Unusual cytochemical reactivity for toluidine blue in granular acute lymphoblastic leukemia: a report of two rare cases

Granüler akut lenfoblastik lösemide toluidin mavisine yönelik olağandışı sitokimyasal reaktivite: İki nadir olgu raporu

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Abstract

Azurophilic granulation of blasts is a feature of acute myeloid leukemia (AML). Granular acute lymphoblastic leukemia (ALL) may mimic AML due to the presence of cytoplasmic granules in lymphoblasts, but cytochemistry and immunophenotyping are helpful in making the correct diagnosis. Toluidine blue (TB) is a metachromatic dye, which stains basophils and myeloid blasts that exhibit basophilic differentiation. Reactivity for TB has not been described in lymphoblasts. We herein report two cases of granular ALL with blasts exhibiting reactivity for TB that caused diagnostic dilemma. Immunophenotyping and cytogenetic studies were helpful in making a correct diagnosis. This report of two rare case highlight the reactivity of lymphoblasts with TB not hitherto described and the importance of a detailed diagnostic work-up in acute leukemia. *(Turk J Hematol 2010; 27: 43-5)*

Key words: Granular ALL, basophilic leukemia, toluidine blue, azurophilic granules

Received: November 26, 2008 *Accepted: April 10, 2009*

Özet

Blastların azurofilik granülasyonu, akut miyeloid löseminin (AML) bir özelliğidir. Granüler akut lenfoblastik lösemi (ALL), sitoplazmik granüllerin lenfoblastlardaki varlığına bağlı olarak AML'ye benzeyebilir, ancak histokimya ve immünofenotipleme doğru tanının konulmasında yardımcıdır. Toluidin mavisi (TB) bir metakromatik boya olup, bazofilik diferansiyasyon sergileyen bazofiller ve miyeloid blastları boyar. TB'ye yönelik reaktivite, lenfoblastlarda tanımlanmamıştır. Bu yayında, tanı ikilemine neden olan TB'ye yönelik reaktivite sergileyen blastlar ile granüler ALL'ye yönelik iki vaka rapor edilmektedir. İmmünofenotipleme ve sitogenetik çalışmalar, doğru tanının konulmasını sağlamıştır. Bu iki nadir vaka raporu ile şimdiye dek tanımlanmamış TB'li lenfoblastların reaktivitesinin ve akut lösemide ayrıntılı tanısal tetkikin öneminin altı çizilmektedir. *(Turk J Hematol 2010; 27: 43-5)*

Anahtar kelimeler: Granüler ALL, bazofilik lösemi, toluidin mavisi, azurofilik granüller

Geliş tarihi: 26 Kasım 2008 *Kabul tarihi: 10 Nisan 2009*

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Introduction

Granular acute lymphoblastic leukemia (ALL) is a morphological variant of ALL and accounts for approximately 2-7% of all cases of acute leukemia. Cytochemistry and immunophenotyping help to differentiate granular ALL from acute myeloid leukemia (AML). Toluidine blue (TB) is a basic dye that helps in identification of myeloid blasts showing differentiation towards basophils, which may be seen in acute basophilic leukemia, basophilic blast crisis of chronic myeloid leukemia (CML) and AML with basophilia. Reactivity for TB is not seen in lymphoblasts. The present report describes an unusual cytochemical staining pattern in lymphoblasts of granular ALL and highlights the importance of immunophenotyping and cytogenetic studies for correct diagnosis in such a scenario.

Case Reports

Informed consent was taken from patients for administering any form of treatment.

Case 1

A 14-year-old boy was admitted with cough and weight loss of two months' duration. On physical examination, there was no lymphadenopathy, organomegaly or symptoms of hyperhistaminemia. His hemoglobin level was 11.6 g/dl, platelet count 1.86 x 10^9 /L and total leukocyte count (TLC) 58x10^9/L, which included 8% blasts, 51% myelocytes and metamyelocytes, 24% neutrophils, 16% lymphocytes, and 1% basophils. Bone marrow was cellular with 90% blasts, which had round to oval nuclei, 1-2 nucleoli and moderate amount of cytoplasm. Prominent 5-12 coarse non-refractile intracytoplasmic granules were seen in 35% of the blasts (Figure 1A). The blasts did not stain with myeloperoxidase but showed metachromatic granules when stained with TB (Figure 1B). On flow cytometric immunophenotyping, the blasts were positive for CD34, CD45, CD10, CD19, and CD22 and negative for CD3, CD5, CD7 CD13, CD33, CD14, CD42a, CD61, and glycophorin A. Cytogenetic studies did not reveal any chromosomal abnormality. A diagnosis of B-lineage granular ALL was made. The patient was treated with MCP-841 protocol of NCI (prednisone, L-asparaginase, vincristine and daunorubicin), but the disease was resistant, remission was not achieved, and the patient died due to intracranial bleed.

Case 2

A 50-year-old male presented with fatigue, weakness and fever of two months' duration. The patient was pale but had no lymphadenopathy, organomegaly or symptoms of hyperhistaminemia. His hemoglobin level was 3.6 g/dl, platelet count 23x10^9 /L and TLC 17.8x10^9/L, which included 89% blasts, an occasional myelocyte, 10% lymphocytes, and 1% neutrophils. Bone marrow was cellular with near total replacement by blasts, which had round to oval nuclei, variable nucleoli and moderate amount of cytoplasm. Prominent coarse non-refractile intracytoplasmic granules were seen in 22% of the blasts. The blasts did not stain with myeloperoxidase but showed metachromatic granules when stained with TB. On flow cytometric immunophenotyping, the blasts were positive for CD34, CD45, CD10, CD19, CD22, and CD33 and negative for CD3, CD5, CD13, and CD14. Cytogenetic studies showed normal male karyotype. A diagnosis of B-lineage granular ALL was made. Treatment was initiated with MCP-841 protocol but the patient did not achieve complete remission and died during the induction phase of treatment.

Discussion

Blasts with coarse azurophilic granules are seen in granular ALL and myeloid leukemia with basophilic differentiation, i.e. acute basophilic leukemia and blast crisis of CML with basophilic differentiation. Granular ALL, morphological variant of ALL, is characterized by presence of at least three clearly defined azurophilic granules (Each 0.5 microns or greater in diameter) in more than 5% blasts and are negative for myeloperoxidase [1]. On immunophenotyping, blasts of granular ALL usually have a precursor B-cell phenotype, although granular ALL with T-cell type has also been reported [1,2]. Blasts of myeloid leukemia with basophilic differentiation may not exhibit reactivity for myeloperoxidase and express myeloid markers such as CD33, CD13 and CD117 along with CD9 and CD25, which are usually associated with basophilic lineage of cells [3,4].

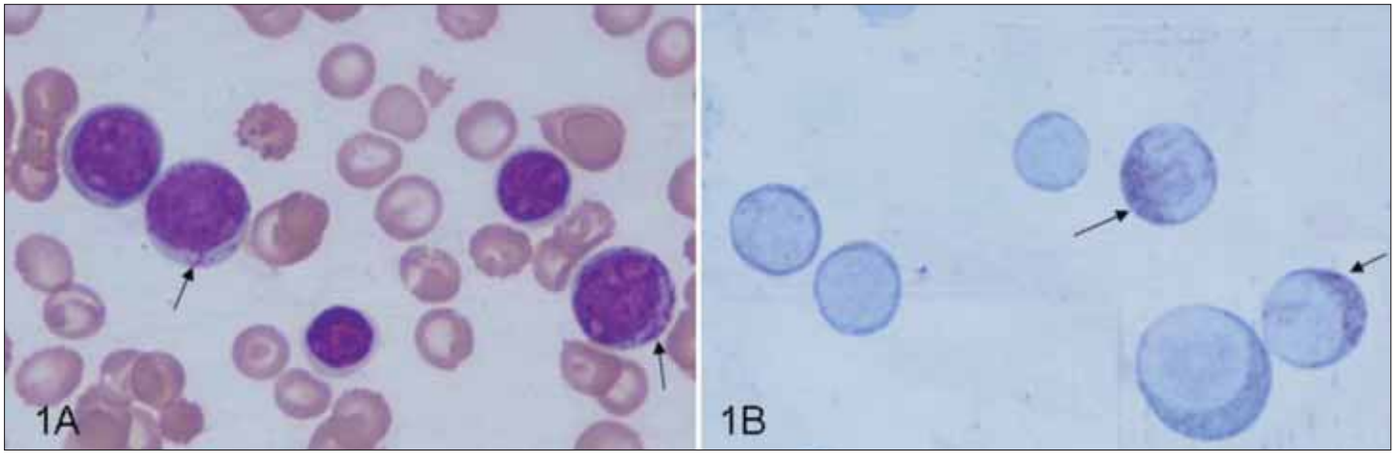

Figure 1. Peripheral blood smear showing blasts with coarse granules (A, Jenner & Giemsa, 1000X), which exhibit metachromasia with toluidine blue (B, 1000X)

Toluidine blue is a metachromatic dye, which stains basophils and is helpful in identifying the basophilic differentiation in myeloid blasts. In the two cases described here, coarse azurophilic granules were present in the blasts, both in peripheral blood smear and bone marrow, which were negative for myeloperoxidase and positive for TB. On the basis of morphology and cytochemistry, the possibility of acute basophilic leukemia and basophilic blast crisis of CML was considered. The B-cell immunophenotype of blasts, absence of Philadelphia chromosome on conventional cytogenetic studies and bcr-abl transcripts on reverse transcriptase-polymerase chain reaction (RT-PCR) were consistent with a diagnosis of B-lineage ALL. In one of the cases, blasts were positive for CD33, but aberrant expression of myeloid markers as CD13 and CD33 is well described in ALL [5].

Toluidine blue reactivity has not been described in lymphoblasts and was the main cause of the diagnostic dilemma in our cases on morphological evaluation. TB reacts with acid mucopolysaccharides of basophils to give metachromatic staining, but the exact mechanism of its reactivity in lymphoblasts, as seen here, is not known. Lysosomes are thought to be the main content of granules in granular ALL and since acid mucopolysaccharides are present in the lysosomes, it is possible that the metachromatic staining observed in our cases was due to the reaction of TB with the acid mucopolysaccharides of the lysosomes [6-10]. This may be one of the possible explanations of TB reactivity in granular ALL, but further studies may be required to elucidate the exact mechanism.

Prognostic value of granular ALL is controversial, with some studies demonstrating no prognostic significance and others attributing it a poor prognosis [6,8,11]. Neither of the patients described here achieved remission and both died despite the best supportive care. Due to the very small number of patients, it remains uncertain at present if patients with granular ALL with TB-positive blasts form a distinct subset with poor prognosis.

To the best of our knowledge, reactivity of granular lymphoblasts to TB has not been described before. It is being reported here to create awareness about the existence of this phenomenon and the need for immunophenotyping in such cases.

Conflict of interest

No author of this paper has a conflict of interest, including specific financial interests, relationships, and/or affiliations relevant to the subject matter or materials included in this manuscript.

References

1. Cerezo L, Shuster JJ, Pullen DJ, Brock B, Borowitz MJ, Falletta JM, Crist WM, Head DR. Laboratory correlates and prognostic significance of granular acute lymphoblastic leukemia in children. Am J Clin Pathol 1991;95:526-31.
2. Tsoi WC, Lai HDH, Feng CS. T-acute lymphoblastic leukemia with cytoplasmic granules. Am J Hematol 1997;56:193-6.
3. Duchayne E, Demur C, Rubie H, Robert A, Dastugue N. Diagnosis of acute basophilic leukemia. Leuk Lymphoma 1999;32:269-78.
4. Gupta R, Jain P, Anand M. Acute basophilic leukemia. Am J Haematol 2004;76:134-8.
5. Brunning RD, Borowitz M, Matutes E, Head D, Flandrin G, Swerdlow SH, Bennett JM. Precursor B lymphoblastic leukemia / lymphoblastic lymphoma. In: Jaffe ES, Harris NL, Stein H, Vardiman JW, WHO-Classification of tumours, editors. Pathology and Genetics of Tumors of Haematopoietic and Lymphoid Tissues. Lyon: IARC Press, 2001: 112-3.
6. Rajnoldi AC, Invernizzi R, Biondi A, Banfi P, Zoia A, Fazio PD, Polli. Biological and clinical features of acute lymphoblastic leukemia with cytoplasmic granules or inclusions. Br J Haematol 1989;3:309-14.
7. Fradera J, Garcia EV, White JG. Acute lymphoblastic leukemia with unusual cytoplasmic granulation. Blood 1986;68:406-11.
8. Darbyshire PJ, Lilleyman JS. Granular acute lymphoblastic leukemia of childhood. J Clin Pathol 1987;40:251-3.
9. Hay CRM, Barnett D, James V, Woodcock BW, Brown MJ, Lawrence ACK. Granular acute lymphoblastic leukemia in adults. Eur J Haematol 1987;39:299-305.
10. Schwarzinger L, Fodinger M, Scherrer R, Woltz M, Mannhatter Ch, Speiser W. Hypergranular acute lymphoblastic leukemia. Ann Hematol 1993;67:301-3.
11. Invernizzi R, Rosanda C, Basso G, Conter V, di Cataldo A, Fenu S, Forni M, Frappampina V, Granchi D, Locatelli F, Miano C, Santostasi T, Cantu-Rajnoldi A. Granular acute lymphoblastic leukemia in children. "Aieop Cooperative Group for Cytology of Acute Leukemias". Haematologica 1992;77:30-4.