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SHORT COMMUNICATION

CML with Megakaryocytic Blast Crisis: Report of 3 Cases

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Abstract

Chronic myelogenous leukemia (CML) is a chronic myeloproliferative neoplasm consistently associated with the *BCR-ABL1* fusion gene located in the Philadelphia chromosome. The Blast Phase is diagnosed when blasts are ≥20% of the peripheral blood white cell count or of bone marrow nucleated cells or when there is an extramedullary blast proliferation. Megakaryocytic blast crisis as the presenting manifestation of CML is extremely rare and only 7 reported cases were found in the literature. Out of 34 cases of CML-Blast Phase between April 2015 and June 2016, 3 cases showed megakaryocytic differentiation. 2 of these presented in Blast phase as the first manifestation of CML and the third case was a known case of CML-Chronic phase. Flow cytometric immunophenotyping was performed on peripheral blood/bone marrow using 6- color flow cytometer Navios. On CD45 vs SSC two distinct populations of blasts were seen in two cases and single population in the third case. All the 3 cases were positive for CD61, cCD41, cCD61 confirming the megakaryocytic lineage. The clinical features, morphologic and cytogenetic findings help in the identification and distinction of megakaryocytic blast phase of CML from Acute Megakayoblastic Leukemia. The diagnosis of such rare presentation of CML is essential for determining the choice of treatment. Therefore including a megakaryocytic marker in the primary flow cytometry panel is important so that these cases are not underdiagnosed as Acute myeloid leukemia because of expression of CD13 and CD33 only.

Keywords Chronic Myeloid Leukemia · Megakaryocytic blast crisis · Flowcytometric immunophenotyping

Introduction

Chronic myelogenous leukemia (CML) is a chronic myeloproliferative neoplasm that originates in an abnormal pluripotent bone marrow (BM) stem cell and is consistently associated with the *BCR-ABL1* fusion gene located in the Philadelphia (Ph) chromosome. Although the initial major finding is neutrophilic leucocytosis with left shift, the *BCR-ABL*1is found in all myeloid lineages as well as in some lymphoid cells and endothelial cells. The natural history of untreated CML is bi- or triphasic: an initial indolent chronic phase (CP) is followed by an accelerated phase (AP), a blast phase (BP) or both. The BP may be diagnosed when 1) blasts are ≥20% of the peripheral blood white cell count or of bone marrow nucleated cells, or 2) when there is an extramedullary blast proliferation [1]. In approximately 70% of cases the blast lineage is myeloid (neutrophilic, eosinophilic, basophilic, monocytic, megakaryocytic or erythroid blasts) and in 20–30%, lymphoid. In BP morphological, cytochemical and immunophenotypic analysis is recommended for characterization. Megakaryocytic BP as the presenting manifestation of CML is extremely rare. BP with features reminiscent of acute megakaryocytic leukemia (AMKL) is rarely encountered, only 7 reported cases were found in the literature. [2–8]. The reason behind the scarcity of such cases might be their rarity or even under-reporting of such cases due to the absence of availability of extensive panel of flow cytometric immunophenotyping (FCM) markers or electron microscopy. In our experience of FCM of around 18 months, we have come across 3 cases of CML in megakaryocytic BP, 2 as the first manifestation of CML and 1 following CML-CP.

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Materials & Methods

A total of 34 cases presented with CML-BP between April 2015 and November 2016 out of which 3 cases showed megakaryocytic differentiation. All cases included in this study were classified according to the World Health Organization guidelines at the time of initial review [1].

The clinical, hematological, radiological, morphological and immunophenotypic data in these 3 cases were reviewed retrospectively. Clinical characteristics of the patients included age, sex, presenting symptoms and clinical examination findings. Hematological data viz. Hemoglobin levels (Hb), Total Leukocyte Counts (TLC), Platelet counts (PLT), peripheral blood smear (PBS), bone marrow aspirate (BMA) & biopsy (BMBx) and FCM findings were noted. Anemia was defined as Hb value <13 g/dL in males and < 12 g/dL in females. Leucopenia and thrombocytopenia were defined when TLC and PLT were < $4.00 \times 10^3/\mu L$ and < $150 \times 10^3/\ \mu L$ respectively. Radiological data included X-ray, CT/MRI and PET findings if any was done. BMA & BMBx were performed on posterior superior iliac crest using Jamshidi needle in all the cases. The BMA slides were stained with Giemsa stain & BMBx were decalcified and H&E stained sections were evaluated. The aspirates and biopsies were examined by hematopathologists to study the cellularity, megakaryocytes, pattern and arrangement of blast cells and fibrosis. Reticulin stain was done on the BMBx sections. FCM was performed on peripheral blood in one case and on bone marrow in two cases using 6- color flow cytometer Navios (Beckman Coulter). Selected antibody combinations were conjugated to fluorescein isothiocyanate (FITC), phycoerythrin(PE), phycoerythrin-Texas Red conjugate(ECD), PE-Cyanine 5 (PC5), PE-Cyanine 7 (PC7) and allophycocyanin(APC). The panel of antibodies to leukocyte-associated markers, included surface CD1a APC, CD2 FITC, CD3 PC5, CD4 PC7, CD5 PE, CD7 APC, CD8 FITC, CD10 PE, CD13 PC7, CD14 PC7, CD15 PC5, CD19 PC7, CD20 FITC, CD22 PE, CD33 PE, CD34 APC, CD38 PC5, HLA-DR PC5,CD45 ECD, CD56 PE, CD61 FITC, CD64 FITC, CD235 PE, CD117 APC, cytoplasmicCD3 PC5, cytoplasmicCD79a PE, myeloperoxidase (MPO) FITC, terminal deoxynucleotidyl transferase (TdT) FITC.

Results

Out of 34 cases of CML-BP between April 2015 and November 2016, 3 cases of BP with megakaryocytic differentiation were identified. The clinical features and hematological parameters are summarized in Table 1. The PBS showed variable percentage of blasts which were small, with high N/C ratio, opened up chromatin, multiple prominent nucleoli, agranular basophilic cytoplasm with some showing cytoplasmic blebs. Bare megakaryocyte nuclei (Fig. 1a), platelet clumping and platelet dysmorphism was also seen. Normocytic normochromic anemia, left shift with leucocytosis, slight basophilia and normal platelet count was seen in all the 3 cases. BMA turned out to be a dry tap in case 1; hemodiluted and aspirated with difficulty in case 2 and was adequate in case 3.The BMBx showed grade 3 fibrosis in all the three cases with increased and dysplastic megakaryocytes (Fig. 1b, c).

On FCM the blast cells were gated using CD45 vs side scatter strategy. In case 1 and 3 on CD45 versus side scatter two distinct populations of blasts, one with negative and other with dim expression of CD45 with low side scatter was seen. The population of blasts with negative CD 45 expression was positive for sCD61, CD7, cCD41, cCD61and the population of blasts dim positive for CD45 was positive for CD34, CD38, CD13(in case 3), CD33(in case 3), HLA-DR. CD13 & CD33 was positive in Case 3 and cMPO was positive in Case 1. Both the blast populations were negative for CD19, CD20, CD22, CD3, CD4, CD1a, CD8, CD14, CD15, CD64, CD117 and CD235a.In Case 2 a single population of blasts with dim CD45 and low side scatter was seen. The blast population was positive for CD34, CD38, CD61, HLADR, cCD41 and cCD61 and negative for all other markers (Fig. 2).

A diagnosis of Acute Megakaryoblastic Leukemia was established. The Case 1 & 2 cases however, on further investigating turned out to be cases of CML in BP at presentation. The presence of BCR-ABL transcript was demonstrated by reverse transcription-polymerase chain reaction (RT-PCR) in all the cases. The Case 3 was a previously diagnosed case of CML-CP turned into BP.

All 3 cases were hence labeled as CML in megakaryocytic blast phase, based on the presence of a BCR/ABL transcript with a protein of 210 kDa together with the expression of platelet-specific antigens CD41, CD61 on blast surface, slight basophilia, splenomegaly and left-shifted leukocytes. The two patients were started on induction therapy with etoposide, cytarabine, and Adriamycin along with Imatinib Mesylate and were maintained on Imatinib. The case 3 was lost to follow up. No further cytogenetic studies for additional abnormalities was done due to money constraint.

Discussion

Owing to the TKI therapy, identification of CML and its variants are very important for patient management. However, in a minority of cases, diagnosing CML can be a challenge, especially when it presents as a transformed disease (accelerated or blast phase).

Our patients presented with features of de novo AMKL and were classified as CML in megakaryocytic BP only after cytogenetic studies. Differentiation between de novo AMKL and megakaryocytic BP of CML is difficult.

Table 1 Clinical features and hematological parameters in cases of CML with megakaryoblastic blast crisis

Parameters	Case 1	Case 2	Case 3
Age(years)/Sex:	31/Female	70/Male	36/Male
Clinical features:	Weakness, abdominal pain, fever	abdominal pain, breathlessness	Abdominal pain, fever, breathlessness
H epatomegaly:	Present	present	Present
Splenomegaly:	Present	present	Present
Previously diagnosed case of CML on Imatinib:	No	No	Yes
Hb(g/dL):	7.4	7.8	5.6
TLC($\times 10^3$/ μL):	37	56	20
Basophils percentage on PS (%):	10	3	7
Platelet count($\times 10^3$/ μL):	267	183	54
Blast percentage on Peripheral Smear (%):	24	20	60

Blast phase with clinical features quite similar to acute leukemia, is the first presentation of CML in 5–10% of patients [4]. Megakaryocytic blast phase in patient with CML is highly unusual, constituting <3% of transformed cases [8]. To our knowledge only 7 cases of megakaryoblastic crisis have been described as first presentation of CML [2–8].

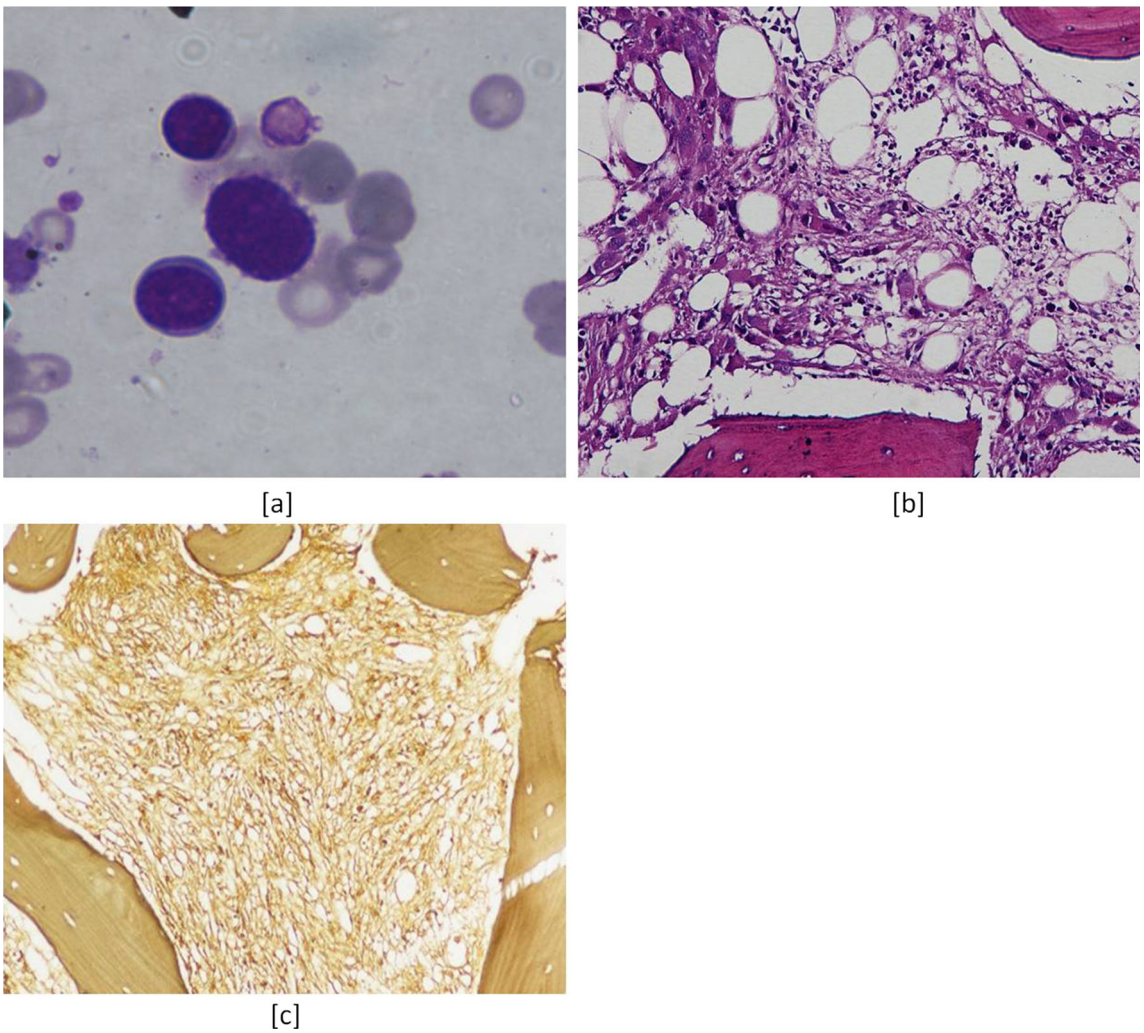

Fig. 1 **a** Peripheral smear (Giemsa stain) showing a megakaryoblast with cytoplasmic blebs and platelet anisocytosis, 40X; **b** Bone marrow biopsy (H&E stain) showing dysplastic megakaryocytes and fibrosis, 40X; **c** Bone marrow biopsy (Reticulin stain) showing diffuse fibrosis, 40X

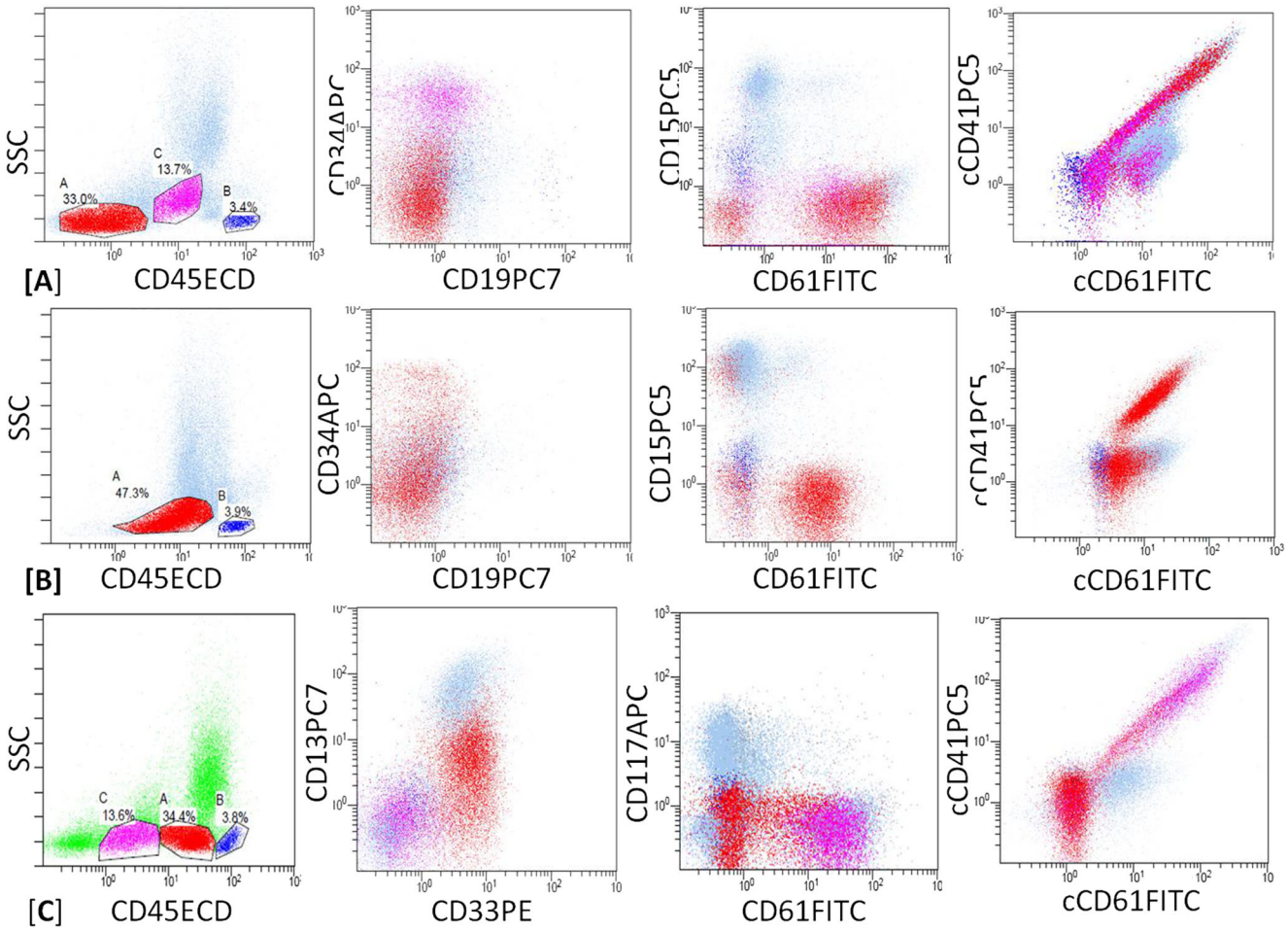

Fig. 2 **a** & **c** Case 1&3:Flowcytometry plots showing 2 distinct clusters of Blasts with positivity for CD61, cCD41, cCD61, CD34 in (**a**) and CD13, CD33 in (**c**). **b** Case 2: Flowcytometry plots showing single cluster of blasts with positivity for CD61, cCD41, cCD61 and partial CD34

Morphologically megakaryoblasts have higher nuclear to cytoplasmic ratio, cytoplasmic budding or vacuoles. The size of the blast cells is quite variable with a round, slightly irregular or indented nucleus with fine reticular chromatin and one to three nucleoli. The cytoplasm is basophilic and usually shows blebs or pseudopod formation [9]. The blast cells showed similar features in all our cases. Bone marrow showed atypical small megakaryocytes, high M: E ratio cells and fibrosis. The role of flow cytometry is important in blast emuneration and lineage assignment in patients in BP. On FCM the megakaryoblasts express one or more of the platelet glycoproteins: CD41 (glycoprotein IIb/IIIa), and/or CD61 (glycoprotein IIIa). The more mature platelet-associated marker CD42 (glycoprotein Ib) is less frequently present. The myeloid associated markers CD13 and CD33 may be positive. CD34, the pan-leukocyte marker CD45, and HLA-DR are often negative; CD36 is characteristically positive. Blasts are negative with MPO antibodies. Lymphoid markers and terminal deoxynucleotidyl transferase (TdT) are not expressed, Cytoplasmic expression of CD41 or CD61 is more specific and sensitive than surface staining; the higher specificity is due to possible adherence of platelets to blast cells in other types of blasts, which may be misinterpreted as positive staining by flow cytometry [9]. Several technical difficulties should be considered while performing FCM in megakaryoblastic leukemias. As CD41 and CD61 are expressed on platelets that can adhere to blasts or other cells leading to a false positive result, repeated washing of the sample prior to labeling so as to release adherent platelets is helpful while evaluating expression of these markers [9]. In our cases samples were washed twice before performing cCD41 and cCD61. Our findings were consistent with the markers of megakaryoblasts, except for CD42 and CD36 which were not available in our set-up. Surface CD61 included in the primary panel was positive in these cases and this was followed by cytoplasmic CD61 and CD41 which were also positive. This confirmed the megakaryocytic lineage of the blasts in our cases. Therefore including a megakaryocytic marker in the primary FCM panel for eg. CD61/CD36 is important so that these cases are not under-diagnosed as Acute myeloid leukemia because of expression of CD13 and CD33 only.

Distinguishing de novo acute megakaryocytic leukemia (AMKL) from megakaryocytic blast phase of CML is important as it has important implications in management of these patients. Although patients with CML present commonly in chronic phase, rarely, they may present in the blast phase, as in our 2 cases. The PBS and BM findings are identical in both groups. Both the groups present with high TLC, fever, anemia, bone marrow dry tap on aspiration due to marked fibrosis, megakaryocytic blasts in both peripheral blood and bone marrow. However, the findings of massive splenomegaly, basophilia, and thrombocytosis point toward CML. Our patients had massive splenomegaly and basophilia but a normal platelet count.

The distinguishing feature between the two groups is the presence of BCR/ABL transcript with a protein of 210 kDa together with the expression of platelet-specific antigens CD41, CD61 on blast surface in patients with CML. However, rare cases of Ph positive de novo AML have been reported [10]. Clinical criteria suggested to differentiate Ph + AML from CML-BP include an absence of a clinical history of a hematologic disorder, lack of evidence of chronic phase or accelerated phase CML after induction chemotherapy, and a lack of clinical and laboratory features of CML, such as splenomegaly and basophilia. Some have suggested that additional cytogenetic aberrations common to CML-BP, such as extra copies of Ph, trisomy 8, trisomy 19 among others; are less common in Ph + AML and that coexistence of normal metaphases along with Ph + metaphases at diagnosis is more characteristic of Ph + acute leukemias than of CML-BP. AMKL is commonly associated with Down Syndrome and with only one other chromosomal abnormality t (1; 22). In addition, return to a normal karyotype following induction chemotherapy is more common in patients with Ph + acute leukemias, whereas the t (9:22) persists in similarly treated CML in blast phase. These patients do not have additional cytogenetic abnormalities, which can be seen in CML with blast phase [11].

Progression of CML signals the development of more aggressive disease with a significantly poorer prognosis when compared to CP patients, even when treated with imatinib. Although hematological responses are seen in up to 50% of patients, the 12-month survival is less than 30% [12]. The prognosis is significantly poor in both de novo AMKL and CML with megakaryocytic blast phase. AMKL by itself is an adverse prognostic factor for disease-free survival. However, the treatment of CML patients in blast phase with a combination of Cytarabine-based induction regimen and the tyrosine kinase inhibitor Imatinib, is less toxic and has a significantly better outcome than when treated with induction therapy alone. Also, the initial use of Imatinib helps to revert from blast phase to chronic phase and further reduction of the BCR-ABL1+ clone achieving a molecular remission prior to stem cell transplantation (SCT) which brings an improved outcome [5, 6].

Hence, AML directed chemotherapy in conjunction with Imatinib; followed by SCT after complete remission promises a better survival outcome in megakaryocytic CML-BP patients than induction regimen alone. An accurate diagnosis is thus important in these patients.

Conclusion

Megakaryocytic blast phase as the primary presentation of CML is very rare and requires Flow cytometric immunopheotyping and additional cytogenetic studies to determine the diagnosis. Use of clinical presenting features, morphologic and cytogenetic findings help in the identification and distinction of megakaryocytic BP of CML from AMKL. Inclusion of one megakaryocytic marker in the primary panel is important so that cases are not under-diagnosed as AML only. Also the diagnosis of such rare presentation of CML is essential for determining the choice of treatment.

Compliance with Ethical Standards

Conflict of Interest None

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