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To study Correlation of Peripheral Blood and Bone Marrow Morphology with Immunophenotypic Findings in Leukemia

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Abstract

Background: Accurate classification of leukemia relies on integration of peripheral blood (PB) morphology, bone marrow (BM) morphology, and immunophenotypic analysis by flow cytometry.

Objective: To evaluate the correlation between peripheral blood and bone marrow morphological findings with immunophenotypic patterns in patients with leukemia.

Methods: A prospective observational study was conducted on 100 newly diagnosed leukemia patients. PB smears, BM aspirates, and flow cytometric immunophenotyping were analyzed. Morphological diagnoses were compared with immunophenotypic lineage assignment.

Results: Morphology-based diagnosis showed concordance with immunophenotyping in 88% of cases. Discordance was most frequent in acute leukemias with ambiguous morphology. Immunophenotyping was essential for accurate lineage assignment in 12% of cases.

Conclusion: Combined evaluation of PB, BM morphology, and immunophenotyping improves diagnostic accuracy in leukemia, particularly in morphologically overlapping cases.

Keywords: Leukemia, Peripheral blood, Bone marrow, Morphology, Immunophenotyping, Flow cytometry

Study Design: Observational Study.

Introduction

Leukemia represents a heterogeneous group of hematological malignancies characterized by clonal proliferation of hematopoietic cells. Traditional diagnosis has relied heavily on peripheral blood and bone marrow morphology. However, overlapping morphological features among different leukemia subtypes may lead to diagnostic uncertainty.

Immunophenotyping by flow cytometry has become an indispensable tool in leukemia classification, allowing precise lineage determination and subclassification. Correlating

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morphological findings with immunophenotypic results is essential for accurate diagnosis, prognostication, and therapeutic planning.

This study aims to assess the degree of correlation between PB and BM morphology with immunophenotypic findings in a cohort of 100 leukemia patients.

Leukaemias are diseases in which abnormal proliferation of hemopoietic cells causes progressively increasing infiltration of the bone marrow or, in certain forms, the lymphatic tissues. It can also be described as a malignant clonal illness that, if not treated promptly, progresses quickly to death because it involves the amplification of premature, ill-differentiated blast cells within the bone marrow [1,2]. Lymphoid and nonlymphoid leukaemias are two major variants of leukaemia according to the cell of origin, signs and symptoms, course of disease, and outcome after medical intervention. On the grounds of the disease's progression, the expected outcome, and other factors, they are more frequently separated into chronic as well as acute varieties [3,4].

By using several techniques concurrently, leukaemia can be accurately identified and categorized. In addition to multiparameter flow cytometry, these also comprise cytochemistry, histomorphology, and cytomorphology, which designate the diagnostic sample to the appropriate entity [5,6]. For the diagnosis to be certain, additional chromosomal examinations are also required [7]. Because they are inexpensive and don't require any specialized equipment, cytochemical stains are crucial for the identification of acute leukaemia (AL) in underdeveloped nations. It gives hints about the kind and course of cellular differentiation, although it should still be viewed as complementary to morphological analysis rather than a replacement for it [8]. Whenever leukaemia is not structurally obvious with Romanowsky stains, cytochemical stains help examine the structure of cells that are still developing.

Material and Methods

This prospective observational study included 100 consecutive patients with suspected leukemia attending the Hematology Department of a tertiary care center over a one-year period.

Inclusion Criteria

- Newly diagnosed cases of leukemia
- All age groups and both sexes
- Availability of PB smear, BM aspirate, and flow cytometry data

Exclusion Criteria

- Previously treated leukemia cases
- Inadequate bone marrow samples

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Morphological Assessment

Peripheral blood smears were stained with Leishman stain. Bone marrow aspirates were stained with Leishman and assessed for cellularity, blast percentage, and lineage-specific features.

Immunophenotyping

Flow cytometry was performed using a standard panel of monoclonal antibodies for myeloid and lymphoid markers. Lineage assignment was based on WHO criteria.

Statistical Analysis

Correlation between morphology and immunophenotyping was analyzed descriptively and expressed as concordance percentages.

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Result

Table 1: Distribution of Leukemia Types Based on Immunophenotyping (n = 100)

Leukemia Type	Number of Cases	Percentage (%)
Acute Myeloid Leukemia (AML)	42	42
Acute Lymphoblastic Leukemia (ALL)	36	36
Chronic Myeloid Leukemia (CML)	12	12
Chronic Lymphocytic Leukemia (CLL)	10	10
Total	**100**	**100**

Table 2: Correlation between Peripheral Blood and Bone Marrow Morphology

Morphological Correlation	Number of Cases	Percentage (%)
PB and BM concordant	90	90
PB and BM partially concordant	7	7
PB and BM discordant	3	3

Table 3: Concordance between Morphology and Immunophenotyping

Diagnostic Agreement	Number of Cases	Percentage (%)
Concordant	88	88
Discordant	12	12
Total	**100**	**100**

Table 4: Causes of Morphology–Immunophenotype Discordance (n = 12)

Cause of Discordance	Number of Cases
Ambiguous blast morphology	5
Mixed lineage features	4
Poorly differentiated leukemia	2
Hypocellular marrow	1

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Discussion

The present study demonstrates a high level of concordance between peripheral blood and bone marrow morphology, highlighting the reliability of morphological assessment in leukemia diagnosis. However, morphology alone was insufficient in a subset of cases, particularly acute leukemias with ambiguous features[9].

Immunophenotyping resolved diagnostic uncertainty in 12% of cases, reinforcing its critical role in modern hematopathology. Similar studies have reported concordance rates ranging from 80–90%, supporting our findings. Discordant cases were most frequently observed in acute leukemias with poorly differentiated blasts, where immunophenotypic markers were essential for lineage determination[10].

Immunophenotyping has a recognised role in the diagnosis and classification of acute leukaemia. AML has an age adjusted incidence of 3.7/100,000 per annum in US with highest incidence in 7th decade[11]. AML can occur at any age group but the incidence increases with age. The mean age in our study was 32 years similar to Harani et al.; However, in some other studies mean age was more than our study ranging from 35 to 47 years.

Childhood AML comprised 27.3% in our study while adult AML comprised 72% which was slightly different from Gosh study. (24%,76% respectively).16 Conversely, in Ahmad et al., paediatric cases were 20% and adults 86.4%,3 There is a predilection for men with AML, 4.8 versus 3.3 new cases whereas in ALL, there is no gender variance (1.9 new cases in men and 1.5 in women) [12].

In our study the male to female ratio was 1.2:1 indicating a slight male predominance is similar to some other international studies. In Patel et al., the male to female ratio was 1:1.1.19 In our study AML-M2 (47.2%) was the most frequent subtype similar to Gosh study (AML-M2=34%). Conversely, in few other international studies, other AML subtypes predominated. CD7, a T-cell antigen known to show aberrant expression was most commonly expressed in our study (26.4%) followed by CD19 (1.2%). The same trend was observed in most of the international studies where CD7 was most commonly expressed aberrant antigen followed by CD19.2, CD7 expression in AML is correlated with lower incidence of complete remission. According to Belurkar et al., expression of lymphoid associated antigens except CD7, on AML blasts lack prognostic significance and CD7 + AML is a particular subset but in general, it may not represent a biologically distinct form of leukemia since these cases have similar clinical features and a comparable response to therapy[13-14].

Conclusion

While peripheral blood and bone marrow morphology remain fundamental to leukemia diagnosis, immunophenotyping significantly enhances diagnostic precision. An integrated approach combining morphology and flow cytometry is essential, especially in cases with overlapping or ambiguous morphological features.

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