# The value of cytochemical stains in the diagnosis of acute leukemia

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**ABSTRACT**

**Background**: acute leukemia(AL) represents a clonal expansion and arrest at specific stage of normal myeloid or lymphoid hematopiosis that result in accumulation of malignant blast cells in the bone marrow and peripheral blood. Cytochemical stains play an important role in the diagnosis, classification and differentiation of acute leukemia.

**Aim:** the aim of the present study is to evaluate the role of cytochemical stains in the diagnosis of acute leukemia compared to immunophenotyping by flowcytometry.

**Materials** and **Methods:** Myeloperoxidase (MPO), Sudan Black B(SBB), Non Specific Esterase(NSE) and Periodic Acid Schiff (PAS) stains were done on thirty newly diagnosed acute leukemia patients admitted at the Alexandria University hospitals. Fifteen age matched healthy controls were included in the study.

**Results**: The present study showed that Cytochemical stains (MPO, SBB, PAS and NSE), when coupled with morphology accurately diagnosed 93.3% of acute leukemia cases. A total of 13/15 (86.7%) ALL cases and 15/15 (100%) AML cases could be diagnosed correctly. MPO showed significant positive association with CD13 and CD33 and significant negative association with CD10, CD19 and CD2. SBB had significant positive association with CD13 and CD33 and negative association with CD 10 and CD19. NSE showed positive association with CD14 and negative association with CD13. PAS had only positive association with CD5.

**Conclusion:** Cytochemical stains( namely MPO and SBB) are useful in the diagnosis of acute leukemia specially AML cases, their importance is particularly of value in developing countries as they are simple and do not need special equipments or highly trained persons.

**Introduction:** Leukemia is a disease resulting from the neoplastic proliferation of haemopoietic or lymphoid cells. It results from a mutation in a single stem cell, the progeny of which form a clone of leukemic cells (blast cells).[1]The annual incidence of ALL is approximately 2500 to 3500 new cases in the United States. [2] In Egypt, according to the National Cancer Registry (NCR), leukemia is constituting 35.6% of cases of childhood cancer diagnosed annually.[3] acute leukemia is classified according to the FAB or WHO classification. In morphological classification (FAB classification) ALL is classified to L1, L2 and L3.[4] AML is classified to M0, M1, M2, M3, M4, M5, M6 and M7.[5] While the WHO classification uses all available information (morphology, cytochemistry, immunophenotyping, genetics and clinical features) to define clinically significant disease entities and to provide a classification that can be used in daily clinical practice as well as to serve as a common language for clinical trials and laboratory investigations. [6] The diagnosis of acute leukemia requires examination of peripheral blood samples and bone marrow aspirates/biopsies. Such examination involves morphology, cytochemistry, immunophenotyping, cytogenetic studies and molecular genetic analysis.[7] the peripheral blood usually shows anemia, thrombocytopenia,

neutropenia and leukocytosis with the presence of blast cells in the circulation.[8] Cytochemical stains on blood and bone marrow smears are helpful in the distinction of AML from ALL and in sub-classification of AML. The combination of myeloperoxidase or Sudan black stain, Non Specific esterase and Periodic acid schiff stain are said to provide the desired information in most cases.[9] Immunophenotyping is used to determine lineage involvement of a newly diagnosed acute leukemia.[10] Combination of cytochemical stains and immunophenotyping data always give more appropriate results.

**Aim of the work:**

The aim of the present study is to evaluate the role of some cytochemical stains namely MPO, SBB, NSE and PAS in the diagnosis of acute leukemia in comparison to immunophenotyping using flowcytometry in the Egyptian patients.

**Materials and Methods. Subjects:** The study was done for thirty patients with de novo acute leukemia (AL) with age ranging from 4 to 60 years, and a control group consisting of fifteen hospitalized patients of matched age and sex with no malignant hematological disease to whom bone marrow aspiration is one of the required investigation. Patients were selected from the outpatient of the Alexandria University Hospitals.,An informed written consent was obtained from each patient or his guardian,and the study protocol was approved by the Alexandria Faculty of Medicine ethics committee.

The laboratory investigations analyzed included (1) examination of peripheral blood(PB) smears stained with Leishman's stain (2) examination of bone marrow(BM) aspirate smears (Leishman's) and trephine biopsy slides (H and E) (3) cytochemical stains-Myeloperoxidase, Sudan Black B, Non Specific Esterase and Periodic acid Schiff (Sigma Aldrich fine chemicals –USA)[11](4). **Immunophenotyping by flowcytometry[12]:** Immunophenotyping of the leukemic blast cells was performed on PB or BM samples using Miltenyi Biotec MACSQuant™ flowcytometry analyzer equipped with MACS Quantify software version 2.4. Monoclonal antibodies (DAKO-USA)[13] labelled with Fluorescein isothicyanate (FITC) or phycoerythrin (PE) were used for immunophenotyping ,the antibodies were arranged in a primary panel that was applied to all cases consisting of CDs:2,5,7,10,13,14,19,33,34,45,56,and HLA-Dr. The confirmatory panel included cytoplasmic CD22, cytoplasmic CD3 and cytoplasmic myeloperoxidase. A secondary panel of certain monoclonal antibodies was used when needed including CDs1a, 4, 8, 11b, 41, 61, 64.

**RESULTS: The final diagnosis of each patient was reached by immunophenotyping & stained slides with MPO, SBB, NSE and PAS were examined for each case**. MPO was negative in 15 cases (100%) of ALL. It was negative in 2/15 (13.3%) and positive in 13/15 (86.7%) of AML cases (table I). SBB was negative in 13/15 (86.7%) and positive 2/15 (13.3%) in of ALL cases. In AML it was positive in all cases (100%) (table1). In case of NSE, it showed positivity in 4/8 (50%) of monocytic AML cases. In ALL, it was positive in 2/15 (13.3%) and negative in 13/15 (86.7%) (Table II). PAS was positive in 7/15 (46.4%) and negative in 8/15 (53.3%) of ALL cases with no apparent difference between B-ALL and T-ALL. In AML, it was positive in 5/15 (33.3%) of cases and negative in 10/15 (66.6%) of cases (table III). When comparing the results of stains we used and immunophenotyping data, MPO had a positive significant association ($P<0.001$) with CD13 and CD33 and a negative significant association with CD10, CD19 and CD2. SBB had a significant positive association ($P<0.001$) with CD13 and CD33 and negative significant association with CD10 and CD19. NSE had a positive significant association with CD14 and a negative significant association with CD13. While PAS had only a significant association ($P<0.001$) with CD5. In conclusion MPO and SBB correlated

with immunophenotyping while PAS and NSE were not showing significant association with immunophenotyping data. (Table IV).

**Table I: Diagnostic performance (sensitivity, specificity and accuracy) for MPO and SBB)**

| | | Diagnosis | | Sensitivity | Specificity | PPV | NPV | Accuracy |
|---|---|---|---|---|---|---|---|---|
| | | ALL | AML | | | | | |
| MPO | Negative | 15 | 2 | 86.67 | 100.0 | 100.0 | 88.24 | 93.33 |
| | Positive | 0 | 13 | | | | | |
| SBB | Negative | 13 | 0 | 100.0 | 86.67 | 88.24 | 100.0 | 93.33 |
| | Positive | 2 | 15 | | | | | |

**Table II: Diagnostic performance (sensitivity, specificity and accuracy) for NSE**

| NSE | Diagnosis | | Sensitivity | Specificity | PPV | NPV | Accuracy |
|---|---|---|---|---|---|---|---|
| | ALL, AML accept M4,5 (n=22) | M4,M5 (n=8) | | | | | |
| Negative | 18 | 4 | 50.0 | 81.82 | 50.0 | 81.82 | 73.33 |
| Positive | 4 | 4 | | | | | |

**Table III: Diagnostic performance (sensitivity, specificity and accuracy) for PAS**

| PAS | Diagnosis | | Sensitivity | Specificity | PPV | NPV | Accuracy |
|---|---|---|---|---|---|---|---|
| | AML | ALL | | | | | |
| Negative | 10 | 8 | 40.3 | 70.0 | 72.73 | 36.84 | 50.0 |
| Positive | 5 | 7 | | | | | |

**Table IV: Relation between cytochemistry and diagnosis by immunophenotype**

| Cytochemistry | Diagnosis | | | | $\chi^2$ | P |
|---|---|---|---|---|---|---|
| | ALL (n = 15) | | AML (n = 15) | | | |
| | No. | % | No. | % | | |
| **MPO** | | | | | | |
| Negative | 15 | 100.0 | 2 | 13.3 | 22.941* | <0.001* |
| Positive | 0 | 0.0 | 13 | 86.7 | | |
| **SBB** | | | | | | |
| Negative | 13 | 86.7 | 0 | 0.0 | 22.941* | <0.001* |
| Positive | 2 | 13.3 | 15 | 100.0 | | |
| **NSE** | | | | | | |
| Negative | 13 | 86.7 | 9 | 60.0 | 2.727 | FEp= 0.215 |
| Positive | 2 | 13.3 | 6 | 40.0 | | |
| **PAS** | | | | | | |
| Negative | 7 | 46.7 | 12 | 80.0 | 3.589 | 0.058 |
| Positive | 8 | 53.3 | 3 | 20.0 | | |

**Discussion:**

Acute leukemia is diagnosed by the presence of blast cells in the peripheral blood or the bone marrow (more than 20%), according to the World Health Organization classification.[14] Based on its origin: myeloid or lymphoid, acute leukemia can be divided into 2 types; acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML)**.** Classification of AL has traditionally been based on a combination of morphology and cytochemical staining. The introduction of immunophenotyping and cytogenetics added important laboratory methods for diagnosis, classification, in a addition to predicting prognosis. [15]

Although cytochemical staining of blast cells is simple, cheap technique that requires no special equipments or extensive training, yet its use in the diagnosis of leukemia is declining. In developing countries re-evaluation of the efficiency of this technique as a tool for the initial diagnosis of leukemia may be of importance. When coupled with morphology cytochemical staining rendered the diagnosis accurate in 93.3% of our acute leukemia cases, it is of particular value in cases of AML. In accordance with our results, Belurkar S et al. [16], who carried out his study on patients with acute leukemia and used morphology,cytochemistry and flowcytometry in the diagnosis. Cytochemistry was able to diagnose 80% of patients correctly 66.7% of ALL and 91.6% of AML cases. Compared with immunophenotyping using flowcytometry there was a complete concordance in 58% of cases, partial concordance

in 22% and non-concordance in 4% of cases between both modalities. In the remaining 16% of the cases, morphology and cytochemistry failed to give a definite diagnosis. Another study by klobusicka et al, [17] showed that the cytochemical analysis of AML subtypes does not sufficiently identify the leukemic blast cell populations and only when connected with immunophenotyping it may help to classify the AML patients to relevant subtypes with more accuracy. In our study MPO diagnosed cases of AML with good sensitivity and specificity and correlated well with the myeloid markers CD13 and CD33.

Sudan black B was expressed in 2/15 patients of ALL, but was sensitive in the diagnosis of AML and correlates with the myeloid markers. In agreement with our results, several studies showed that SBB may be expressed in ALL. In the study of Charak BS et al,[18] 1.3% of their ALL patients showed positive reaction with SBB in blast cells with no reactivity to any other myeloid markers. In our study SBB reaction had significant positive association with CD13 and CD33 and a significant negative association with CD10 and CD19. In contrast, van den Ancker W et al, [19] preferred to use cytoplasmic MPO by flowcytometry in a cut off value of 10% as an independent marker to diagnose AML than SBB expression .

NSE is stain of monocytic elements (M4 and M5).In our study it showed low diagnostic performance in the staining of monoblasts, it was also positive in some ALL cases. These results go with those of Sharma P et al, [20] who found positive staining with NSE in some patients with acute lymphoblastic patients. Aberrant cytochemical nonspecific esterase/α-naphthyl acetate esterase (NSE/αNAE) positive B-lymphoblasts can cause confusion with monoblasts Also it was recorded that such cases are associated with relatively poorer outcome of acute lymphoblastic leukemia (ALL).Among the monocytic markers NSE had a positive association with CD14.

Although the study of lilleyman JS et al [21] concluded that PAS positivity is important for the diagnosis of new cases of ALL , yet in our study PAS had a little or no value in the diagnosis of ALL or AML furthermore it could not differentiate B-ALL from T-ALL

**Conclusion:** Being cheap, simple and require no special instruments or highly trained personnel, cytochemical stains are particularly important in developing countries for the diagnosis of acute leukemia specially AML where MPO and SBB correlate well with immunophenotyping markers.On the other hand , NSE and PAS have little or no value in the diagnosis of acute leukemia cases.

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