Original Article

Evaluation of Clonality in Patients with B-cell Lymphoid Malignancies by Immunohistochemistry Panel and Flow Cytometry Analysis

Clonality Detection with B-cell Lymphoblastic Leukemia

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ABSTRACT

Objective: To assess various methods for clonality detection in patients with B-cell lymphoblastic leukemia.
Study Design: Descriptive diagnostic study
Place and Duration of Study: This study was conducted at the Department of Pathology and Forensic Medicine, Faculty of Medicine, University of Kufa, from October 2023 to June 2024.
Methods: This descriptive diagnostic study was carried out with cases (67 patients) sourced from the Hematology Department of Baghdad Medical City/Educational Lab. Diagnoses were based on the gold-standard flow cytometry test for B-cell acute lymphoblastic leukemia. Age, gender, clinical features, complete blood count, blood smear, and bone marrow morphology reports were collected. Cytochemical tests (Periodic acid-Schiff & Sudan Black B) were performed on fresh bone marrow aspirates.
Results: The mean patient age was 30.74 years, with a male-to-female ratio of 1:2.04. Cytochemical tests, Sudan Black B exhibited high specificity (100%), while periodic acid-Schiff had high sensitivity (65.67%).
Conclusion: The Periodic acid-Schiff cytochemical test, with its high sensitivity, offers valuable diagnostic utility in low-resource laboratories lacking access to immunohistochemistry and flow cytometry.
Key Words: Clonality detection, B-cell lymphoblastic leukemia, Flow cytometry, Immunohistochemistry, Cytochemical stain

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INTRODUCTION

Hematologic cancer, such as acute lymphoblastic leukemia (ALL), is on the rise in healthcare systems as global populations age.[1] This is a fast-growing, aggressive cancer characterized by the proliferation of immature lymphoid cells that infiltrate the blood, bone marrow, and other body tissues. Immunophenotypic profiles can be used to classify ALL into two types: B-cell and T-cell. B-cell ALL (B-ALL) has Early Pre-B ALL (10%), Common ALL (50%), and Mature B-cell ALL (4%), but T-cell ALL subtypes have Pre-T ALL (5-10%), and Mature T-cell ALL (15-20%).[2]
The French-American-British (FAB) system is a classification that all cells are classified according to their morphology, where the L1 cells are small, L2 cells are larger with irregular nuclei, and L3 cells are similar to the Burkitt leukemia. Leukemia refers to the cancer of blood and bone marrow, and it is described as the uncontrolled growth of the abnormal white blood cells (leukemic blasts). These immature cells proliferate and surround the normal cells, causing bone marrow failure and possibly spreading of the disease to other organs like the lymphocytes, liver, and spleen, among others. Leukemia is classified into four major groups which depend on the rate of progression (acute and chronic) and the lineage of the affected blood cell (lymphocytes, lymphocytes): acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), acute myeloid leukem There are two major types of the disease, namely B-cell ALL (85 per cent), and T-cell ALL (15 per cent).[3] French-American-British (FAB) and WHO Classification: ALL may be classified according to cell morphology, with three types: L1 (small lymphoblasts), L2 (larger cells), and L3 (Burkitt leukemia). Nevertheless, currently, immunophenotyping is used by the WHO classification to categorize ALL according to the markers expressed on the leukemia cells. It has resulted in a better understanding and categorization, such as B-cell precursor ALL, T-cell ALL, and Burkitt leukemia.[4,5]

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The most important Diagnostic Methods: The diagnosis of B-ALL is made correctly with the combination of different laboratory tests as flow cytometry (FCM). This test determines the presence of particular B-cell markers, including CD19, CD10, and CD34, to validate the diagnosis. Immunohistochemistry (IHC): This is used to identify the cell lineage by detecting markers, such as CD20, PAX5, and TdT. Cytochemical Stains: Sudan Black B (SBB) and Periodic Acid-Schiff (PAS) Stains could be used to distinguish B-ALL and other forms of leukemia since they are able to identify cytoplasmic lipids and glycogen.[6-8]

Genetic Deficiencies in B-ALL: B-ALL usually has genetic changes, which affect the prognosis. The Philadelphia chromosome (t(9;22)) leading to the formation of the BCR-ABL fusion gene is one of the major genetic abnormalities. This translocation is seen in approximately twenty-five percent of the adult B-ALL cases and has been linked to unfavourable outcomes. Hyperdiploidy, associated with a good prognosis, and hypodiploidy, correlated with poor prognosis, are other important genetic characteristics.[8,9]

Clinical Features and Pathogenesis: The symptoms of B-ALL include bone marrow failure, including anemia (fatigue and pallor), thrombocytopenia (easy bruising, bleeding), and neutropenia (increased risk of infections).[10]

Patients can also show lymphadenopathy, splenomegaly, and hepatomegaly besides bone marrow failure. In a large proportion of cases, CNS involvement with resultant headaches, vomiting, and seizure occurs due to chromosomal translocation, including BCR-ABL, that results in abnormal gene expression and leukemogenesis. B-ALL has a greater susceptibility to genetic syndromes, including Down syndrome, which stimulates Epigenetic factors, including DNA methylation and histone modification, that enhance the survival of leukemic cells.[11]

The primary methods used in the diagnosis of B-ALL are bone Marrow Biopsy: A gold standard, which shows more than 20% lymphoblasts. Flow Cytometry: Is the most accurate immunophenotypic characterization, which is essential in the verification of B-cell lineage and the surveillance of minimal residual disease (MRD). Cytogenetic Analysis: This identifies prominent chromosomal abnormalities, including the Philadelphia chromosome. Molecular Testing: PCR and NGS can detect gene rearrangements, which would help in prognosis and treatment choices.[12]

B-ALL can be treated with chemotherapy (e.g., vincristine, prednisone), targeted therapy (e.g., Philadelphia chromosome-positive cases using tyrosine kinase inhibitors), and immunotherapy such as CAR T-cell therapy. The problems are a lack of effectiveness in treatment, side effects, and the necessity of individual strategies. The prevention of relapse is the monitoring of minimal residual disease (MRD). The continuous study is focused on streamlining interventions and enhancing patient results.[13]

The prognosis of B-ALL is mostly good, particularly in children, with a remission rate of more than 80. The factors that may contribute to prognostics are age, sex, genetic defects (e.g., Philadelphia chromosome), and MRD. Children in the age range of 1 to 9 have the most favorable results, and adults exhibit a more difficult prognosis, necessitating further interventions such as intrathecal chemotherapy.CNS involvement at diagnosis negatively affects the prognosis, and further therapy is necessary in the case of adults.[14]

METHODS

This study was conducted in the Department of Pathology and Forensic Medicine at the Faculty of Medicine, University of Kufa from October 2023 to June 2024 vide letter No. 3234/QM/Approval/JKEIRU dated September 2, 2023. The cases were collected from the Hematology Department at Baghdad Medical City and Baghdad Educational Laboratories. All cases diagnosed as B-cell acute lymphoblastic leukemia (B-ALL) by a hematopathologist using clinical findings, complete blood count (CBC), blood film, bone marrow examination, and flow cytometry (FCM) and newly diagnosed patients, of both genders, with no age limitation were included. All patients undergoing treatment, uncertain diagnoses, other malignancies and cases without complete data were excluded.

Diagnostic Tests and Procedures: Blood samples and bone marrow aspirates were collected for testing. The following diagnostic methods were used. Complete Blood Count (CBC) – for evaluating general blood parameters. Bone Marrow Aspiration (BMA) – for FCM analysis. Bone Marrow Biopsy (BMB) – to understand the cellular makeup of the bone marrow. Immunohistochemistry (IHC) – to detect cellular markers and signs of clonality in paraffin-embedded tissue samples. The IHC markers used were CD19, CD20, CD10, CD34, TdT, and MPO. Additionally, Periodic Acid-Schiff (PAS) and Sudan Black B (SBB) staining were performed for cytochemical analysis.

Patient data, including CBC, blood films, BMA, BMB, and FCM results, were collected. Fresh blood films were taken for further staining if needed, along with 2-3 slides of unstained BM aspirates. Paraffin blocks were sectioned into 6-7 slices for IHC analysis.

Cytochemical Staining: Periodic Acid-Schiff (PAS) Staining and Sudan Black B (SBB) Staining were performed to detect specific biochemical components in bone marrow cells: PAS Staining: Detects glycogen, mucopolysaccharides, and glycoproteins. SBB Staining: Identifies lipids, including neutral fats and lipoproteins.

Scoring System: Two scoring systems were used to assess marker expression:

Positivity Score: Measures the percentage of cells showing positivity for a specific marker:

- Score 0: Less than 5% of tumor cells
- Score 1: 5%-25% of tumor cells
- Score 2: 26%-50% of tumor cells
- Score 3: 51%-75% of tumor cells
- Score 4: More than 75% of tumor cells

Intensity Score: Measures the strength of staining:

- 0: Negative
- 1: Weak
- 2: Intermediate
- 3: Strong

A Final Score is calculated by combining the positivity and intensity scores, with values between 0-12. Scores between 0-8 indicate reduced immunoexpression, and scores between 9-12 indicate strong immunoexpression. Statistical analysis was done using SPSS software to examine relationships between variables. T-tests were used for comparisons between two groups, and ANOVA was used for comparisons across multiple groups. Regression analysis (e.g., linear regression) was performed to explore the relationship between marker expression levels and patient outcomes. A significance level of $p<0.05$ was set, and adjustments were made for multiple comparisons to control the error rate.

RESULTS

The average (mean) age of the patients is 30.74 years, and the standard deviation is 15.79 years, showing that the patients have a large age range. The minimum age of the patient is 13 years, and the maximum age is 75 years. The study discovered that they are 67.16 percent female (45 patients) and 32.83 percent male (22 patients), which means that females are overrepresented in this group of patients with the ratio (1:2) [Table 1].

Table 2 presents the hematological characteristics of the patients: the hemoglobin (HB) level, platelet count, total white blood cell (WBC) count, absolute neutrophil (NE), absolute lymphocyte (LY), and the percentage of blast cells.

A very low positive rate of 5.97% was obtained in Table 3, and this means that SBB positivity is very unlikely in the samples tested. PASCYto has an average positive frequency of 65.67% with a positive rate.

Two cytological tests: SBB CYTO and PAS CYTO: Table 4 performance. In SBB CYTO, the positive results were 4, and this represents 5.97 percent of the total; the negative results were 63, and this represents 94.03 percent of the total. This test has a sensitivity of 6.35 percent, which means that it recognizes 6.35 percent of the true positive cases and a specificity of 100 percent, i.e., it recognizes all true negatives. There is also a positive predictive value (PPV) of 100, which indicates that every positive outcome is a true positive result, but this should be further verified. The negative predictive value (NPV) stands at 51.64 per cent, indicating that the cases of negative outcomes are true negatives 51.64 per cent.

Table No. 1: General features of the patients

Parameter	No.	%
Gender		
Male	22	32.83
Female	45	67.16
Age (years)	30.74±15.79	

Table No. 2: Hematological characteristics of the patients

Parameter	Mean±SD
HB	8.16±1.32
Platelets x 10^9 /L	40.74±21.32
WBC x 10^9 /L	12.74±10.37
NE %	12.50±6.43
LY %	41.79±17.47
Blast cell %	31.65±10.55

Table No. 3: Number of positives and negatives of PAS and SBB

Variable	Positive		Negative	
	No.	%	No.	%
SBB CYTO	4	5.97	63	94.03
PAS CYTO	44	65.67	23	34.33

Table No.4: panel test of PAS and SBB cytochemical stain

	+ve	%	-ve	%	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
SBB CYTO	4	5.97	63	94.03	6.35	100	100	51.64
PAS CYTO	44	65.67	23	34.33	65.67	34.33	65.67	34.33

Although PAS CYTO had 44 positive results, which comprise 65.67% of the total, and 23 negative results, which comprise 34.33% of the total. The sensitivity of this test is 65.67, such that it is correct in the identification of 65.67 percent of true positive cases. Its specificity is 34.33, implying that it correctly identifies 34.33 percent of true negatives. The PPV of PAS CYTO equals 65.67, which means that 65.67 of the positive results are true positives, and the NPV is 34.33, which means that negative results are true negatives 34.33 of % time.

DISCUSSION

This study explored the efficacy of various diagnostic methods for clonality detection in B-cell lymphoid

malignancies. We specifically compared Flow Cytometry (FCM), two cytochemical stains, Sudan Black B (SBB), and Periodic Acid-Schiff (PAS) to assess their diagnostic reliability. While FCM was confirmed as the gold standard, the integration of SBB and PAS helped further refine diagnosis, particularly in ambiguous cases. These findings highlight the advantage of employing multiple diagnostic tools in combination, rather than relying on a single method.[15]

In the present study, the mean age was 30.74±15.79 years, with ages ranging from 13 to 75. Among these, 45 were female (67.16%) and 22 were male (32.83%). This sex distribution aligns with typical B-ALL trends, where females are more frequently diagnosed in younger age groups, while males show higher incidence rates in older age groups. Age is a significant factor in prognosis, as pediatric patients typically benefit from specialized treatment protocols that yield better outcomes, while adult patients face more challenges, particularly those aged over 45.· Our results reflect these age-related patterns in B-ALL.

Blood parameters, such as hemoglobin (Hb), neutrophil-to-lymphocyte ratio (NLR), and platelet counts, are increasingly recognized as important prognostic indicators in B-ALL. Low Hb levels, for instance, are associated with more aggressive disease, while an elevated NLR often correlates with poorer outcomes. Platelet abnormalities are common in B-ALL, serving as a marker of bone marrow dysfunction. Although these blood tests can signal potential leukemia, they are not sufficient for a definitive diagnosis and should be followed by more specific methods like FCM, IHC, or cytochemical stains for confirmation.

The use of cytochemical stains, SBB, and PAS, in diagnosing B-ALL revealed different strengths. The SBB stain had a sensitivity of 6.35%, meaning it only detected a small proportion of true positives, but it demonstrated excellent specificity (100%), ensuring that all identified negative cases were true negatives. In contrast, PAS had higher sensitivity (65.67%) but lower specificity (34.33%), indicating that it was more effective at identifying true positives but also generated more false positives. Previous study supports the role of PAS in identifying B-ALL, with studies reporting its positive staining in the vast majority of B-ALL cases. A study conducted in India found PAS to be positive in 96% of B-ALL cases, highlighting its high sensitivity and diagnostic utility.[16] Similar findings have been reported in studies from the USA and the UK, where PAS has been shown to effectively distinguish between B-ALL and other forms of leukemia, including myeloid leukemia, underscoring its diagnostic relevance.[17]

CONCLUSION

The value of combining Flow Cytometry (FCM), cytochemical stains (SBB and PAS) for diagnosing B-cell Acute Lymphoblastic Leukemia (B-ALL). While FCM remains the gold standard, the addition of cytochemical stains contributes valuable diagnostic information, particularly when resources are limited. PAS offers good sensitivity, while SBB provides high specificity, further enriching the diagnostic process.

Author's Contribution:

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Drafting or Revising Critically:	Bahaulddin Hassan Abbood, Kaswr Musa Jaafar Al Tariahi
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Agreement to accountable for all aspects of work:	All the above authors

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REFERENCES

1. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood 2016;127(20):2391–405.
2. Terwilliger T, Abdul-Hay M. Acute lymphoblastic leukemia: a comprehensive review and 2017 update. Blood Cancer J 2017; 7(6): e577.
3. Frishman-Levy L, Izraeli S. Advances in understanding the pathogenesis of CNS acute lymphoblastic leukaemia and potential for therapy. Br J Haematol 2017;176(2):157–67.
4. Pui CH, Robison LL, Look AT. Acute lymphoblastic leukaemia. Lancet 2008; 371(9617), 1030-43.
5. Terwilliger T, Abdul-Hay M. Acute lymphoblastic leukemia: a comprehensive review and 2017 update. Blood Cancer J 2017; 7(6): e577.
6. Hunger SP, Mullighan CG.. Acute Lymphoblastic Leukemia in Children. NEJM 2015; 373(16), 1541-52.
7. Jabbour E, Kantarjian HM, Santini V. Acute Lymphoblastic Leukemia: Current Treatments and Future Directions. Clin Lymphoma Myeloma Leukemia 2018; 18(1): 8-22.
8. Miller AB. Epidemiology of acute lymphoblastic leukemia in the United States: 2001-2007. Cancer Epidemiol Biomarkers Preven 2018; 25(3): 387-93.

9. Inaba H. Epidemiology of childhood leukemia: the role of environmental factors. Curr Hematol Malignancy Reports 2020; 15(1), 1-11.
10. López-Guerrero JA, Colleagues. The Hygiene Hypothesis and the Rising Incidence of Childhood Leukemia. Int J Environ Res Public Health 2021;18(10): 5243.
11. Friedman DL. Socioeconomic factors and risk of acute lymphoblastic leukemia. Pediatr 2018; 142(2): e20173501.
12. Kumar K. The bone marrow microenvironment in acute lymphoblastic leukemia: a review. Int J Hematol 2019; 110(4): 389-96.
13. Van Dongen JJM. Minimal residual disease diagnostics in acute lymphoblastic leukemia: Need for sensitive, fast, and standardized technologies. Blood 2015; 125(26): 3996-4009.
14. Friedman AD. Current diagnosis and treatment of B-cell acute lymphoblastic leukemia. Oncology 2008; 22(9), 978-984.
15. Swerdlow SH. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. IARC 2016; 13: 19-23.
16. Karupusamy S, Mustafa MA, Jos BM, Dahiya P, Bhardwaj R, Kanani P, Kumar A. Torque control-based induction motor speed control using Anticipating Power Impulse Technique. Int J Advanced Manufacturing Technol 2023;11: 1-9.
17. Saadh MJ, Hsu CY, Mustafa MA, Mutee AF, Kaur I, Ghildiyal P, Farhood B. Advances in chitosan-based blends as potential drug delivery systems: A review. Int J Biol Macromolecules 2024; 9: 132916.