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Immunophenotypic Characterisation of Blast Crisis in Chronic Myeloid Leukemia : Experience at a Tertiary Care Centre

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims and Objectives: Chronic myeloid leukemia (CML) is a clonal stem cell disorder that is hallmarked by the presence of a t(9;22), also known as the Philadelphia chromosome. The natural history of CML is typically triphasic: an initial indolent chronic phase, followed by an accelerated phase and usually a terminal, highly aggressive blast phase. Determination of the cell lineage of CML blasts is clinically important for a better response to chemotherapy and longer survival. Hence, it becomes essential to correctly classify the nature of BC preferably by immunophenotyping for further course of management and survival.
Materials and Methods: This study retrospectively analyzed cases of CML-BC for 5 years and lineage of blasts were determined in each case depending upon the expression of markers by comprehensive immunophenotyping on flow cytometry. EDTA peripheral blood samples or bone marrow aspirates were used for immunophenotyping using 19 antibody panels.
Results: 15 cases of CML-BC were reported for 5 years with a male to female ratio of 2:1 and a median age of 38.3 yrs. Flow cytometry revealed 8 cases were of lymphoid BC and 7 cases were of myeloid BC. Blast percentage ranged from 16% to 90%. Aberrant myeloid antigen expression was

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common in cases with lymphoid BC. Out of 7 cases of myeloid BC, 2 cases of monocytic lineage were seen. CD7 positivity was common in cases with myeloid BC.
Conclusion: Immunophenotyping is important in distinguishing between a myeloid and lymphoid blast crisis, thus providing clinically useful information for further treatment protocols and prognosis.

Keywords: Blast crisis; chronic myeloid leukemia; immunophenotyping.

1. INTRODUCTION

Chronic myeloid leukemia (CML) is a clonal stem cell disorder that is hallmarked by the presence of a t(9;22) BCR-ABL chromosome translocations. Natural history of CML typically triphasic: an initial indolent chronic phase, followed by an accelerated phase and a terminal, highly aggressive blast phase (BC). CML-BC phase of CML is defined as >20% blasts in peripheral blood/bone marrow. Consistent with the early stem cell nature of CML, blastic transformation may be myeloid, lymphoid, or undifferentiated/ mixed, with myeloid blast crisis being about two times more common than lymphoid [1].

Morphological analysis of blast cells to determine the lineage of blasts is inadequate, thus we need special stains and immunophenotyping for better characterization of blast cells. Immunophenotyping is a technique that couples specific antibodies to fluorescent compounds to measure specific protein expression within a cell population. The protein expression is used to identify and categorize the tagged cells, thus determining the lineage of cells. Though flowcytometry has a limited role in CML diagnosis, it is increasingly being used for lineage characterization in the blast crisis phase. Determination of the cell lineage of CML blasts is clinically important for a better response to chemotherapy and longer survival. Additionally, aberrant antigen expression is associated with adverse prognosis [1,2].

Although the definition of CML in the blastic phase has not changed from the pre- to post-imatinib era, the treatment history of the current CML patient population who has managed exclusively with imatinib or other ABL kinase inhibitor therapy, (a significant change in therapeutic approach) will almost certainly affect the kinetics and molecular phenotype of CML-BC [3].

This study was planned to evaluate immunophenotyping of blasts by flow cytometry in cases of CML-BC at a tertiary care institute in North India.

2. MATERIALS AND METHODS

A retrospective analysis was conducted in the Department of Pathology, in the tertiary care institute of north India over a period of 5 years to select all cases of CML-BC. CBC with differential was performed using an Automatic Hematological Analyzer Sysmex XE-5000 (Sysmex America, Inc., Lincolnshire, IL, USA). Peripheral blood film and Bone marrow aspiration smears were stained with Leishman-Giemsa (LG) stain. Blast crisis was defined as >20% blasts in peripheral blood or bone marrow aspirate. Additionally, cytochemical stains like Periodic Acid Schiff (PAS), Myeloperoxidase (MPO), Sudan black were done in these cases wherever possible.

Flowcytometry: Immunophenotyping was performed on 8 Colour Flow cytometer BD FACS Canto II (Becton Dickinson, San Jose, CA) using 19 monoclonal antibody panels to determine the blast lineage on peripheral blood/ bone marrow. Common antibodies used in acute leukemia panel were CD34, HLA-DR, terminal deoxynucleotidyl transferase (Tdt), myeloid markers (cMPO, CD13, CD33, CD117), monocytic markers (CD64), B lymphoid markers (CD19, CD10, CD20, cCD79a), T lymphoid markers (CD3, CD5, CD7, CD4, CD8). All the standard protocols were followed. All these cases were cytogenetically confirmed cases of CML showing the Philadelphia chromosome or BCR-ABL fusion gene. This is a retrospective study that is conducted from data obtained for clinical purposes.

3. RESULTS

In our study, 15 cases were diagnosed as CML-BC by morphology and immunophenotyping for five years. Males outnumbered females with a male to female ratio of 2:1. The median age was 38.3 yrs. Blast percentage ranged from 16% to 90%. Out of 15 cases of CML-BC, 8 cases (53.3%) were categorized as Lymphoid BC and 7 cases (46.6%) as Myeloid BC by immunophenotyping listed in Table 1.

Among the 8 cases of Lymphoid BC, all of them showed positive expression for CD10, CD19, CD20, CD79a, and CD34. Followed by HLA-DR (87.5%), Tdt (75%) and CD4 (25%). Aberrant myeloid antigen expression was common in cases with Lymphoid BC. CD 13 (50%) being the most common, followed by CD33 (25%) and CD 7 (12.5%). (Fig. 1: A case of CML in Lymphoid Blast).

Out of 7 cases of Myeloid BC, 2 cases showed monocytic differentiation. CD13 and CD33 were positive in all the cases (100%), followed by CD34, HLA-DR, cMPO (85.7%), CD117 (71.4%) and CD64 (28.6%). CD7 (42.8%) was the most common aberrant lymphoid antigen seen in Myeloid BC. (Fig. 2: A case of CML in Myeloid Blast Crisis).

4. DISCUSSION

The pathophysiology of CML-BC is incompletely understood. There are various theories for the mechanistic model of CML-BC. i) Progressive genomic instability or epigenetic changes occurs due to BCR-ABL translocation, either at the CML stem cell level and/or in later CML progenitor cells; ii) BCR-ABL kinase activity is directly proportional to the degree of genomic instability; iii) CML stem cells are resistant to ABL-targeted therapy and may act as reservoirs for occult CML progression. In correlating all the possible hypotheses, it is considered that there is an acquired loss of hematopoietic cell differentiation and clonal evolution likely facilitated by the dysregulation of normal apoptotic pathways by BCR-ABL resulting in acute leukemia [3-5].

Table 1. Clinicohematological profile of patients

S. N.	Age (Years)	Sex	Hemoglobin gm%	TLC $\times 10^9$/L	Platelet count $\times 10^9$/L	Blasts %	Type of Blast Crisis
1	28	M	13.6	150	18	85	Myeloid
2	40	M	4.8	80	10	80	Lymphoid
3	30	F	7.4	81	9	80	Myeloid
4	53	M	7.0	47	31	82	Myeloid
5	58	F	7.9	8	200	84	Myeloid
6	36	M	4.8	110	18	85	Lymphoid
7	50	F	4.6	12	4	43	Lymphoid
8	46	M	9.7	75	43	84	Myeloid
9	28	M	9.4	24	91	33	Myeloid
10	45	F	4.0	32	30	90	Lymphoid
11	25	M	11.4	200	150	65	Myeloid
12	38	F	11.6	28	20	72	Lymphoid
13	29	M	8.3	7	250	26	Lymphoid
14	34	M	8.7	100	20	40	Lymphoid
15	35	M	6.0	2	2	90	Lymphoid

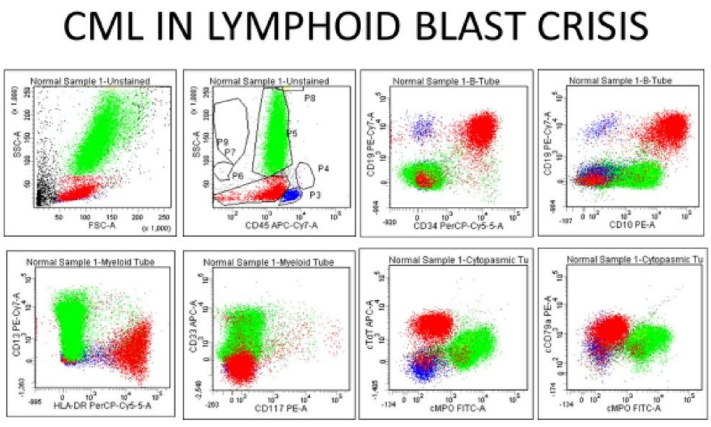

Fig. 1. A case of CML in Lymphoid Blast Crisis; Dot plot SSC/CD45 gating showing dim CD45, coexpression of CD10 & CD19, positive for CD34, CD79a, Tdt, aberrant expression of CD13, negative for CD117 and CD33

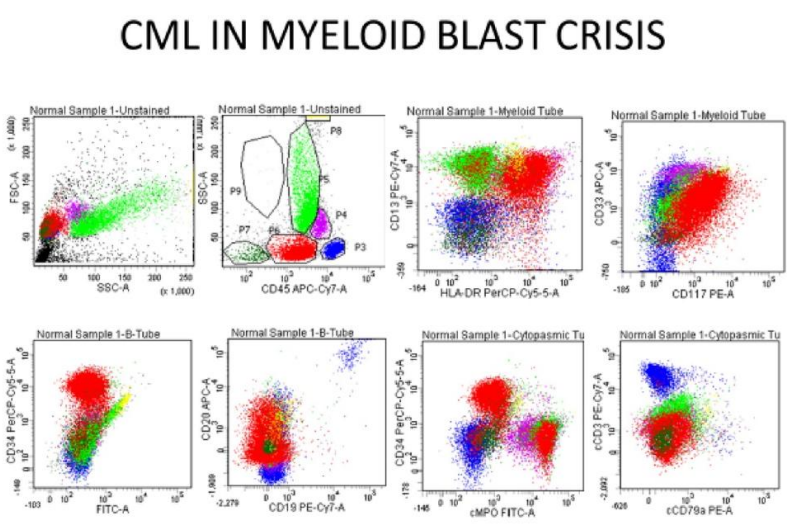

Fig. 2. A case of CML in myeloid Blast Crisis; Dot plot SSC/CD45 gating showing dim CD45, positive for CD34, CD13, CD33, CD117, HLA-DR, cMPO, aberrant expression of CD20, negative for CD19, CD79a and cCD3

Immunophenotyping has a definite and important role in the classification of the blasts. The morphology and the routine cytochemical stains have a high failure rate in the determination of the lineage differentiation particularly in patients with CML-BC. Lineage-specific monoclonal markers have improved the detection of the differentiation pattern in CML-BC [6].

Our study was conducted to analyze the immunophenotyping results of flow cytometry in cases of CML-BC at our center. Out of the 15 cases evaluated, 8 cases (53.3%) were categorized as Lymphoid BC and 7 cases (46.6%) as Myeloid BC. On the contrary *Narang et al* reported 14 cases of Myeloid BC and only 1 case of lymphoid BC [2]. *Shubeilat et al* found 12/14 cases of myeloid BC and 2 cases of lymphoid BC [7]. This discrepancy may be due to the small sample size.

Our data suggest that the majority of myeloid blast crises show minimal myeloid differentiation due to early arrest of maturation associated with asynchrony between cytoplasmic and cell membrane maturation.

The majority of cases did show cross lineage antigen in our study. Our findings are in concordance with the studies done by Narag et al and Nair et al. [2,6]. The malignant event triggering in CML-BC cases occurs in pluripotent stem cells, leading to differentiation along different cell lineages, thus explaining the heterogeneity of the blast population. CD7 was the most common aberrant antigen seen in 42.8% of Myeloid BC in our study. Similar findings were observed by other authors [2,6,8]. Expression of CD7 indicates arrest at an early stage of immature myeloid progenitors which are difficult to detect under normal conditions [9].

Immunophenotyping not only helps characterize the lineage of blasts but also demonstrates the alterations in the expression profile of surface markers on blasts after chemotherapy. Such immunophenotypic shifts might occur in CML-BC patients due to the emergence of a new clones or previously minor clones, resistant to the therapy. This carries prognostic information regarding initiating chemotherapy and response to treatment.

Ascertaining the blast phenotype has its therapeutic implication, since the treatment protocol of lymphoid blast crisis is different from that of the myeloid type. In other words, linking these abnormalities to the lymphoid crisis, in this case, had therapeutic implications since this directed the treatment toward vincristine-and prednisone-based protocols (i.e., lymphoid blast crisis protocol [4,10,11].

5. CONCLUSION

CML BC is a stem cell disorder and the nature of the blast crisis is determined by disorders of the maturation and differentiation. With changing

blast pattern in recent TKI era, immunophenotyping becomes necessary for assigning specific lineage to blasts. This provides clinically useful information for deciding the treatment protocols and prognosis of the patient in addition to clinical features, morphological and cytogenetic findings.

CONSENT

No clinical intervention was performed; only the retrospective data audit was done; individual consent was not obtained as each information was anonymized and the submission did not include any image of any person.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, Bloomfield CD, Cazzola M, Vardiman JW. The 2016 revision to the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia. Blood. 2016;127:2391–2405. Available:https://doi.org/10.1182/blood-2016-03-643544
2. Narang V, Sachdeva MUS, Bose P, Varma N, Malhotra P, Varma S. Immunophenotyping in chronic myeloid leukemia blast crisis: Looking beyond Morphology. J Postgrad Med Edu Res 2016;50(4):181-184.
3. Ilaria RL. Pathobiology of lymphoid and myeloid blast crisis and management issues. American Society of Hematology. 2005;188-194.
4. Al-Khallaf H, Alali H, Alkhatti A. Precursor B cell lymphoid blast crisis of chronic myeloid leukemia with novel chromosomal abnormalities: A case report. Oncology Letters. 2018:16;6691-6. DOI: 10.3892/ol.2018.9497
5. Calabretta B, Perrotti D. The biology of CML blast crisis. Blood. 2004;103: 4010-22.
6. Nair C, Chorpa H, Shinde S, Barbhaya S, Kumar A, Dhond S, et al. Immunophenotype and Ultrastructural Studies in Blast Crisis of Chronic Myeloid Leukemia. Leukemia & Lymphoma. 1995 19:3-4, 309-13. DOI: 10.3109/10428199509107903
7. Shubeilat T, Oudat R, Fares A. Immunophenotypic characteristics of the blast crisis in chronic myelogenous leukemia: Experience at princess Iman research and laboratory sciences center. JRMS. 2006;13(1):5-8.
8. Tang X, Wu D, Xue, Zhu M, Lu D, Ruan C. The morphological, immunophenotypical and cytogenetic characteristics study of blast crisis in chronic myeloid leukemia. Zhonghua Nei Ke Za Zhi. 2002;41(10):685-7.
9. Hirose MY, Shimoyama K, Furkushima T, et al. Immunophenotypic analysis of various blastic crisis in chronic myeloid leukemia: Correlation between CD7 expression and response to chemotherapy. Int J Hematol. 2003 ;77:420-22.
10. Khemka R, Gupta, Jena NK. CML with Megakaryocytic Blast Crisis: Report of 3 Cases. Pathology & Oncology Research. 2018;1-11. Available:https://doi.org/10.1007/s12253-018-0484-7
11. Morris EL, Dutcher JP. Blastic phase of chronic myelogenous leukemia. Clin Adv Hematol Oncol. 2005;3:547–52.

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