheral smear reveal anaemia, leukocytosis/leucopenia, and thrombocytopenia with the presence of 20% or more circulating blasts. It is not uncommon to find < 20% blasts in the peripheral smear, however, bone marrow shows >20% blasts. This is known as "sub-leukemic leukemia." In aleukemic leukemia, there are no blasts in the peripheral smear, but marrow or imprint smear would show >20% blasts [2, 3].

Due to advancements in molecular genetics, there are very few situations in which this general rule is not applicable. The World Health Organization has classified some cases of acute myeloid leukemia (AML) as such, regardless of blast count, based on their molecular results. It covers instances where translocations like t(8:21), t(15:17), inv(16), or t(16:16) have been found, together with the corresponding transcripts, as shown by fluorescence in situ hybridization or molecular tests like polymerase chain reaction.[4, 5]

The diagnosis of acute leukemia requires integration of clinical history, morphology, immunophenotype, and molecular analysis, However, the results of molecular genetic and cytogenetic investigations might not be accessible right away, therefore morphology, in addition to immuno-phenotyping, plays a role in quickly identifying a likely diagnosis.[6]

The initial point to classify acute leukemia starts with the morphological identification of blasts as lymphoblasts or myeloblasts. Acute Lymphoblastic Leukemia (ALL) is, however, more common than Acute non-lymphoblastic Leukaemia. (kishor m) The morphological hallmark to identify the myeloblasts is the presence of "Auer rods" which are defined as needle-like crystals, comprised of condensation of azurophilic granules present in the cytoplasm [7].

Lymphoblasts are described as small to medium-sized blasts, with a high nuclear-cytoplasmic ratio, moderately condensed to dispersed chromatin, inconspicuous nucleoli, and scant basophilic and agranular cytoplasm. The presence of fine or coarse azurophilic granules, vacuoles, or inclusions in the cytoplasm does not eliminate it as lymphoblasts, if the cytochemistry or Immunophenotype is consistent with their diagnosis, however Auer rods are not found in lymphoblasts [8, 9].

Myeloblasts are delineated as large-sized blasts, with high nuclear-cytoplasmic ratio, opened up nuclear chromatin, and presence of 2 to 3 prominent nucleoli. The cytoplasm is scant to moderate and mostly shows the presence of azurophilic and secondary granules [4]. Acute Lymphoblastic Leukemia (ALL) is, however, more common than Acute non-lymphoblastic Leukaemia.

However, in practice sometimes morphological opinion does not correlate with flowcytometry and cytogenetics. In this article, we present two cases that showed unconventional morphological features, which may lead to wrong morphological interpretation.

Case History

Case 1- A 39-year-old male presented to the emergency outpatient department with fever, abdominal pain, and lethargy. On examination, moderate hepato-splenomegaly was present. His complete blood count revealed a total leucocyte count of 17500/mm^3, hemoglobin 7.8gm%, and platelet count of 60000/mm^3. Differential leucocyte count showed 66% blasts with, myelocytes 06%, neutrophils 12%, and lymphocytes 16%. These blasts were 3-5 times the size of small mature lymphocytes, having a high N:C ratio, opened up chromatin, and 1-2 prominent nucleoli in many blasts. Cytoplasm was scant to moderate and revealed granules in a few blasts. Morphological opinion favored myeloblasts. Cytochemistry was performed for MPO, which was negative for blasts. A provisional diagnosis of MPO-negative acute leukemia was made.

Flow cytometry was performed with peripheral blood. 62% of blasts (CD 45 dim) were gated, which showed moderate expression of CD 19 & CD 10, dim expression of CD 20, and dim to moderate expression of HLA-DR. Markers for T-cell lineage and myeloid lineage were negative. Impression of Acute lymphoid leukemia was made. Karyotyping of patient revealed 68 chromosomes with +X+Y+1+1+4+4+5+6+8+9+10+11+13+14+18+19+20+20+21+21+22+22.

Case 2- A 49-year-old male presented with fever and

weakness. On examination, mild hepatosplenomegaly was seen. His complete blood count showed a total leucocyte count of 13,600/mm^3, hemoglobin 9.3gm%, and platelets 81,000/mm^3. Differential leucocyte count revealed 86% blasts and 10% lymphocytes. These blasts were 1.5 to 2 times the size of small lymphocytes, having a high N:C ratio, irregular nuclear membrane, coarse chromatin, 0-1 inconspicuous nucleoli, and scant agranular cytoplasm. Cytochemistry for MPO was negative.

Flow cytometry was performed with peripheral blood on which, 82% of blasts were gated which expressed dim CD13, moderate to bright CD33, and dim to moderate CD34. Markers for T and B cell lineage were negative. A diagnosis of Acute myeloid leukemia with minimal differentiation was given. Cytogenetic study could not be performed since we lost patient follow-up.

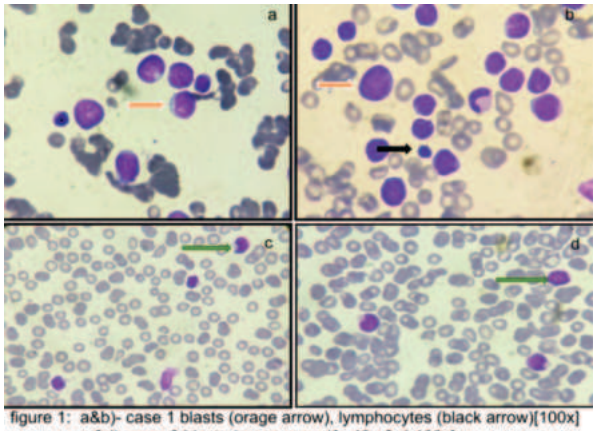

figure 1: a&b)- case 1 blasts (orage arrow), lymphocytes (black arrow)[100x]
c &d)- case 2 blasts (green arrow)[c-40x & d-100x]

DISCUSSION

Diagnosis of acute leukemia starts with the complete blood count and morphological examination of either peripheral blood smear or bone marrow. "Auer rods" are considered the morphological hallmark of the myeloblast [7]. Hand-mirror cells is cytoplasmic pseudopods, which are seen in some cases of ALL but it has also been described in rare instances of AML.[8]

Cytochemical stains such as myeloperoxidase, Sudan Black B, periodic acid Schiff stain, nonspecific esterase, and Perls' stain complement the morphological diagnosis especially in resource-limited settings as cytochemistry improves diagnostic accuracy.[8]

The recent World Health Organization classification still validates the utility of morphology which requires either 20% lymphoblasts, myeloblasts/or its equivalents (monoblasts, promonocytes, or megakaryoblasts) and integrates it with the clinical features, immunophenotyping (IP), and molecular genetics for making the diagnosis of acute leukemia. Morphology can give clues to the specific diagnosis of acute myeloid leukemia like acute promyelocytic leukemia, monocytic, and monoblastic leukemia. There are some attractive features such as blasts with "hand-mirror" morphology, nuclear cleavage, prominent cytoplasmic vacuoles, differentiating lymphoid and myeloid leukemias. Morphological examination in acute leukemia is not only helpful for diagnosis but also useful for predicting the prognosis in post-treatment cases [5]. However in a few cases, like in these cases morphology contradicts the immunophenotype and final diagnosis. In the first case, morphology revealed myeloblasts due to their large size, prominent nucleoli in many blasts, and granules in few blasts. On immunophenotype, it turned out to be Acute lymphoid leukemia.

In the second case small-sized blasts with inconspicuous nucleoli, scant cytoplasma and many hand mirror blasts suggested lymphoid origin of blasts which on flowcytometry unfolded as Acute myeloid leukemia.

During our literature search, we came across a few examples that had peculiar morphology. Similar to the findings in our single case, David C. et al. also discovered a case exhibiting B cell immunophenotype with shape resembling myeloid leukemia.[10]

Few cases with misleading morphology are described in literature like Cytoplasmic vacuoles were discovered by Kiran K. et al in a case of acute undifferenciated leukemia.[11] AML with a 5q deletion instance was discovered to have misleading APML morphology by Shoraya W et al.[12]

Hua J et al found a case of AML resembling acute promyelocytic leukemia on morphology.[13] Because the lymphoblasts have cytoplasmic granules, granular acute lymphoblastic leukemia (G-ALL) can be mistakenly diagnosed as acute myeloid leukemia (AML). Although it is typically observed in youngsters, adults may also have this ALL variation.[14]

As morphology can be deceiving in certain circumstances, the discrepancy between morphology and immunophenotype and ultimate diagnosis emphasizes the significance of flowcytometry and cytogenetics for the latter. In resource-constrained settings where flow cytometry and cytogenetics are unavailable, morphology becomes extremely significant. Nevertheless, given the foregoing conditions, these investigations become extremely crucial to prevent incorrect diagnosis and therapy. Since every case is different, getting the right diagnosis is crucial to the patient's course of care. If APML is treated early, the prognosis is favorable. Few studies indicate that prognosis of G-ALL is poor as compared to ALL. [14]

CONCLUSION

The morphology can give clues for diagnosis of acute leukemias, however with the availability of immunophenotype and molecular genetics it should not be entrusted fully. Morphology is considered a starting point for the analysis of acute leukemia and in some cases, it gives information about specific cytogenetic abnormalities too, but in a few cases, it may contradict the final diagnosis. In view of the above cases immunophenotype or cytogenetics is necessary for the final diagnosis and should always be used as an adjunct to the morphology.

Authorship Contribution:
All authors contributed to the study's conception and design. Shaily Goyal searched the literature and written the article and Chintamani Pathak and Sunil Ranga has reviewed, critically revised, approved and granted permission for the article. All authors read and approved the final manuscript.

Conflict of Interest:
None

Funding:
None

REFERENCES

1. Kansal R. Toward integrated genomic diagnosis in routine diagnostic pathology by the World Health Organization classification of acute myeloid leukemia. J Clin Haematol. 2020;1:33–53. DOI:https://doi.org/10.33696/haematology.1.006.
2. Singh T. Atlas and Text of Hematology. New Delhi: Avichal Publishing Company; 2010:136
3. Bates I, Bain BJ, eds. Approach to the Diagnosis and Classification of Blood Diseases. Dacie and Lewis Practical Haematology. China: Elsevier; 2012:549.
4. Swerdlow SH, Campo E, Harris NL, eds. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Lyon, France: IARC Press; 2017
5. Sekar, Mithraa & Raj, Manasa & Manivannan, Prabhu. (2023). Role of Morphology in the Diagnosis of Acute Leukemias: Systematic Review. Indian Journal of Medical and Paediatric Oncology. 44. 10.1055/s-0043-1764369.

6. Barbara J. Bain, Marie C. Béné; Morphological and Immunophenotypic Clues to the WHO Categories of Acute Myeloid Leukaemia. Acta Haematol 15 May 2019; 141 (4): 232–244. https://doi.org/10.1159/000496097
7. Ladines-Castro W, Barragán-Ibañez G, Luna-Pérez MA, et al. Morphology of leukaemias. Rev Med Hosp Gen (Mex) 2016; 79:107–113
8. Löffler H, Gassmann W. Morphology and cytochemistry of acute lymphoblastic leukaemia. Baillieres Clin Haematol 1994;7(02): 263–272.
9. Bashir, Dr. (2019). Bone Marrow Spectrum of Acute Leukaemia's at a Tertiary Care HospitalAn Experience. journal of Medical Science And clinical Research. 7. 10.18535/jmscr/v7i4.126.
10. David C. et al, Challenging diagnosis in a patient with clear lymphoid immunohistochemical features and myeloid morphology: Mixed phenotype acute leukemia with erythrophagocytosis,Leukemia Research,Volume 35, Issue 5,2011,Pages 693-696,ISSN 0145-2126, https://doi.org/10.1016/j.leukres.2011.01.024.
11. Kiran K, Narender Kumar, Man Updesh Singh S, Subhash Varma (2013) An Uncommon Morphology of Acute Undifferentiated Leukemia: Report of a Rare Case. J Bone Marrow Res 1: 103. doi:10.4172/2329-8820.1000103
12. Soraya Wuillème, Yannick Le Bris; Misleading acute promyelocytic leukemia morphology. Blood 2015; 125 (24): 3815. doi: https://doi.org/10.1182/blood-2015-02-616904.
13. Hua J, Bao X, Xie Y. A Rare Morphology Resembling APL with t (11;12) (p15;q13) in Acute Myeloid Leukemia: Case Report and Literature Review. Clin Lab. 2019 Oct 1;65(10). doi: 10.7754/Clin.Lab.2019.190236. PMID: 31625367.
14. Kishore M, Kumar V, Marwah S, Nigam AS. Unusual Presentation of Acute Leukaemia: A Tripod of Cases. J Clin Diagn Res. 2016 Oct;10(10):ED04-ED08. doi: 10.7860/JCDR/2016/21472.8715.