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Flow Cytometry in Chronic Myelogenous Leukemia Blast Crisis

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ABSTRACT

Background: Chronic myelogenous leukemia (CML) is a myeloproliferative disorder due to BCR-ABL1 translocation. Patients showing transformation into blast crisis (BC) have poorer treatment response and prognosis.
Methods: A retrospective study was conducted in the department of Pathology, Maulana Azad Medical College, New Delhi, India, over a time period of 5 years (2014-2019) to evaluate the immunophenotypic features of the blast population. Twenty-one Cases of CML in BC were subjected for multiparametric flow cytometry. Data of the subjects in CML blast crisis was compiled and analyzed for the immunophenotypic categorization of the blast population.
Results: The mean age of the patients at presentation was 39.84 years. Male to female ratio was 1:1.3. Out of 21 cases, 5 (23.8%) showed blasts of myeloid lineage, 8 (38%) myelomonocytic, 6 (28.5%) B lymphoid and 2 (9.5%) showed mixed lineage blast population.
Conclusion: Blast lineage in blast crisis of CML is heterogeneous and may show antigens of more than one lineage. Hence, it is necessary to evaluate the immunophenotypic nature of the blasts for the best appropriate management of the patients accordingly.

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Introduction

Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm, accounting for about 15-20% of all leukemia cases in adults.[1] It is a clonal expansion of hematopoietic stem cells in which the major proliferative component is granulocyte lineage. CML is characterized by the balanced chromosomal translocation between the long arms of chromosome 9 and 22, t (9;22) (q34.1;q11.1), leading to the formation of Philadelphia chromosome, containing BCR-ABL 1 fusion gene.[2]

The usual clinical course of CML is triphasic; chronic phase (CP) followed by accelerated phase (AP) and blast phase (BP) or blast crisis (BC). However, direct transformation to the BC could be expected. CP is a stable stage which may last for more than 10 years, but the median duration is around 3.5 years.[2] BC is usually a terminal event in the course of CML. The criteria for the blast phase is the presence of ≥20% blasts in the peripheral blood or bone marrow nucleated cells and/ or extramedullary blast proliferation in most of the cases blast lineage is composed of myeloid, albeit the blasts may show neutrophilic, monocytic, megakaryocytic, basophilic, eosinophilic, or erythroid phenotypes. Approximately 20-30% of patients with CML develop lymphoblastic crisis, usually B and rarely T or NK cell transformation is described.[3, 4] CML blast crises are often heterogeneous and may show expression of antigens of more than one lineage. So it is important to do the immunophenotypic analysis of the blast population in the BC phase of CML. This study was carried out to evaluate the immunophenotype of the blasts in patients with CML in BC using flow cytometry.

Materials and Methods

A retrospective study was conducted in the department of

Pathology, Maulana Azad Medical College, New Delhi, over a period of 5 years (2014-2019). Peripheral blood of 21 cases of CML were analyzed. Criteria for diagnosis of CML in blast crisis was considered as presence of ≥20% of blasts in the peripheral blood or bone marrow. Clinical, hematological and immunophenotypic data were reviewed.

Hematological parameters including hemoglobin, total leukocyte count, platelet count and blast count done in peripheral blood and bone marrow aspirate smears stained with Giemsa were evaluated. 200 cells count and differential was performed. Immunophenotyping was performed in all cases. Peripheral blood samples collected in EDTA was used for immunophenotyping. Flowcytometry was performed on five color two laser Beckman Coulter FC500. Selected antibodies were conjugated to fluorescein isothiocyanine (FITC), phycoerythrin (PE), phycoerythrin-Texas Red conjugate (ECD), PE-cyanine 5 (PC5) and PE-cyanine 7 (PC7). Standard procedure for cytoplasimic and surface antigens were used. Antibody panel comprised of: CD45, CD19, CD20, CD10, cCD79a, CD3, cCD3, CD4, CD7, CD8, HLA-DR, CD34, CD117, CD56, CD33, CD14, CD38, CD41, CD123, CD16, CD64, CD11c and cMPO.

Results

Demographic, hematologic and immunophenotypic data of 21 cases of CML-BC are shown in Table 1. Mean age of the patients at presentation was 39.84 years (18-54 years). There were 12 female and 9 male patients (F:M,1.3:1). Out of 21 cases, 8 showed blasts of myelomonocytic phenotype (Figure 1), 5 myeloid, 6 B-lymphoid (Figure 2A) and 2 cases showed mixed phenotypic features (Figure 2B) (Table 2), accounting for 38%, 23.8%, 28.5% and 9.5%, respectively. None of the cases expressed antigens indicating T cell differentiation. Two patients with blast crisis of mixed phenotype showed features of both myeloblasts and megakaryocytes confirmed by the expression of CD13, CD33, CD117, HLADR, CD34, CD41 and cMPO on flowcytometry.

Discussion

In the present study, 21 patients with CML -BC on which immunophenotyping by multiparametric flow cytometry was performed at the time of diagnosis were studied over the period of 5 years. The predominant blast phenotype in the BC was myelomonocytic followed by B-lymphoid and myeloid. Two cases showed mixed phenotypic features comprising of myeloid and megakaryocytic

Table 1: Demographic, hematologic and immunophenotypic characteristics of patients with CML-BC

Number	Age	Sex	TLC ($x10^9$/l)	Blast count (%)	Immunophenotyping
1	40	F	60.8	31	Myeloid
2	18	M	173.7	35	B lymphoid
3	42	M	35	65	Myeloid
4	40	F	6.6	20	Mixed
5	32	F	98.3	28	Myelomonocytic
6	45	F	81.9	78	Myelomonocytic
7	40	M	14.7	30	Myelomonocytic
8	21	F	11.6	30	B lymphoid
9	34	F	171.1	41	B lymphoid
10	54	F	479.2	46	B lymphoid
11	32	M	36.1	60	Myelomonocytic
12	55	F	300	49	Myelomonocytic
13	48	M	390.3	45	Mixed
14	50	F	74.3	23	Myeloid
15	25	F	407.4	57	Myelomonocytic
16	35	M	18.3	26	Myelomonocytic
17	48	M	137.9	69	Myeloid
18	50	F	514.6	22	Myelomonocytic
19	38	M	236.8	22	Myeloid
20	40	F	11.8	23	Myelomonocytic
21	35	M	223.4	98	B lymphoid

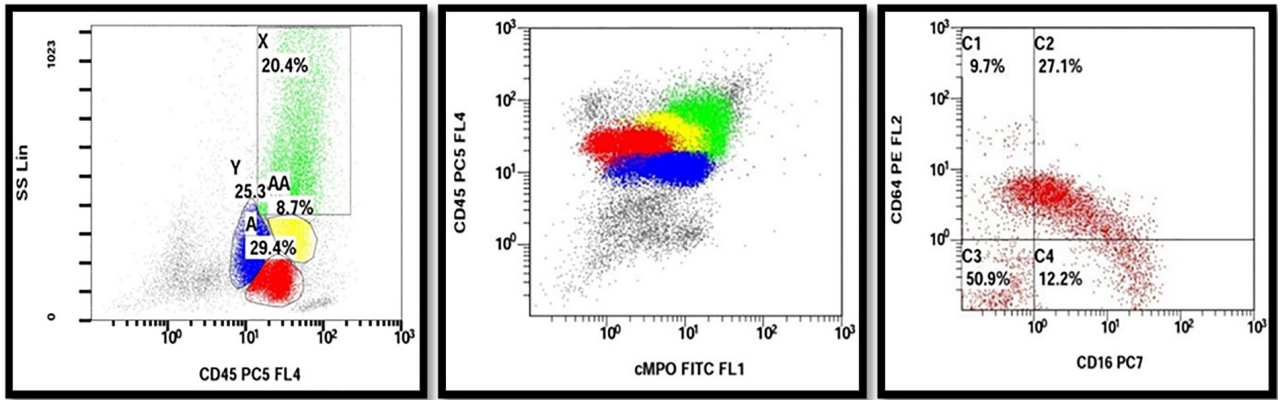

Figure 1: Myelomonocytic blasts showing positivity for CD 16 and CD 64

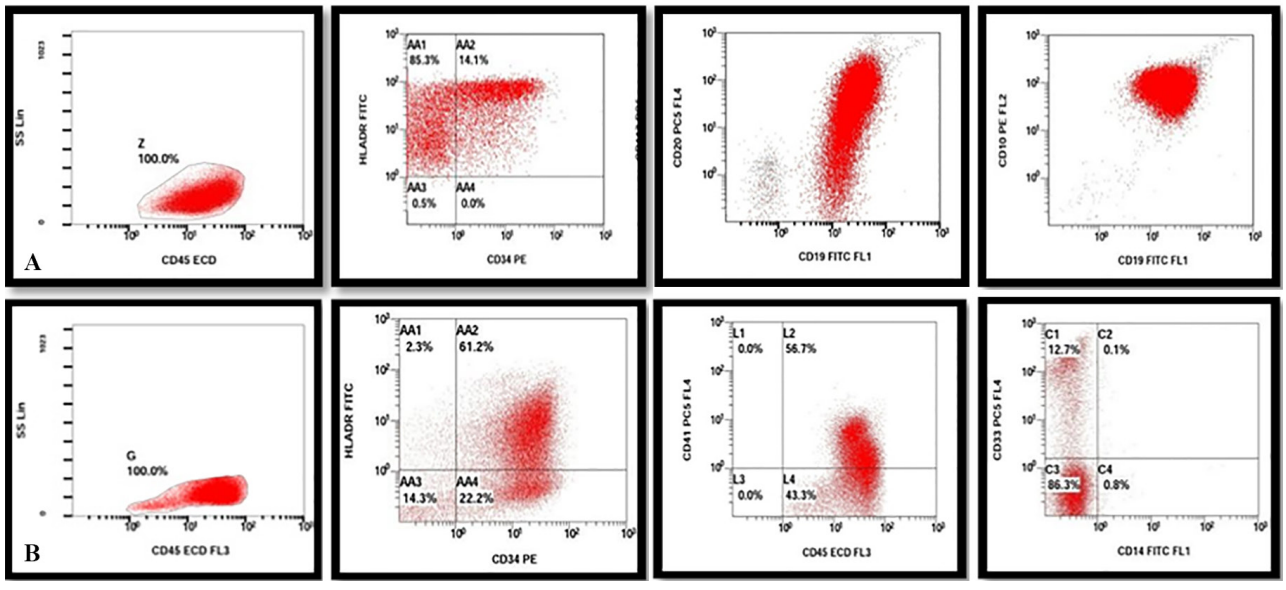

Figure 2: A) Lymphoid blasts showing positivity for CD34, HLA-DR, CD10, CD19 and CD20. B)Megakaryoblast showing positivity for CD34, HLA-DR and CD41.

Table 2: Lineage determination by flow cytometry in patients with CML-BC

Blast phenotype	n=21	%
Myelomonocytic	8	38
Myeloid	5	24
B lymphoid	6	28.5
Mixed	2	9.5

differentiation.

A study conducted by Padmanabhan et al. showed female, while other studies showed male predominance.[5,6] Saikia et al. analyzed 60 cases of CML-BC where predominance blast population was myelomonocytic (50%), followed by mixed phenotypic in 15% and erythroid in 2 (0.3%) of the cases.[7]

In another cohort study on 15 cases of CML, the majority showed myeloid differentiation (80%). No case of mixed phenotype was identified in their study.[6] Khemka et al. reported 3 cases with megakaryocytic blast crisis. [4] Interestingly, there is a case report describing concurrent megakaryocytic and erythroid phenotype in a 65-year-old male patient with CML who developed blast crisis.[1]

Mean survival after the diagnosis of BC is estimated around 2-4 months.[1] It is well known that malignant cells in the blast crisis are different from those in chronic phase so that marked alterations in the proliferation, differentiation, adhesion and apoptosis is described. It is postulated that blast transformation triggered by new molecular or genetic mutations which are non-random.[8] The most common genetic aberrations are reported as trisomy 8, trisomy 19, isochromosome 17, mutations in p53, RB, or RAS pathway. Inactivating mutations of p53 and RUNX1 are indicated in myeloid BC; while in lymphoid blast crisis, inactivating mutations in CDKN2A/B have been observed.[8] CML-BC with unusual blast phenotype is highly resistant to standard induction therapy with response rates less than 20-30%. Treatment for patients with erythroid or megakaryocytic blast phenotype is similar to other cases of CML with BC. However, Cases with lymphoblastic differentiation are treated as acute lymphoblastic leukemia.[1]

According to European Leukemia Net (ELN) 2018, newly diagnosed cases of CML-BC are treated with first generation TKIs (imatinib, nilotinib, dasatinib and bosutinib). Patients who fail to respond to the first generation TKIs should be treated with second or third generation TKIs. Ponatinib is the TKI of choice for the patients with BCR-ABL coding domain T315I mutation. These patients also are suggested to receive polychemotherapy. Patients who have been previously treated with TKI and progress to AP or BC are considered to be treated with TKI which was not administered before. The best option for patients in CML-CP who have developed resistance to at least two TKIs is to undergo allogenic stem cell transplantation.[9]

Conclusion

Chronic myelogenous leukemia in blast crisis has a poor response to standard therapy. The presence of unusual blast phenotypes further decreases the treatment response. Since, presence of the unusual blast phenotypes affect the treatment plan and prognosis of the disease, it is important to identify the blast lineage of the patients in CML-BC. A comprehensive panel of antibodies used in multiparametric flow cytometry helps in lineage determination and detection of aberrant expression of antigens.

Conflict of Interest: None declared.

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