ROLE OF FLOWCYTOMETRY IN EARLY DIAGNOSIS OF ACUTE PROMYELOCYTIC LEUKEMIA: REPORT OF THREE CASES

Dr. Vijay Kumar, *Dr. Manjari Kishore, Dr. Sadhna Marwah, Dr. A.S. Nigam and Dr. Minakshi Bhardwaj

Department of Pathology, PGIMER, Dr. RML Hospital, New Delhi, India

ABSTRACT

Acute promyelocytic leukemia (APML) is a type of acute myeloid leukemia with genetic abnormalities [t (15;17)]. The patients with APML can present with varied and unusual sign and symptoms. If treated timely, this subtype of APML has good prognosis; hence early diagnosis is important in such cases. Flowcytometric (FCM) immunophenotyping provides an accurate and early method of diagnosing this disease entity, especially in developing countries where molecular diagnosis is still not a feasible option for majority of the population. We present a triad of cases of APML with unusual clinical features where FCM played a significant role in giving an early diagnosis and management of APML.

CASE REPORT

A total of 6 cases of APML were retrieved from the departmental records during the 3-year study period (January 2015-December 2017). However, flowcytometry was done in 3 cases. Herein, we present these three cases where FCM played a key role in giving an early diagnosis of this subtype of AML (APML). The cases are described as below.

Case 1: A 45-year-old male presented with fever for last 20 days along with loose stool and vomiting. Physical examination showed bilateral pitting edema, pallor and minimal crepitations in left axillary region. No history of bleeding, rashes, double vision, lymphadenopathy or splenomegaly was noted. Laboratory investigations revealed hemoglobin of 5 g/dl, total leukocyte count of 23,200/μl, total differential leukocyte count of 23,200/μl with differential count being polymorphs-2%, lymphocytes-20%, promyelocytes-74% and blasts-2%. Abnormal promyelocytes showed high nuclear-cytoplasmic ratio, scanty to moderate granular cytoplasm, opened-up chromatins and evident nucleoli. Few cells showed Auer rods. Platelets were markedly reduced. Serum urea was increased (162 mg/dl). Mild derangement of liver function test was noted. On admission, coagulation profile was within normal limit. The patient was admitted as a case of dengue for which 8 units of platelets were transfused. However, later dengue serology was found to be negative and subsequently after 1 day of admission, coagulation profile was mildly deranged. Further bone marrow examination was advised;
however, we already started with FCM procedure. On FCM, an abnormal cluster with negative to dim expression of CD34 was noted and it constituted 30% of all acquired events. These abnormal cells showed bright positivity for CD13 and heterogenous expression of CD33 [Figure 1]. Negative expression for CD117, HLA-DR, CD5, CD7, CD22, CD10, CD19, CD64, CD14, cCD3 was also noted [Figure 1]. A diagnosis of APML was suggested. Subsequently, cellular bone marrow aspirate smears also showed marked prominence of promyelocytes constituting 65% of all nucleated cells. Marked paucity of normal hematopoietic elements were noted. Bone marrow biopsy showed similar features. Based on peripheral blood smear, FCM and bone marrow findings, a diagnosis of APML (FAB, AML-M3) was suggested.

Case 2: A 20-year-old female presented with history of generalised weakness for last 3 months. She had 2-3 episodes of hematemesis along with menorrhagia for which she was taken to nearby hospital and her Hb was found to be 2 gm/dl. She had received multiple blood transfusions over 3-4 months in multiple centres before coming to our hospital. On admission, physical examination revealed pallor. No lymphadenopathy or hepatosplenomegaly was found. Serum urea (68 mg/dl) and creatinine (2.1 mg/dl) was increased. Liver function test was deranged along with marked increased in serum lactate dehydrogenase (7052 U, normal range being 220-600 U). Coagulation profile was mildly deranged at the time of admission. Complete blood count revealed haemoglobin of 4.8 gm/dl, leucocytosis- 43,500/µL

Figure 1. FCM analysis showing abnormal cluster marked with red colour and revealing bright positivity for CD13 & heterogenous expression of CD33. Also noted are negative expression for CD117, HLA-DR, CD5, CD7, CD22, CD10, CD19, CD64, CD14, cCD3.
Differential leukocyte count: Blasts - 8%, Abnormal promyelocytes - 49%, Myelocyte and metamyelocytes - 26%, lymphocytes - 06%, polymorphs - 11%, along with marked reduction in platelet count (<20,000/µL). Abnormal promyelocytes were large cells with round to oval to bilobed nuclei, moderate amount of cytoplasm with some cells showing Auer rods. FCM analysis showed abnormal cluster myeloid cells constituting 75% of all acquired events and revealed low to intermediate forward scatter and intermediate to high side scatter on FSC/SSC analysis. These abnormal cells showed bright positivity for CD13 along with heterogeneous expression of CD33 [Figure 2]. A dim expression of CD64 was also noted along with MPO positivity. Cells were found to be negative for CD117, CD19, CD22, CD34, HLA-DR, CD5, CD7, CD10, CD64 [Figure 2].

Peripheral blood smear cytomorphology and FCM analysis suggested a diagnosis of APML. These findings were subsequently supported by the bone marrow examination.

**Case 3:** A 10-year-old female presented with high grade fever for last 2 months and left hemiparesis for last 3 days before admission. Physical examination showed pallor and hepatomegaly (2cm below right costal margin). There was no history of bleeding, rashes, double vision, lymphadenopathy or splenomegaly. Complete blood count showed hemoglobin of 3.2 g/dl, total leukocyte count of 2400/µl with differential count being polymorphs-54%, lymphocytes-45%, eosinophil-01%, and platelets of 30,000/µL. No atypical cells or blasts were found on peripheral blood smear; however, platelets were markedly reduced. Liver and renal function tests were within normal limits.
Coagulation profile was also normal in this patient. NCCT head showed a lesion in right temporo-parieto-occipital region, suggestive of infarction. In this case, bone marrow biopsy imprints were cellular showing a marked increase in leukemic promyelocytes constituting 77% of all nucleated cells. Many Auer rods and faggots were also seen. Blasts constituted 7-8% of all nucleated cells. Blasts were intermediate to large in size with high nuclear-cytoplasmic ratio, opened up chromatin and scanty to appreciable amount of cytoplasm containing granules. In the meantime, FCM showed an abnormal cluster of 74% of abnormal promyelocytes. This cluster had a broad base narrowing upwards and showed intermediate to high SSC on FSC/SSC analysis. These cells were brightly positive for CD13 & moderate expression of CD33. A dim expression of MPO was noted. These cells were negative for CD117, HLA-DR, CD34, CD14, CD64, CD5, CD7, CD10, CD19, CD22, cCD3.

A dim expression of MPO was noted. These cells were negative for CD117, HLA-DR, CD34, CD14, CD64, CD5, CD7, CD10, CD19, CD22, cCD3 [Figure 3]. Further, bone marrow biopsy (BMB) revealed cellular marrow spaces showing marked increase in leukemic promyelocytes and scattered blast cells with marked paucity of normal haematopoietic elements. Based on the hematological and FCM findings, a diagnosis of APML was given.

**DISCUSSION**

APML is a subtype of AML and is commonly seen in adults. The incidence appears to rise during the second decade of life, with a plateau during adulthood, and then a decline in...
incidence after age 60 (Vogt, 2007; Kotiah and Besa, 2012). We noted 1 case of APLM in adult and 2 cases in young individuals (20-years female and a 10-years male). APM may also arise as a secondary leukemia, following chemotherapy and radiotherapy. APM was first recognized in the 1950s, with t (15;17) and it continues to be one of the most studied and researched leukemias (Huang, 2007; McKenzie, 2007). It is classified under the French-American-British system of nomenclature as AML-M3 and the World Health Organization as a subtype of AML with characteristic genetic abnormalities. APM arises out of a defect in normal granulocytic cell maturation and apoptosis. Approximately 1,000 new cases are diagnosed each year, with equal incidence among males and females (Huang, 2007; McKenzie, 2007). APL with t (15;17) presents with several clinical symptoms, including fatigue, anemia, shortness of breath, thrombocytopenia, and severe bleeding syndrome.

In our study also, we noted varied clinical presentations in the patients of APM like menorrhagia, infarct, fever, lose stool, vomiting, hematemesis, generalised weakness (Vogt, 2007; Huang, 2007; McKenzie, 2007; Henderson et al., 1996). The mortality rate is high if not treated early. Complications with secondary fibrinolysis and disseminated intravascular coagulation, or DIC syndrome, often occur due to the release of cellular content from promyelocytic cells in the blood (Huang, 2007; McKenzie, 2007; Henderson et al., 1996; Yates, 2010). This risk is more often associated with APL with t (15;17) than other acute myeloid leukemias. Due to the risk of rapid hemolysis, early diagnosis and aggressive treatment is important in ensuring the patient's survival. Many factors increase the likelihood of developing fatal hemorrhages in patients with APM like active bleeding, low levels of fibrinogen (<100 mg/dl), or elevated fibrin degradation products or D-dimers combined with elevated prothrombin time or activated partial thromboplastin time, and those patients with increased WBC or circulating blast counts, abnormal creatinine values, or poor performance status (Yates, 2010; Licht, 2009; Ravindranath et al., 2004; Betz and Hess, 2010; Leu and Mohassel, 2009 Pizzo and Poplack, 2002). Two morphologic variants of APM have been described: typical (hypergranular) type and the microgranular (hypogranular) type. Typical APM has hypergranular abnormal promyelocytes with irregular shaped nuclear contours that frequently have a bilobed appearance (Licht, 2009; Ravindranath et al., 2004; Betz and Hess, 2010; Leu and Mohassel, 2009 Pizzo and Poplack, 2002). Their cytoplasm usually contains numerous large azurophilic granules and Auer rods, some of which can be so abundant that they completely obscure the nucleus.

The cytochemical myeloperoxidase stain is strongly positive in the abnormal promyelocytes. Cells with many prominent Auer rods are often present and have been referred to as term "fagott cells" due to the resemblance to a bundle of straw. Occasionally, more typical myeloblasts with Auer rods can also be seen. In the microgranular type, the abnormal promyelocytes mostly have bilobed nuclei with very few or complete absence of granules. (Ravindranath et al., 2004; Betz and Hess, 2010; Leu and Mohassel, 2009 Pizzo and Poplack, 2002). However, usually a few of the characteristic cells described in the hypergranular type can be found. Flow cytometry (FCM) is emerging as an important tool for the diagnosis of acute leukemia. With increasing numbers of monoclonal antibodies for haematopoietic cell markers becoming commercially available, routine flow cytometry panels are expanding up and can be very easily utilised for giving an early diagnosis of APM (Betz and Hess, 2010; Leu and Mohassel, 2009 Pizzo and Poplack, 2002). The classical PMLI/RARA fusion protein is responsive to all trans-retinoic acid (ATRA) therapy, which restores the intracellular signalling that results in maturation of cells to mature neutrophils (Betz and Hess, 2010; Leu and Mohassel, 2009 Pizzo and Poplack, 2002). Although remission with ATRA alone can be achieved, this does not remove the underlying leukemic clone and relapse occurs. Therefore, additional treatment with more typical anthracycline-based therapy is also needed. Survival rates for patients diagnosed with APL with t (15;17) are good. This type of leukemia is considered preferable or favorable compared to the other variations of AML (Ravindranath et al., 2004; Betz and Hess, 2010; Leu and Mohassel, 2009 Pizzo and Poplack, 2002). However, patients are at higher risk of death by bleeding. Approximately 10% of patients will die from hemorrhage and 40% of untreated cases will show cerebral or lung bleeding (Betz and Hess, 2010; Leu and Mohassel, 2009 Pizzo and Poplack, 2002). This underscores the need to begin therapy early. Statistics show a survival and remission rate of approximately 70% to 90% can be achieved in patients who receive therapies for APL with t (15;17) (Leu and Mohassel, 2009 Pizzo and Poplack, 2002). Keeping in view the good prognosis of this leukemia variant, an early diagnosis is very much essential. In our study, 2 young individuals who presented with chief complaints of menorrhagia and cerebral infarct respectively, were diagnosed with APM on FCM.

Conclusion

APML is a disease characterized by abnormal proliferation of promyelocytic cells in the peripheral blood and bone marrow, along with coagulopathy and thrombocytopenia. The varied and unusual clinical presentations like hemiparesis, stroke, menorrhagia, etc., should be kept in mind while investigating a suspected case of leukemia or thrombocytopenia. In a developing country with paucity of resource and infrastructure for molecular studies, it is very much difficult for the patients to bear costly molecular investigative modalities. In such situation, FCM is lifesaving with early initiation of therapy.

REFERENCES


Vijay Kumar et al. Role of flowcytometry in early diagnosis of acute promyelocytic leukemia: report of three cases