

The Role of Cytochemistry in the Diagnosis of Acute Leukemias

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ABSTRACT

Introduction: Acute leukemias are clonal disorders of the hematopoietic tissue, characterized by proliferation of immature cells in the bone marrow and maturative block.

Objective: To verify if the cytochemical stains offer contributions for the diagnosis of Acute leukemias.

Materials and methods: Sixty-seven patients with suspected clinical and hematological acute leukemia (AML), attended the Department of Hematology and Hemotherapy of the Federal University of the Triângulo Mineiro / UFTM. Patients were aged from two to ninety-three years old, with median of 29.0. The study used morphological analysis, cytochemical staining of myeloperoxidase (MPO), Sudan Black (SBB), Schiff's Periodic Acid (PAS) and alpha-naphthyl acetate esterase (ANAE), as well as immunophenotyping.

Results: Cytochemical staining (associated with morphology) was diagnosed in 89.5% of cases. MPO was positive in 30 of the 36 AML cases (83.33%). SBB was positive in 29 of 35 cases (82.9%). Both reactions (MPO and SBB) were negative for the 31 cases (100%) with acute lymphoblastic leukemia (ALL). The PAS was positive in 31 of cases (100%) of ALL and in one of AML (3.2%) who was also positive for MPO and SBB with monocytoid blasts to morphological analysis.

Immunophenotyping confirmed that the staining of ANAE processed in 10 cases of suspected monocytic leukemia cases showed positivity in 7 of the 10 cases (70%).

Conclusion: Significantly, cytochemistry associated with morphology differentiates most of the myeloid lymphoid leukemias. Thus, based on this study and considering the initial impact on prognosis and therapeutic choice, we believe that cytochemistry continues to play an important role in the distinction between these leukemias.

Key words: Leukemia; Cytology; Cytochemistry; Immunophenotyping

1. INTRODUCTION

Acute leukemias are a heterogeneous group of hematopoietic cell neoplasms characterized by clonal expansion and

maturative block, with predominance of immature cells constituting more than 20% of the nucleated elements of the bone marrow.^[1,2] These cells compromise normal

hematopoiesis, causing anemia, neutropenia and thrombocytopenia, with abrupt outbreak of and signs and symptoms of weakness, fever/infection and hemorrhage [3] (FARIAS et al, 2004). Frequently these blasts are present in the peripheral blood, and may also infiltrate other tissues and organs such as liver, spleen and lymph nodes. [4]

There are differences in cellular type in acute myeloid leukemia (AML) and acute lymphoid leukemia (ALL), with a predominance of lymphoid type in childhood in 70% of cases (with a higher occurrence between 2 to 7 years) and myeloid type in 80% of adults. [5] A minority (about 5% of the cases) present characteristics of both the lineages (myeloid and lymphoid) and are defined as biphenotypic. [6]

The lymphoid and myeloid subtypes differ substantially, as well as in terms of therapy and clinical course. Thus, morphological and cytochemical analysis associated with more sophisticated techniques such as immunophenotyping, cytogenetics and molecular biology allow this differentiation, contributing to a more precise diagnostic and therapeutic approach. [2,7]

Preliminary techniques for morphology and cytochemistry of peripheral blood and bone marrow form the basis for diagnosis, [8] and follow criteria established since 1976 by the American British Franco Group (FAB), which initially classified myeloid leukemias in six subtypes (M1, M2, M3 M4, M5, M6) and the lymphoid cells in three (L1, L2 and L3), subsequently incorporating the M0 and M7 subtypes. [7,9] Since the 1980's, leukemia of ambiguous or biphenotypic lineage has been identified. However cytochemical staining techniques are rarely positive in these cases, and when present, are less than 3% and only diagnosed via immunophenotyping. [10]

The cytochemical stains include myeloperoxidase (MPO), Sudan Black B (SBB), Schiff Periodic Acid (PAS), Alpha Naftil Acetate Esterase non-specific esterase (ANAE), Chloroacetate Esterase (CAE),

Alpha naphthyl butyrate esterase (ANBE). These techniques allow the distinction between lymphoid and myeloid lineages in the majority of cases. [5,11] With the advent of flow cytometry, immunophenotypic analysis of bone marrow and peripheral blood became standard because of the differentiation it provides in relation to the lineage and the maturative stage of the cell, and because it can be used in conjunction with cytochemistry. [7]

The cytochemical stain technique (whilst declining) is a simple and low-cost technique for the diagnosis of acute leukemias. We believe that the re-evaluation of this technique in distinguishing these leukemias may still play an important role in localities that do not have advanced diagnostic techniques, which justifies this study. [11]

2. METHODS

The study included 67 patients with acute leukemia, aged between two and ninety-three years old, who attended the Service of Hematology and Hemotherapy of the Federal University of the Triângulo Mineiro (UFTM) from August 2009 to February 2013 (excluding those in treatment and / or relapse). The study was approved by the Ethics and Research Committee of the UFTM under protocol number 1696. Samples of peripheral blood and bone marrow were analyzed and after making the smears were stained by Leishman and cytochemistry. Samples of bone marrow were sent for immunophenotyping. Blade readings were performed by two hematologists and one biomedical. The diagnosis of acute leukemia was confirmed when >20% of the nucleated elements of the bone marrow were blasts and / or these were identified in the peripheral blood. The cytochemical reactions, Sudan Black B (SBB) and Myeloperoxidase (MPO), were processed by the Sheehan technique and the DAB Plus Liquid Chromogen / Substrate kit and Biosystems kit, Pleasanton, CA (USA) respectively, and PAS by the Hotchkiss technique. Leukemias were classified as

AML and ALL according to cytochemical reactions, when 3% or more of the blasts in the bone marrow or peripheral blood were positive. For MPO and SBB, positivity was defined as presence of brown and black granules, respectively present in 3% or more in the cytoplasm of blasts in the bone marrow or peripheral blood. [12] Positivity for PAS was defined as presence of coarse and fine granules (purple or magenta) in blasts in the bone marrow or peripheral blood³. The ANAE reaction with and without inhibition of fluoride was performed in ten cases with morphology suggestive of the monocytoid lineage. Immunophenotyping by flow cytometry was performed on a Calibur Facsimile apparatus using the panel of monoclonal antibodies: D13, CD33, HLA-DR, CD117, CD34, CD38, CD45, Anti-MPO, CD11b, CD14, CD64, TdT, CD42a, CD79a, CD56, CD2, CD3 and cCD3, CD4, CD8, CD10, CD19, CD22, Anti-Kappa and Anti-Lambda. The results of the cytochemistry were compared with those of the immunophenotyping and

agreement between the methods was verified by the statistical tests of Kappa and McNemar Square Qi.

3. RESULTS

Results were derived from a group of 67 patients, aged from two to ninety-three years old, with a median of 29.0. The majorities were aged over 20 years old and white (56.7%), 73.7% were adults and 61.42% of the 67 patients were male. MPO was positive in 30 (83.3%) cases of AML. The SBB in 29 cases (82.85%) and both reactions were negative for 31 (100%) cases of ALL. The SBB in one reaction was not processed due to marrow scarcity. The PAS staining was positive in 31 (100%) cases of ALL and positive in 1 of 36 cases (2.77%) of AML with diffuse and granular standard positivity concomitantly, with morphology suggestive of the monocytic lineage. Six cases (8.95%) were negative for the three reactions (Table 1). The ANAE staining was positive in 7 of 10 cases with inhibition of fluoride.

Table 1: Cytochemical diagnostic performance

		Diagnóstico		Sensitivity	Specificity	PPV	NPV	Accuracy
		AML	ALL					
MPO	Positive	30	0	83,3	100	100	83,8	91
	Negative	6	31					
SBB	Positive	29	0	82,9	100	100	83,8	90,9
	Negative	6	0					
PAS	Positive	1	31	96,9	100	100	100	96,9
	Negative	30	0					

MPO: Sensitivity: Se = 83.8%; Specificity: Sp = 100%; Positive Predictive Value: PPV = 100%; Negative Predictive Value: NPV = 83.8%, SBB: Sensitivity: Se = 82.9%; Specificity: Sp = 100%; Positive Predictive Value: PPV = 100%; Negative Predictive Value: NPV = 83.8%, SBP: Sensitivity: Se = 96.9%; Specificity: Sp = 100%; Positive Predictive Value: PVV = 100%; Negative Predictive Value: NPV = 96.9%.

Table 2: Concordance of cytochemical results with immunophenotyping								
		Immunophenotyping				McNemar	Kappa	Accuracy
		Mieloide (n= 36)		Linfoide (n=31)				
Cytochemistry		No.	%	No.	%			
MPO						P= 0,041	0,82	91,0
Positive	30	83,3	0.0	0.0				
Negative	6	16,7	31	100				
SBB						P= 0,041	0,82	90,9
Positive	29	80,55	0.0	0.0				
Negative	6	16,7	31	100				
PAS						P= 0,0001	0,97	98,4
Positive	1	2,77	31	100				
Negative	0	0.0	0.0	0.0				

MPO: myeloperoxidase; SBB: Sudan Black B; Kappa coefficient: k = 0.82); McNemar's concordance: p = 0.041; Considering Myeloid (+) and Lymphoid (-); PAS: (kappa coefficient: k = 0.97), McNemar agreement: p = 0.0001; Considered lymphoid (+) and myeloid (-).

Of the 67immunophenotyping cases, 30 (44.77%) were AML, 31 (46.26%) ALL, and 3 (4.47%) were biphenotypic.

When comparing the results of the cytochemical staining with the immunophenotyping, the MPO and SBB

had significant agreement ($p = 0.041$; kappa = 0.82) with CD13, CD33 and anti-MPO of the myeloid and negative lineage with markers of the lymphoid lineage. The positive PAS presented significant agreement ($p = <0.0001$; kappa = 0.97) with CD2, CD3, CD4, CD8 CD 19, CD10 and CD22 and negative with CD13 and CD33. (Table 2). The ANAE, positive in 7 of 10 (70%) of cases analyzed, showed strong positivity for CD14 in six cases and weak positivity in one case.

4. DISCUSSION

Cytochemistry, together with morphology, identified 85.9% of the cases with an accuracy of 91.0% of myeloid leukemias and 98.5% of lymphoid leukemia which is supported by the study by Akran et al which found an accuracy of 93.3%. In our study, MPO and SBB correctly diagnosed 83.8% of myeloids and 100% of lymphoid SBP. MPO and SBB were concordant in 83.3% and 80.5% of AML cases, particularly in more differentiated AML subtypes such as M2, M3, M4 and M5, similar by the study found by Klobuzicka [13] (2005) and 100% of ALL. In this study MPO diagnosed cases of AML with high sensitivity and specificity correlated significantly with CD13 and CD33. The presence of 3% of positive MPO already confirms the diagnosis of AML, considered of high sensitivity and specific of the myeloid lineage. [14-16] In addition, a study by Matsuo et al, with 491 patients, revealed that the percentage of positive MPO is a highly significant prognostic factor for AML patients, and especially useful for stratifying patients with normal karyotype. [17]

According to Klobusická [15] in 2000 the MPO reaction may be superior to the CD13 and CD33 markers in the identification of the myeloid lineage, as in some cases of AML-M2 with t (8:21) (q22:q22) in which these markers are negative, the MPO is strongly positive.

SBB was expressed in 83.8% of AML, with good sensitivity and also

significant agreement with CD13, CD33 and negative for lymphoid markers. It is important to emphasize that SBB is not specific for myeloid series, and may be positive in ALL, as reported by Stass et al., [18] which demonstrated 1.6% of 350 patients with ALL and Srishti et al which demonstrated 13.3% of 15 cases. However, SBB demonstrated high sensitivity for the diagnosis of AML, correlated with CD13 and CD33 [11] as demonstrated in our study.

ANAE is a cytochemistry to identify monocytic blasts (M4 and M5). Of the ten cases evaluated by us, seven were ANAE positive, supported by MPO and SBB and CD14 also positive and three negative were AML of the M2 subtype. One of the seven cases was also positive for standard granular and concomitant diffuse SBP, but with an evident presence of monocytoid blasts for morphological analysis, MPO, SBB positive, and weak CD14 positive. These findings reinforce the importance of cytochemistry in this subtype. Klobusická [15] in 2000 reports that although CD14 is considered specific to monocytic lineage, it is not restricted to all stages of maturation of monocytes, such as nonblasts and promonocytes, generally negative for this marker, but strongly positive for ANAE. [15] The final diagnosis was then based on the junction of morphology, cytochemistry and immunophenotyping. Studies have reported the positivity of ANAE in lymphoid blasts, a fact not observed by us, since this reaction has not been tested in these cases. [19]

PAS was positive in 100% of ALL, with a significant positive correlation with CD.4, CD3, CD10, CD19 and CD20. Unlike the results found by Samir et al, in which this staining was negative in 20% (9/44) of the lymphoid cells. [20] This reaction has granular positivity for lymphoblasts, although studies have already demonstrated negativity in some cases of T-ALL, [21] this was not observed in our study. Granular deposits have also been occasionally described in AML, such as M4, M5 and M6. [22] In our study, PAS was granular and concomitant diffuse positive in a case of

AML-M4, but with positivity for MPO and SBB. The sensitivity of the combination of PAS staining with MPO and SBB was 96.4%, but the specificity for ALL was 100%. Thus, SBP, in combination with negative MPO and SBB, continues to play an important role in the distinction between lymphoblastic and myeloblastic leukemia and immunophenotyping supports this diagnosis. [11,19]

Cytochemistry was negative in six cases (8.95%) and two morphological analysis revealed immature blasts suggestive of AML-M0. In the M7 case, (whose morphology is rarely distinguishable and cytochemical staining is always negative) immunophenotyping reveals an absence of most markers of myeloid and lymphoid lineage, expressing those specific for megakaryocytic lineage. [23] In the remaining three cases of acute biphenotypic leukemias that rarely present positivities for cytochemical staining (or less than 3%), immunophenotyping becomes indispensable for the diagnosis of undifferentiated leukemias. [1]

6. CONCLUSION

In our study, the cytochemistry associated with morphology (considering the low cost, easy execution and the impact on the prognosis and therapeutic choice in the first instance) demonstrated high sensitivity and specificity of the differential diagnosis of acute myeloid lymphoid leukemias. We believe that cytochemistry continues to play an important role in the distinction between these leukemias and can be used in places that do not yet have the most advanced diagnostic resources and / or immunophenotyping, cytogenetics and molecular biology.

REFERENCES

1. Subhash CJ, Muzaffar MA, Anju S, Amod K, Shahab R, Ramesh PD. Flowcytometric Evaluation and Morphological and Cytochemical correlation of 150 cases of Acute Leukemia. *Int J Biomed Adv Res.* 2015; 6(12): 844-852.

2. Gupta S, Chatterjee BT, Sharma CS, Sharma BA, Ganguly CP, Singh CJ, Satyaranjan SC. Flowcytometric comparative analysis in acute leukemias between Indian and proposed minimal screening panel. *Medical J Armed Forces India.* 2016; 72(3):220-230.
3. Farias MG, Castro, SM. Diagnóstico Laboratorial das Leucemias Linfóides Agudas. *J Bras Patol Med Lab.* 2004; 40 (2): 91-98.
4. Estey EH. Acute myeloid leukemia: 2012 update on diagnosis, risk stratification, and management. *Am J Hematol.* 2012; 87(1):89-99.
5. Silva G. Diagnóstico Laboratorial das Leucemias Mielóides Agudas. *J Bras Patol Med Lab.* 2006; 42 (2): 77-84.
6. Béné MC, Porwit A. Acute leukemias of ambiguous lineage. *Semin Diagn Pathol.* 2012; 29(1): 8-12.
7. Silveira NA, Arraes, SMAA. A imunofenotipagem no Diagnóstico Diferencial das Leucemias: uma revisão. *ArquivMudi.* 2008; 12(1): 5-14.
8. Loffler H, Gassmann W. Morphology and cytochemistry of acute lymphoblastic leukaemia. *Baillieres Clin Haematol.* 1994; 21(7):263-272.
9. Cortes JE, Kantarjian HM. Acute lymphoblastic leukemia: a comprehensive review with emphasis on biology and therapy. *Cancer.* 1995; 76(12): 2393-2417.
10. Batinić D, Dubravčić K, Rajić L. Biphenotypic and bilineal acute leukemias. *Acta Med Croatica.* 2008; 62(4):387-390.
11. Deghady AAM, Mansour AR, Elfahham BAAAE. The value of cytochemical stains in the diagnosis of acute leukemia. *Int J Res Health Sci Nurs.* 2016; 2(5):1-7.
12. Pefaut de Latour R, Legrand O, Moreau D, Perrot JY, Blanc CM, Chaoui D, et al. Comparison of flow cytometry and enzyme cytochemistry for the detection of myeloperoxidase in acute myeloid leukemia: untests of a new positivity threshold. *Br J Haematol.* 2003; 22(2): 211-216.
13. Klobusicka M, Kusenda J, Babusikova O. Myeloid enzymes profile related to the immunophenotypic characteristics of blast cells from patients with acute

- myeloidleukemia (AML) at diagnosis. *Neoplasma*. 2005; 52(3):211-218.
14. Tan S, Wang G, Peng M, Zhang X, Shen G, Jiang J, Chen F. Detection of myeloperoxidase activity in primary leukemic cells by an enhanced chemiluminescent assay for differentiation between acute lymphoblastic and non-lymphoblastic leukemia. *ClinChimActa*. 2009; 403(1-2): 216–218.
 15. Klobusická M. Reliability and limitations of cytochemistry in diagnosis of acute myeloid leukemia. Mini review. *Neoplasma*. 2000; 47(6): 329-334.
 16. Qadir M, Barcos M, Stewart CC, Sait SN, Ford LA, Baer MR. Routine Immunophenotyping in Acute Leukaemia: Role in lineage Assignment and Reassignment. *Cytometry Part B. Clinical Cytometry*. 2006; 70B(5): 329-333.
 17. Matsuo TKK, Miyazaki Y, Yoshida S, Tomonaga M, Emi N, Kobayashi T, et al. For the Japan Adult Leukemia Study Group. The percentage of myeloperoxidase-positive blast cells is a strong independent prognostic factor in acute myeloid leukemia, even in the patients with normal karyotype. *Leukemia*. 2003; 17(8):1538-1543.
 18. Stass SA, Pui CH, Melvin S, Rovigatti U, Williams D, Motroni T, Kalwinsky D, DahlGV. Sudan black B positive acute lymphoblastic leukaemia. *Br J Haematol*, 57(3):413-21, 1984.
 19. Snower DP, Smith BR, Munz UJ, McPhedran P. Reevaluation of the periodic acid-Schiff stain in acute leukemia with immunophenotypic analyses. *Arch Pathol Lab Med*. 1991; 115(4):346-350.
 20. Kheiri SA, MacKerrell T, Bonagura VR, Fuchs A, Henny H, Billett HH. Flow Cytometry With or Without Cytochemistry for the Diagnosis of Acute Leukemias? *Cytometry*. 1998; 34(2):82–86.
 21. Oliveira BM, Diniz MS, Viana MB. Leucemias Agudas na Infância. *Rev Med Minas Gerais*. 2004; 14(sup11):33-39.
 22. Abdul-hamid, G. Classification of Acute Leukemia. In: *Acute Leukemia - The Scientist's Perspective and Challenge* [Internet]. 2011 [revisado 2011 Dez. 22; citado 2016 Oct 7]. Disponível em: <https://www.intechopen.com>.
 23. Farias MG, Bierman MB. Análise morfológica, imunofenotípica e molecular na identificação da leucemia megacariocítica aguda (LMA-M7). *Rev Bras Hematol Hemoter*. 2007; 29(4):387-393.

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