IMMUNOPHENOTYPIC CHARACTERISTICS OF THE BLAST CRISIS IN CHRONIC MYELOGENOUS LEUKEMIA: EXPERIENCE AT PRINCESS IMAN RESEARCH AND LABORATORY SCIENCES CENTER

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ABSTRACT

Objective: To characterize the Immunophenotypic of the blast cell population in 14 patients with Philadelphia chromosome-positive chronic myelogenous leukemia in blastic crisis (CML-BC) at Princess Iman Research and Laboratory Sciences Center.

Methods: Bone marrow or peripheral blood smears from 14 CML-BC patients diagnosed at Princess Iman Research and Laboratory Sciences Center during the years 2001 to 2004 were studied by morphological and cytochemical stain analysis. Immunophenotypic analysis was performed by flow cytometry using a panel of 22 monoclonal antibodies.

Results: Cytochemical stain analysis correlated with immunophenotyping results. Twelve patients (86%) were found to have myeloid blast crisis and two patients (14%) had lymphoid blast crisis. The blast crisis were divided into subtypes as de novo acute leukemias: Of the twelve myeloid blast crisis six were differentiated, one had myelomonocytic differentiation, another one had monocytic differentiation and two had megakaryocytic differentiation. In the lymphoid blast crisis one case displayed a mature B cell phenotype while the other case displayed a precursor B cell phenotype. There were two cases transformed to acute myeloid leukaemia after bone marrow transplantation and were diagnosed by cytochemistry only. No T-lymphoid, mixed lineage or undifferentiated blast crisis were observed. The blast cells in both myeloid blast crisis and lymphoid blast crisis displayed the same marker profile as those seen in acute leukemias. CD34 was expressed in all cases of myeloid blast crisis. Coexpression of CD34 and CD7 was observed in one case of myeloid blast crisis with megakaryocytic differentiation.

Conclusion: our findings are comparable to those found in the literature. Immunophenotyping is important in distinguishing between a myeloid and lymphoid blast crisis, thus providing clinically useful information for the designing of treatment protocols and the judgment of prognosis. A sub classification as in acute leukemia is possible. A study of cytogenetic characteristics of CML-BC is planned.

Key words: Flow cytometry, Immunophenotyping, Chronic myelogenous leukemia, Myeloid blast crisis, Lymphoid blast crisis, CD markers.

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Introduction

Chronic myelogenous leukemia (CML) is a clonal myeloproliferative disorder, characterized by the constitutively activated BCR-ABL tyrosine kinase. The course of CML is generally triphasic. The disease is usually in chronic phase; it changes spontaneously after a variable interval to an accelerated phase, and then proceeds to a phase of blastic transformation or blast crisis (BC) [(1)].

BC of CML is characterized by cell line heterogeneity, since the blast cells can express a myeloid, lymphoid, megakaryocytic and, less commonly, an erythroid phenotype [(2,3)].

Immunophenotypic studies using flow cytometry have

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a limited role in the diagnosis of CML but are increasingly being used in chronic myelogenous leukemia in blast crisis cases [4-7].
The primary aim of this study was to characterize the immunophenotype of 14 patients with Philadelphia chromosome – positive CML in BC at Princess Iman Research and Laboratory Sciences Center over 4 years.

Methods

A total of 43 cases of CML were reviewed in the Hematopathology department at Princess Iman Research and Laboratory Sciences Center between March 1, 2001, and December 31, 2004. This included 14 cases (10 males and 4 females) of CML in transformation to acute leukemia. The ages of the CML-BC patients ranged from under than 2 years to 62 years (mean, 32 years; median, 40 years). (Table I)

Morphologic Examination

The diagnosis of CML was made using morphological examination of May-Grünwald-Giemsa stained peripheral blood and bone marrow aspirate smears and the special stain leukocyte alkaline phosphatase. Detection of the Philadelphia chromosome was done by reverse transcriptase polymerase chain reaction (RT-PCR). The specific leukemic fusion transcript BCR/ABL (P210) protein was detected by multiplex RT-PCR System (BIO-RAD mD_x® hema Vision™ kit).

Cases of CML in transformation to acute leukemia were diagnosed by morphological examination of May-Grünwald –Giemsa stained peripheral blood and bone marrow aspirate smears, in addition to special stains including Sudan black B (SB), Naphthol AS-D chloroacetate esterase (NAS-D.CE), α-Naphthy1 acetate esterase (α-NAE); (Sigma-Aldrich, Procedure No. 91) and Acid phosphatase (AcP) (Sigma- Aldrich, Procedure No. 181). BC was defined as the presence of at least 20% blast cells in peripheral blood white cells or bone marrow mononuclear cells.

Flow cytometry

Bone marrow aspirates or peripheral blood were anticoagulated with K_3EDTA and transported immediately to the Hematology department.

Bone marrow or whole blood cells were stained for 10 minutes with various combinations of monoclonal antibodies conjugated by fluoroscein isothiocyanate (FITC) and phycoerythrin (PE). After that, cells were lysed for 10 minutes, using FACS lysing solution from Becton Dickenson(BD), centrifuged and the pellets were washed with cell wash (BD), then the centrifuged pellets were resuspended in FACS flow diluent (BD). Two-color flow cytometry was performed on FACS Calibur, (BD).

For the detection of cytoplasmic CD3, CD79a, MPO, TdT and Lysozyme, cells were lysed for 10 minuets, then washed with cell wash, and were fixed with 100 micoliters of fixative solution A (Caltag) for 15 minutes. After that cells were incubated with monoclonal antibodies and permeabilization medium, reagent B (Caltag), for another 15 minutes, washed, then cells resuspended in FACSflow for flow cytometry.

Cells were analyzed by flow cytometry (FACS Calibur; BD) using the Cell – Quest-software from BD by collecting 10,000 ungated list mode events, selecting an appropriate blast gate on the combination of forward and side scatter and analyzing cells with the most appropriate blast gate. Cell samples were considered positive for a specific antigen if the antigen was expressed on at least 20% of gated leukemic cells. (Table II)

Results

In our patients population 43 cases of CML were diagnosed throughout the period of study. Fourteen cases (33%) of CML were transformed to acute leukemia; twelve (86%) cases were diagnosed as acute myeloid leukemia (AML) and 2 cases as acute lymphoblastic leukemia (ALL), which were analyzed separately. All cases were assigned the French-American-British (FAB) classification [8], and this included FAB M1/M2 (6 cases) expressing CD34, CD13, CD33, CD117, and myeoperoxidase, M4 (1 case) expressing CD13, CD14, CD11b, CD11c, and lysozyme, M5 (1 case) expressing CD11b, CD13, CD14, CD15, CD33, CD34, and lysozyme, M7 (2 cases) expressing CD13, CD33, CD34, CD41, CD61 and CD7 in one case.

Cases of M1 and M2 were combined because no prognostic difference exists based on morphologic differentiation alone. The lymphoid blast crisis demonstrated a B-lineage origin: mature B –cell ALL (TdT^-, $SmIg^+$) in one case and precursor B-cell ($CD10^+$, $CD19^+$, TdT^+, SmIg) in the other, both cases were adults. No undifferentiated, T-lymphoid, or mixed lineage BC was observed.
The flow cytometric Immunophenotypic results correlated with the pattern of results obtained by cytochemical analysis in those cases. There were two cases that transformed to AML after bone marrow transplantation and they were diagnosed by cytochemistry alone. (Table III)

Discussion

The CML-BC has a poor prognosis due to marked refractoriness to treatment, and an overall median survival of 3-6 months [1,9-11].

Evolution of CML into BC is usually of myeloid phenotype (70-85%) and less commonly of B-lymphoid phenotype (16-30%). In this study, twelve out of fourteen patients (86%) displayed a myeloid phenotype and two out of fourteen patients (14%) displayed a lymphoid phenotype. These figures are comparable with the figures reported in other studies including patients diagnosed consecutively at single institution [1,6,910].

The morphologic, immunologic, and cytogenetic characteristics of CML-BC are quite heterogeneous. They could be predominantly myeloid, lymphoid,

erythroid, monoblastic, basophilic and megakaryoblastic [2,3]. In our study all the patients were divided into subtypes as de novo acute leukemia using FAB classification according to the morphological, conventional cytochemical reactions and lineage-specific immunological markers. The blast cells displayed the same marker profile as those seen in de novo acute leukemia. Our results are comparable with the results obtained in other studies [5,10,12]. CD34 was expressed in all blast cells of myeloid blast crisis, other studies showed variable expression of CD34 [5,6,7]. This variability of CD34 expression may be related to the sensitivity of monoclonal antibodies used, technical factors (e.g. flow cytometry sensitivity and gating strategy), and the criteria used to define a positive result [13].

CD34 expression predicts shorter BC duration, which could be related to resistant to treatment due to over expression of P-glycoprotein [4,14].

Cervantes *et al* [10] and Denderian *et al* [11] pointed out that patients with lymphoid blast crisis have a favorable response to treatment and longer survival than other non-lymphoid BC patients.

Coexpression of CD7 (a non specific T-cell marker) and CD34 was observed in one case (a child) of myeloid blast crisis with megakaryocytic differentiation. This coexpression may represent the maturation arrest of immature myeloid progenitor cells when CD7 is transiently expressed which would reflect the early hematopoietic cell nature of the blast cells in CML-BC [4,6,7,15,16].

Hirose *et al* [7] in their study suggested a poorer response to chemotherapy in $CD7^+$ BC patients than those with $CD7^-$ blast cells.

The results obtained in this study in which the CML-BC was established by immunological methods confirmed the importance of immunophenotyping in distinguishing between myeloid blast crisis and lymphoid blast crisis, thus providing clinically useful information for the designing of treatment protocols and the judgment of prognosis.

A sub classification as in acute leukemia is possible. A study of cytogenetic characteristics of CML-BC is planned.

Table I. Characteristics of the 14 patients at blast crisis.

Case No.	Age (year)	Gender (F/M)	FAB-classification	% of blasts in bone marrow
1	32	M	ALL	90
2	45	M	M4	75
3	52	M	M1/M2	87
4	23	F	M1/M2	72
5	62	M	M1/M2	83
6	60	M	ALL	82
7	56	F	M5	54
8	62	M	M1/M2	62
9	38	F	M7	56
10	2	M	M7	20
11	2.5	M	M1/M2	40
12	47	M	M1/M2	80
13*	18	M	M1/M2	35
14*	42	F	M1/M2	52

*The last two cases were diagnosed as AML post bone marrow transplant by cytochemistry alone.

Table II. Monoclonal antibodies.

Markers	Relative Lineage
Isotype controls	--
CD2, CD3, cyCD3, CD5, CD7	T-cell lineage
CD10, CD19, CD20, CD22, CD79b, cyCD79a	B-cell lineage
CD13, CD33, CD14, CD15, CD117, MPO, Lysozyme	Myeloid lineage
CD34, CD45, HLA-DR,TdT	Non lineage restricted

Table III. Immunophenotyping and cytochemistry prophiles of the transformed Leukemia cases.

FAB classification	Antigen expression	NAS-D.CE ‡	α-NAE †	SB ‡‡	AcP §
M1/M2 6 cases	CD13, CD33, CD34, CD117, HLA-DR MPO	6/6	0/6	6/6	0/6
M4 1 case	CD11b, CD11c, CD13, CD14, CD33, CD34, CD11, HLADR, MPO, Lysozyme	1/1	1/1	1/1	0/1
M5 1 case	CD11b, CD13, CD14,CD15, CD33 CD34, HLA-DR, Lysozyme	0/1	1/1	0/1	0/1
M7 2 cases	CD13, CD33, CD34, CD41, CD61, CD117, HLA-DR, (CD7 in one case)	0/2	0/2	1/2	0/2
B-ALL* 1 case	CD10, CD19, CD20, CD22, HLA- DR. SmIg.	0/1	0/1	0/1	0/1
pB-ALL** 1 case	CD10, CD19, CD34, HLA-DR.TdT	0/1	0/1	0/1	0/1

* Mature B-cell ALL ** Precursor B-cell ALL ‡ naphthol AS-D chloroacetate
† α- Naphthol acetate esterase ‡‡ Sudan black B § Acid phosphatase

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