Case Report-V

Acute Myeloid Leukemia Presenting with an unusual Phenotype

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ABSTRACT

The diagnosis of acute myeloid leukemia (AML) requires expression of myeloid markers, CD13 and 33, positivity for myeloperoxidase and /or anti- MPO. AML with an unusual phenotype represented by myeloperoxidase positivity and absence of myeloid antigen expression is seen in less than 10% of cases. Myeloid antigens may be detected in the cytoplasm where they may be present before they are expressed on the cell surface.

Here we present two rare cases of AML where the blasts were positive for myeloperoxidase and anti-MPO but negative for surface CD13 and CD33. Although AML-M2 with t(8; 21) is the subtype usually seen in these cases, one of our patients had biphenotypic leukemia with del[9]

INTRODUCTION

The blast cells in acute leukemia express antigens on the cell surface which may be either myeloid or lymphoid. In recent years, immunophenotyping has been increasingly used for the diagnosis and classification of acute leukemias. It is useful in the detection of minimal residual disease and is also reported to have prognostic significance. However, there have been few reports in the literature where the myeloblasts in occasional cases of acute myeloid leukemia do not express myeloid antigens, CD13 or CD33. Most of these cases belong to the AML-M2 subtype. These cases would be incorrectly diagnosed according to the immunologic classification if a myeloperoxidase or anti-MPO stain is not performed. About 5-15% of leukemic blasts have morphologic, cytochemical, immunophenotypic or genetic evidence of more than one hematopoietic lineage.[1] In addition to the myeloid antigens, the blasts in case of AML may express T or B lymphoid antigens. Here we report two rare cases of acute leukemia which were myeloperoxidase positive but myeloid antigen negative. One of the cases was diagnosed as AML-M2, whereas the other was a case of biphenotypic leukemia.

Case 1 – A thirteen year old male presented with swelling in the left side of the neck for a period of one month. He also complained of abdominal pain, swelling in the groin, fever, weight loss and cough for the same duration. On examination the patient was found to have generalised lymphadenopathy with involvement of cervical, axillary, trochlear, abdominal and inguinal nodes. The nodes were enlarged (1-6 cm), firm and non tender. The spleen was enlarged and of 6 cm size. Liver was not palpable. An FNAC of the cervical lymph node done in a local hospital was diagnosed as Non Hodgkins Lymphoma. The patient was referred to this institution for further treatment. The blood counts were as follows: WBC – 3.93 X 10 9/ L, hemoglobin- 9.52gm/dl, platelet – 111 x 10 9/ l. There were 2% blasts in the peripheral blood.

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Wright stained smears of the bone marrow aspiration were examined. The marrow was hypercellular with 85% blasts. Suppression of the erythroid and myeloid series was noted. There were two populations of blast cells. Some of the blasts were larger with slightly more cytoplasm compared to the smaller blasts which had scanty cytoplasm and denser chromatin. Myeloperoxidase activity was present in only 9% of the blasts. In addition the blasts were positive for anti MPO. Immunophenotyping was preformed by flow cytometry on the bone aspiration after Ficoll-Hypaque separation of cells. A routine panel of twelve antibodies was used which included the following markers – CD34, HLA-DR, tdt, CD13, CD33, CD14, CD36, CD19, CD10, CD 22, CD2, CD3, CD4, CD5, CD7, CD8. At least 20% of the blasts were required to be stained for the surface marker to be considered positive. On analysis the tumour cells were positive for stem cell markers, CD34 + 40%, HLA-DR + 50%, tdt + 49% and the T-lymphoid markers – CD2+40%, CD3+41%, CD7+60%, cytoCD3+57%. The B lymphoid markers and the myeloid markers were negative. The immunophenotyping for the myeloid markers, CD13, CD33 and cytoCD13 was repeated on the bone marrow aspiration. Although CD13 and CD33 expression remained negative, 81% positivity for cytoCD13 was noted. (Fig. 1) A diagnosis of biphenotypic leukemia was made according to the St. Judes criteria, based on the dual blast cell population and the positivity for both myeloid and lymphoid markers.[2] Conventional karyotyping was performed on the bone marrow cells. Out of the ten metaphases analysed, del[9] (q21.2; q22.3) was seen in four cells (Fig. 2), three cells showed 45X, -Y and three cells showed the normal male karyotype. The patient was put on the IOSG protocol for AML. Bone marrow aspiration was repeated on day 14 of induction. At this time the marrow was hypocellular with 15% blasts, 40% lymphocytes, 44% atypical lymphocytes and 1% myelocytes. The blasts were MPO negative. Subsequently the patient developed overwhelming infection and died within one month.

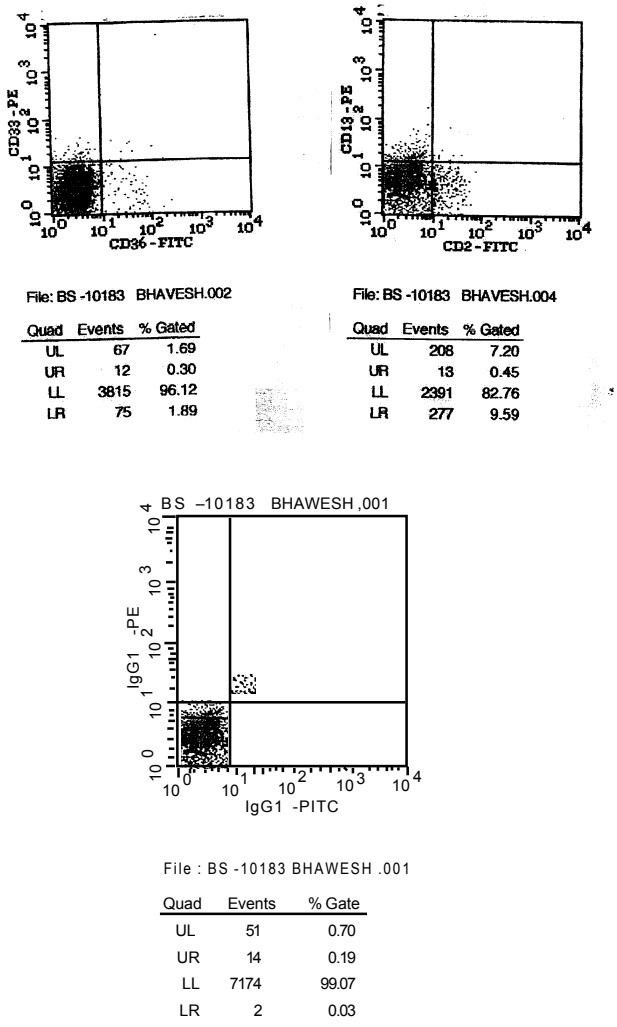
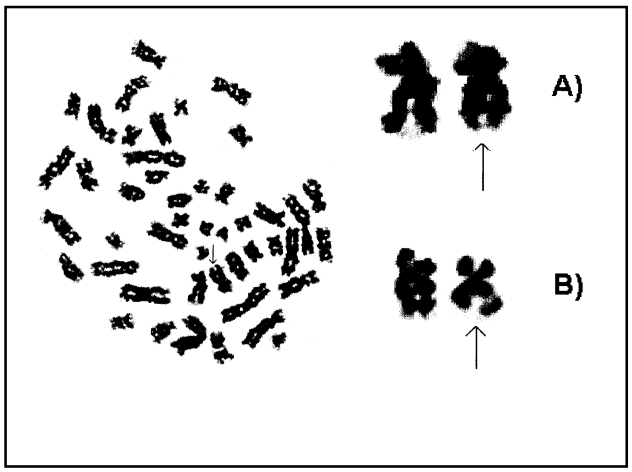


Fig. 1. Immunophenotyping showing negativity for myeloid markers, CD13 and CD33.

Case 2 – A 13 year old male presented with fever off and on for one month. He also complained of giddiness and fatiguibility. There was mild pallor and axillary nodes were palpable on the left side. The liver was 5cm palpable and the spleen was 7cm palpable. The WBC count was 7.43 x 10 9/l, hemoglobin was 8.62 gm/dl and platelet count was 10.7 x10 9/l. The peripheral blood had 57% blasts. The bone marrow aspiration was dilute with 40% blasts. The MPO positivity was 68%. Immunophenotyping done by flow cytometry showed positivity for CD34+68% and HLA-DR+79%. The myeloid markers and the lymphoid markers were



Fig, 2 a & b Partial karyotypes showing del (9) (q21.2; q22.3)

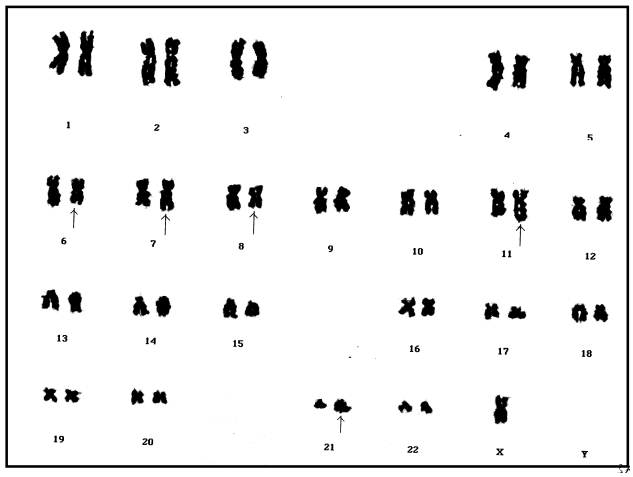


Fig. 3 A

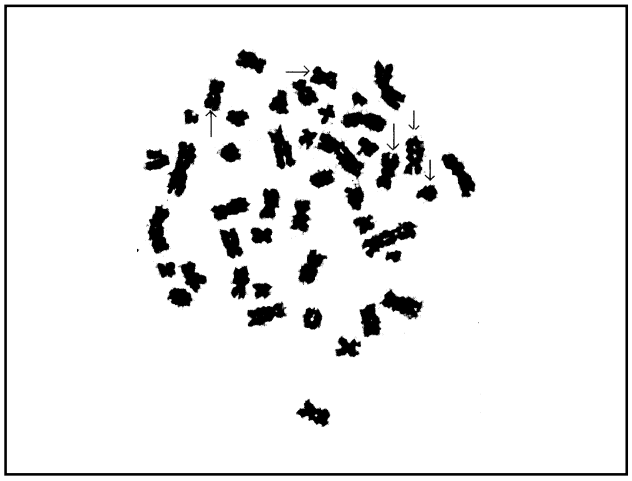


Fig. 3 B G- banded karyotype showing t(8;21) (q22; q22), t(7;11) (q32; qq23) and loss of Y

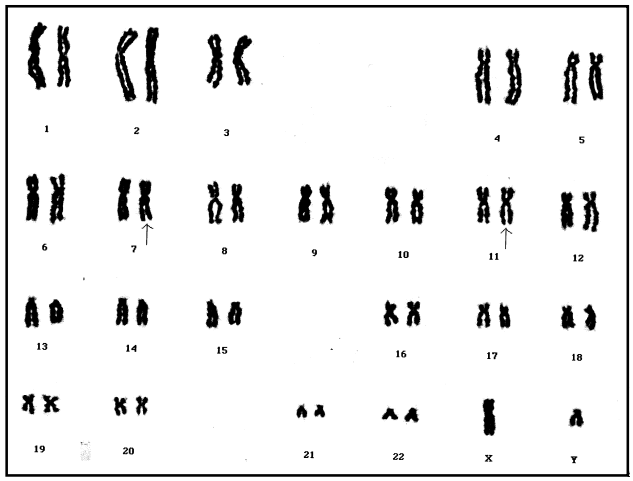


Fig. 4. Karyotype showing t(7;11) (q32; q23)

negative. The immunophenotyping was repeated for myeloid markers as well as cytoCD13. While CD13 and CD33 remained negative, cytoCD13 was positive, 54%. A diagnosis of AML, M2 was made. The cytogenetic abnormality by conventional cytogenetics was found to be t (8; 21)(q22; q22) (Fig. 3). In addition, an unusual karyotypic anomaly in the form of t(7; 11)(q32; q23) was identified. (Fig. 4) Loss of Y was seen in few cells with t(8; 21) / t(7; 11).

DISCUSSION

Acute myeloid leukemia is characterised by the expression of myeloid markers, CD33, CD13, CDw65, myeloperoxidase and/ or anti MPO. Recently, CD117 has been demonstrated to be a more reliable marker of cells committed to the myeloid lineages.[3] Anti-MPO is now accepted as the gold standard in the diagnosis of AML.[4]

Cases of AML where the myeloid antigens are not expressed by the leukemic blasts are extremely rare. Out of 15 such cases reported by Specchia, nine were of the AML-M2 subtype, four were of the M1 subtype and one belonged to the M4 category.[3] Coexpression with CD19 was seen in two cases.[5] Literature reports indicate that most cases of AML with absent myeloid antigen expression belong to the AML-M2 subtype. To the best of our knowledge this unusual phenotype has not been reported in a case of biphenotypic leukemia. (Table 1.)

Table 1. A profile of the Myeloid antigen negative AML cases reported in the literature.

No	Author/ Year of study	No of AML cases	No of myeloid Ag neg cases	FAB subtype
1	Seshi B, 1992	1	1	AML, M1
2	Arber DA,1997	4	4	AML, M2
3	Hirai K, 1999	1	1	—
4	Garcia VJA, 1999	2	2	AML,M2
5	Kragulic N, 2000	220	8	AML, M1 (5), AML, M2 (3)
6	Speechia G, 2000	1	1	AML, M2
7	Present Study	2	2	Biphenotypic (1), AML, M2 (1)

The leukemic blasts in case of t(8;21)(q22:22) associated AML-M2 have a distinct phenotype. They exhibit CD34, HLA-DR, CD65 but CD33 and CD13 expression is very weak or sometimes even absent.[2] The lack of myeloid antigen expression may be due to an asynchronous differentiation or a block in antigen expression at the translational level.[6]

Over the years, immunophenotyping by flow cytometry has become popular and in many institutions is being used as a first line diagnostic modality. The two cases of AML reported here as well as the other cases of AML with this unusual phenotype reported in the literature, underscores the importance of routine peroxidase cytochemical staining. In the absence of cytochemical staining for MPO, the first case with cyto CD3 expression would have been wrongly diagnosed as T-ALL rather than biphenotypic leukemia. It has been reported that some of the antigens express earlier in the cell cytoplasm than in the membrane.[6,11] Positivity for cyto CD13 in the absence of surface CD13 and CD33 in our cases indicates that the antigen appears first in the cytoplasm to be followed by its expression on the membrane.

Approximately, 10-15% of AMLs have demonstrated lymphoid antigen expression.[1] Among the B lymphoid markers, CD20 is most often expressed (20%) followed by CD19 expression (9.8%).[12] In one of our patients, in addition to CD 2, 3, 4 and 7, cyto CD3 was also positive (57% positivity). Two populations of blast cells were seen both on morphology and on immunophenotyping. Whereas one population of blasts was positive for MPO and anti-MPO another group showed cyto CD3 positivity. The MPO positivity was only 9% which is compatible with the degree of MPO positivity seen in cases of biphenotypic and biclonal leukemias. It has been reported that the low MPO reactivity (4 -15%) may demonstrate both lymphoid and myeloid differentiation as seen by a dimorphic blast population as well as immunophenoptyping.[13] The pathogenesis of mixed lineage leukemia is poorly understood. It may be due to the leukemic transformation in a primitive stem cell or the aberrant expression of a lymphoid gene in a myeloid leukemia. [1] The first patient did not respond well to induction chemotherapy on the AML protocol. The day 14

marrow showed 15% blasts. Interestingly these blasts were MPO negative. This case underscores the controversy regarding treatment modalities in patients with mixed lineage leukemias. Whereas some authors are of the opinion that an AML regimen would be in order, others believe that these patients merit lymphoid as well as myeloid directed therapy for remission induction.

The cytogenetic abnormality seen in case 2, with the diagnosis of AML- M2, was t(8; 21). In addition to this, occurrence of an unusual translocation t(7;11)(q32; q23) was an interesting finding. It was identified as a sole anomaly as well as in association with t(8;21). The breaks in chromosome 7 at q32 and chromosome 11 at locus q23 are frequently seen in patients with acute myeloid leukemia.[14]

The karyotypic abnormality associated with most cases of AML presenting with MPO positivity and aberrant myeloid antigen expression has been reported to be t(8; 21). This was also the cytogenetic abnormality reported in our case with the diagnosis of AML- M2. Karyotypic abnormalities involving chromosome 9 are less commonly seen in such cases. The clonal abnormality seen in case 1, was del (9)(q21.2; q22.3), an unfavourable karyotype associated with AML.

To conclude, the two cases showing MPO positivity and absent myeloid antigen expression represent a rare AML phenotype. In certain leukemias, such as AML - M0, MPO is negative cytochemically and the myeloid markers and anti MPO helps determine the myeloid lineage. We wish to report that there are cases where the blasts of myeloid lineage, which are positive for MPO can be negative for surface antigens detected by CD13 and CD33. There is a need to be aware of these aberrations and include CD117 and cytoplasmic CD13 especially in the biphenotypic/ bilineage leukemias.

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