CASE REPORT

Mixed Phenotypic Acute Leukemia Presenting as Mediastinal Mass-2 Cases

Rig Vardhan¹ · Jyoti Kotwal¹ · Prosenjit Ganguli¹ · Rehan Ahmed¹ ·
Ajay Sharma² · Jasjit Singh²

Received: 4 March 2015 / Accepted: 19 October 2015 / Published online: 29 October 2015
© Indian Society of Haematology & Transfusion Medicine 2015

Abstract Mixed phenotype acute leukemia symbolizes a very small subset of acute leukemia that simply cannot be allocated as lymphoid or myeloid lineage. The 2008 World Health Organisation classification established stringent standard for diagnosis of mixed phenotype acute leukemia, accentuating myeloperoxidase for myeloid lineage, cytoplasmic CD3 for T lineage and CD19 with other B markers for B lineage obligation. Mixed phenotype leukemia is rare and 3–5 % of acute leukemias of all age groups, is associated with poor outcome with overall survival of 18 months. We wish to present two cases of mixed phenotypic acute leukemia who presented with mediastinal masses, were suspected to be T cell lymphoma/leukemia clinically and radiologically. In one case, tissue diagnosis was given as lymphoma for which treatment was given. These cases show that patients diagnosed as lymphoma on histopathology can be cases of mixed phenotype acute leukemia and varying specific treatment protocols and follow up are required. Awareness of these entities will help in proper diagnosis and treatment.

Introduction

Mediastinal masses are characteristically associated with Precursor T cell lymphoblastic leukemia/lymphomas and sometimes with Precursor B cell lymphoblastic

leukemias/lymphomas [1]. Rare cases of acute myeloid leukemia presenting as mediastinal mass are reported [2]. Mixed phenotype acute leukemia presenting as mediastinal mass are rare. These represent a very small subset of acute leukemia that simply cannot be assigned as lymphoid or myeloid lineage. This is because of ambiguous phenotype exhibited by the leukemic cells and varying treatment protocols. It encompasses leukemias having separate populations of blasts of more than one lineage or a single population of blasts which co-express antigens of more than one lineage. The 2008 World Health Organisation classification established strict criteria for diagnosis of mixed phenotype acute leukemia, emphasizing myeloperoxidase for myeloid lineage, cytoplasmic CD3 for T lineage and CD 19 with other B markers for B lineage assignment [1, 3]. Mixed phenotype leukemia are rare and are associated with poor outcome compared to other leukemias and clinically presents challenges in diagnosis and treatment. Mixed phenotype acute leukemias represent only 3–5 % of acute leukemias of all age groups, and 2.4–3.7 % in children [4]. However, the true incidence is difficult to establish due to problems with definition, and perhaps variation between different laboratories and also non existence of flow cytometry in most laboratories. It affects both adults and children, more frequently adults and has slight male preference [5]. The prognosis of MPAL is poor comparing to other acute leukemias, with overall survival of 18 months [3]. We wish to present two cases who presented with mediastinal masses and were thought to be Precursor T cell leukemia/lymphomas clinically but who turned out to be mixed phenotypic Acute Leukemia on Flow cytometry thereby changing the treatment protocol and outcome.

✉ Jyoti Kotwal
atuljyot@hotmail.com

¹ Department of Pathology, Army Hospital (Referral & Research), Delhi, India

² Department of Hematology, Army Hospital (Research & Referral), Delhi, India

Flow Cytometric Analysis and Diagnostic Criteria

Flow cytometry analysis (4 colour) was performed on blast cell population identified by CD45 versus light side scatter properties using Beckman Coulter FC 500 instrument and standard staining and analytical methods. The cases were characterized with a panel of antibodies to leukocyte associated markers. A marker was considered positive by this method when more than 20 % or more of the blasts reacted with antibodies to that marker with definite intensity shift greater than a corresponding negative control. All samples were stained as per manufacturer's protocol. Cocktails were used wherever possible. All reagents were CE/IVD approved. Diagnostic criteria as laid down by WHO 2008 were applied.

Case History

Case-I: A 30 years old male patient presented with low grade fever and backache, referred to civil hospital with suspected Lymphoma. MRI spine revealed increased marrow signals on L1-L5 vertebrae and bilateral hip bones. He was referred to higher medical centre of further evaluation. Contrast enhanced computed tomography (CECT) chest revealed anterior mediastinal mass (Fig. 1), mediastinal and left supraclavicular lymphadenopathy with left sided pleural effusion. A tissue diagnosis of lymphoma without subtype definition was given based on cell blocks made from pleural fluid and cyclophosphamide, adriamycin, vincristine, prednisolone (CHOP) 1cycle was exhibited but the patient did not show any improvement. At this juncture patient was referred to this centre. At this hospital on routine peripheral blood smear (PBS) examination we found hemoglobin (Hb) 10.8 gm/dL, total leucocyte count (TLC) 10,940/cumm with 12 % blasts (not picked by cell counter) and platelet count of 133,000/cumm. The blasts were large cells with greyish blue cytoplasm and

Fig. 1 CT scan chest showing anterior mediastinal mass

nuclei with prominent nucleoli. Occasional blasts showed granules. Due to 12 % blasts on PBS examination bone marrow was asked for. Bone marrow aspirate smears were cellular and showed myeloid preponderance with 30 % blasts with morphology same as PBS. 2 % blasts were myeloperoxidase (MPO) positive. Thus opinion of Spill from either lymphoblastic leukemia or undifferentiated myeloid leukemia was offered and flow cytometry (FCM) was undertaken. Flow Cytometry was performed on bone marrow aspirate sample. The gating strategy was FSC versus SSC, SSC versus CD45. Once the blast region was ascertained back gating was done considering the dual morphology of blasts. Backgating was done with CD34 positive population and sequential gating for ontogeny of cells revealed gated cells were positive for CD45 (Dim), CD34, HLA-DR, cMPO, CD33, cCD3, CD7, TdT, CD5 and of these cells 51 % of gated cells were co-expressing cMPO and cCD3. The markers profile was consistent with T/Myeloid Acute Leukaemia (T/My-MPAL) according to WHO 2008. When patient was not responding to therapy then FCM was repeated on 29 Jan 2014 with 3, 44,890/cumm TLC and found gated cells were positive for CD45 (Dim), CD34, cMPO, CD33, CD15, cCD3, CD7 and TdT (Fig. 2a–d).

Case-II: A 38 years old male patient presented at zonal hospital with chronic cough, episodic high grade fever and head ache since 1 month. CECT showed evidence of mediastinal mass and hepato-splenomegaly and suggested a diagnosis of Lymphoma versus Acute Leukemia. TLC was 82,400/cumm, Hb was 14.5 gm/dL and Platelets were 72,000/cumm. PBS revealed 42 % blasts. Bone marrow was done which revealed 90 % blasts. These were categorised into two types morphologically, large blasts with abundant cytoplasm, round to convoluted nucleus, fine lacy chromatin and prominent nucleoli which are 66 % of all nucleated cells (ANC) and 24 % blast which were smaller, had coarse chromatin and inconspicuous nucleoli with scant cytoplasm (Fig. 3). MPO positivity was seen in more than 3 % blasts and thus diagnosis on morphology was Acute Myeloid Leukaemia according to FAB. Flow Cytometry was performed on bone marrow aspirate sample. The gating strategy was FSC versus SSC, SSC versus CD45, back gating with CD34 positive population and sequential gating for ontogeny of cells revealed gated cells were positive for CD45 (Dim), CD34, HLA-DR, cMPO, CD33, cCD3, CD7, TdT, CD5 (Fig. 4). Thus, the diagnosis was MPAL.

Discussion

Morphologically, MPAL is heterogenous and most patients either present as ALL or as AML with or without lymphoid or myeloid or monocytic differentiation features. Thus, a

Fig. 2 **a** Gated cells were positive for CD34, **b** Gated cells were positive for CD33 and negative for HLA DR, **c** Gated cells were positive for CD7, **d** 63.6 % of gated cells were co-positive for Cytoplasmic MPO and CD3

Fig. 3 **a** Two distinct population of blasts in case II, **b** CT scan chest showing anterior mediastinal mass

Fig. 4 **a** SSC versus CD45 and gated cells, **b** Gated cells were positive for CD34, **c** Gated cells were positive for CD33 and HLA-DR, **d** 50.8 % of gated cells were co-positive for Cytoplasmic MPO and CD3

diagnosis of MPAL is unlikely to be suspected by morphology except for the small subset in which there is evidence of a distinct dual blast population with either lymphoid or myeloid features. Therefore, the diagnosis of MPAL always relies on immunophenotyping and exclusion by cytogenetics of AML cases with recurrent abnormalities and by morphology that rules out the presence of a dysplastic background [5].

For practical perspective and to establish a diagnosis of MPAL, it is recommended to use a minimal battery of monoclonal antibodies that includes: anti-CD3, anti-CD19 plus three other B cell associated markers (CD22, CD79a, and CD10), anti-MPO and 2 or 3 monocytic associated markers such as CD14, CD11c, CD64, CD36 or anti-lysozyme [6]. Lysozyme cannot be considered fully specific for the monocytic lineage as it may also be expressed by neutrophil-committed cells and its use is not common in flow cytometry laboratories; however, it can be assessed by immunohistochemistry or the other monocytic markers can establish the monocytic commitment [6].

Before the publication of WHO 2008 classification, the diagnosis and classification of MPAL were based on

European Group for Immunological Classification of Leukemias (EGIL) scoring system [7]. The EGIL classification scheme used to assign score points to major antigens to ascertain if a certain lineage is present.

The most recent 2008 WHO classification has established new and strict criteria for the diagnosis of MPAL. The T lineage is recognized by the presence of specific T-lymphoid antigens, cytoplasmic CD3 or surface CD3 which are best demonstrated by flow Cytometry. The myeloid lineage is established by presence of myeloperoxidase (MPO) by flow cytometry, immunohistochemistry or cytochemistry or monocytic differentiation (requiring at least 2 of the following: non-specific esterase (NSE), Cd11c, CD14, CD64, lysozyme. For B cell lineage multiple antigens are required, including strong expression of CD19 with one of the other B cell markers (CD 79a, cytoplasmic CD 22, CD100, or weak CD 19 expression with at least two other B cell markers [1]. There are four major categories listed under MPAL in the 2008 WHO classification: B/myeloid, NOS; T/myeloid, NOS; MPAL with t (9; 22) (q34; q11.2); BCR-ABL 1; and MPAL with t (v; 11q23); MLL rearranged [6].

Fig. 5 **a** SSC versus CD45 and gated cells, **b** gated cells were positive for CD34, **c** gated area now shows positivity for cytoplasmic MPO but cells which were positive for cytoplasmic MPO and CD3 were now reduced to 8.5 % only

Fig. 6 **a** SSC versus CD45, **b** Gated cells were positive for CD34, **c** Gated cells were positive for CD33 and HLA DR, **d** 48.3 % gated cells were co-positive for cytoplasmic MPO and CD3

Case 1 who was initially treated with CHOP regimen for NHL at peripheral centre was initially treated with ALL induction protocol after being diagnosed as MPAL at this centre. D + 33 bone marrow revealed 8 % blasts following which the patient was treated with hyper CVAD. Repeat marrow showed blasts which were now of only myeloid lineage as per flow cytometry (Fig. 5). AML induction

protocol was instituted. At the end of induction repeat marrow study showed 18 % blasts. Patient then received salvage chemotherapy, mitoxantrone and etoposide but the patient did not respond.

Case 2 was initially treated with ALL induction protocol with which the patient did not respond. Thereafter AML protocol was instituted but the patient failed to respond and

bone marrow still showed persistence of 31 % blasts. Flow cytometry undertaken at this stage. The blast population still showed persistence of all the markers which were present earlier (Fig. 6).

Although the putative cell of origin in MPAL is unknown, it is possible that this leukemia arises in a very early hemopoietic progenitor with potential to undergo either on myeloid or lymphoid differentiation or rarely T- or B-cell differentiation. The recognition of MPAL cases with features of B- and T-cell commitment would support the proposed model of adult BM hemopoiesis where a common lymphoid (B & T) progenitor is present [8].

Extensive data on response to therapy and clinical outcome in MPAL is not available; however cases reported in the literature show unfavourable response both in children and adults. Zheng et al. showed that out of 12 MPAL cases at first diagnosis treated with ALL or ALL based induction regimen, 75 % (9/12) achieved complete remission. Of the 7 patients who were exhibited AML regimen only 2 achieved complete remission. Of the latter group 3 of 5 patients achieved complete remission after switching to ALL based induction therapy. However disease free survival and overall survival was 28.3 and 32.4 % respectively [9].

Matutes et al. analysed clinical, laboratory features and outcome in 100 cases defined as per WHO 2008 and documented poor outcome of MPAL in terms of achieving complete remission and overall survival. Studies have suggested that these are related to association of unfavourable markers like unfavourable karyotype and over-expression of p-glycoprotein. However because of small numbers included and patient heterozygosity the results should be taken with caution [10]. They also suggested that ALL directed therapy was more effective with higher response rate and comparatively better outcome. In the prognostic risk assessment two other variables might be considered as strong predictors for outcome, presence of Philadelphia chromosome and age [5].

We wish to present two cases who presented with mediastinal mass and were thought to be Precursor T cell lymphoma/leukemia but turned out to be MPAL. Both the patients did not attain remission in spite of various

chemotherapy regimen. Thus prospective studies are warranted analyzing the global gene profiling in a large cohort as it will result in better study of pathogenesis of the disease and to provide important information and data of dysregulated genes which in turn may be therapeutic targets.

Compliance with Ethical Standards

Conflict of interest All authors have none to declare.

References

1. Borowitz MJ, Chan JKC (2008) T lymphoblastic leukemia/lymphoma. In: Swerdlow SH, Campo E, Harris NL et al (eds) World Health Organisation classification of tumours: pathology and genetics of tumours of hematopoietic and lymphoid tissues. IARC Press, Lyon, pp 176–178
2. Manjusha N, Kusumakumary P, Sindhu NP (2014) Acute myeloid leukemia presenting as mediastinal mass. *Indian J Paediatr* 81(8):824–825
3. Chen G, Sands AM, Jianlan S (2012) Mixed phenotype acute leukemias. *N A J Med Sci*. 5(2):119–122
4. Rubnitz JE, Onciu M, Pounds S et al (2009) Acute mixed lineage leukemia in children: the experience of St. Jude Children's Research Hospital. *Blood* 113(21):5083–5089
5. Matutes E, Pickl WF, van't Veer M et al (2011) Mixed phenotype acute leukemia (MPAL): clinical and laboratory features and outcome in 100 patients defined according to WHO 2008 classification. *Blood* 117(11):3163–3171
6. Borowitz M, Been MC, Harris NL, Profit A, Matutes E (2008) Acute leukemias of ambiguous lineage. In: Swerdlow SH, Campo E, Harris NL et al (eds) World Health Organisation classification of tumours: pathology and genetics of tumours of hematopoietic and lymphoid tissues. IARC Press, Lyon, pp 150–155
7. Bene MC, Castoldi G, Knapp W et al (1995) Proposals for the immunological classification of acute leukemias. European Group for Immunological Characterization of Leukemias (EGIL). *Leukemia* 9(10):1783–1786
8. Kawamoto H (2006) A close developmental relationship between the lymphoid and myeloid lineages. *Trends Immunol* 27(4):169–175
9. Zheng CC, Wu JS, Liu X et al (2009) What is the optimal treatment for biphenotypic acute leukemia? *Haematologica* 94(12):1778–1780
10. Legrand O, Perrot JY, Simonin G et al (1998) Adult biphenotypic acute leukemias: an entity with poor prognosis which is related to unfavourable cytogenetics and p-glycoprotein over-expression. *Br J Haematol* 100(1):147–155