Clinical, Immunophenotypic and Cytogenetic Features of Megakaryocytic Blast Crisis of Chronic Myeloid Leukemia: A Single Institution Study

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ABSTRACT

We present 15 patients with megakaryocytic (Mk) blast crisis (BC) of a Philadelphia (Ph) chromosome positive CML confirmed by immunophenotype analysis between 1989-2000. The primary aim of this study is to define clinical, immunological, cytogenetic and laboratory characteristics of Mk BC in Ph positive CML. We have done retrospective analysis regarding basic clinical findings, immunologic phenotype, cytogenetic studies and platelet functions. All patients had significant expression of CD61 (14/14) and CD34 (13/13) antigens, and a high frequency of expression of CD13 (9/12), CD33 (10/12) and CD11b (9/11). The BC in 6/15 patients was presented with thrombocytosis, 7/15 had a normal platelet count and two patients had thrombocytopenia. A grade IV myelofibrosis was present in 8/10 patients. Six patients evolved additional karyotypic abnormalities. Two patients had extramedullary BC. The serum activity of LDH (med. 1095.6) was elevated in all patients. A platelet dysfunction was documented in 4/5 patient tested. There are no clinical and hematological characteristics specific for Mk BC of CML. Normal or elevated platelet count (med. 427.4 x 10^9/L) in BC of CML with prominent expression of CD34 and CD61 antigens, and significant myelofibrosis (grade IV) are the most consistent clinical findings.

Key Words: Chronic myeloid leukemia, Megakaryocytic blast crisis, CD61, CD34, Platelet count.

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INTRODUCTION

Chronic myeloid leukemia (CML) inevitably undergoes blast crisis (BC), which is characterized by blast stemline heterogeneity (myeloid, lymphoid, megakaryocytic, erythroid) and fatal outcome[1]. The megakaryocytic (Mk) type of BC occurs in 5-31% of patients in BC of CML[1-3]. The criteria for Mk type of acute leukemia was incorporated in the FAB classification in 1985[3]. There are reports about significant diversity in clinical behaviour between lymphoid and nonlymphoid BC[4]. It is critical to make a distinction between lymphoid and nonlymphoid blast crisis, because patients with lymphoid blast crisis respond to treatments that are effective against acute lymphoid leukemia[5]. Subgrouping of nonlymphoid BC in CML by the frequency and intensity of expression of immunologic markers on the malignant cells don't make any difference in regard of survival or prognosis.

MATERIALS AND METHODS

Patients and Diagnostic Criteria

The criteria for diagnosis of Mk BC of Ph positive CML were met by 15 adult patients treated at the Institute of Hematology, in Belgrade, between 1989-2000[8]. There were 158 patients with BC of CML during this period. The diagnosis of BC was based on one or more of the following criteria:

1. Blast cells were > 20% in blood or bone marrow

2. Blasts plus promyelocytes in peripheral blood > 30% or > 50% in the bone marrow: or

3. Extramedullary blastic infiltration[6].

All of the patients with diagnosis of BC of CML were not systematically immunophenotyped, and only those with uninformative cytochemical and morphological findings were sampled and analyzed at the Immunophenotyping and Flow Cytometry Unit of the Institute of Hematology. In a cohort of 40 patients analyzed between February 1, 1989, and October 25, 1999, distribution of CML-BC types were as follows: Myeloid BC, 10; Monoblastic BC, 5; Megakaryoblastic BC, 12; Erythroid BC, 1; B-Lymphoblastic, 9; and Hybrid BC, 3. Diagnosis of CML-BC Mk in another three patients was established by immunohistochemistry.

Treatment

All patients with Mk BC received chemotherapy including doxorubicine and cytosine-arabinoside, except for one (No 4, Table 2) who was treated with cytosine-arabinoside alone and the other (No 14, Table 2) who was treated with large doses of busulfan and hydroxyurea palliation.

Morphological and Cytochemical Characteristics

The blast cells were analyzed on May-Grunwald-Giemsa stained bone marrow smears. The cytochemical reactions for: Sudan black B (SBB), myeloperoxidase (MPO), Periodic acid-Schiff (PAS), nonspecific esterases and Acid phosphatase (AcP) were performed in all patients[7]. The bone marrow biopsies were performed using Jamshidi needles and the samples were fixed in B5 decalcinated and embedded in paraffin. The sections were stained with routine histological stainings (H&E, Giemsa and reticulin).

Immunologic Studies

The immunophenotype analysis was performed on heparinized bone marrow aspirates (5 pts) or peripheral blood samples (7 pts) in 12/15 patients. Mononuclear cells (MNC) were isolated by density gradient centrifugation (1.077 g/mL, Lymphoprep, Nycomed). Blast cell enriched samples were used for immediate immunophenotype analysis using a standard indirect immunofluorescence method, a panel of monoclonal antibodies (MoAb) (Table 1) and GAM-FITC (Coulter) as a second-step reagent[8]. Labeled cells were examined by flow cytometry (EPICS-C, Coulter or FACScalibur, Becton Dickinson). The results were recorded on blast cell gated po-

Table 1. Panel of monoclonal antibodies (MoAb) used in the characterization of leukemic immunophenotype in 15 patients with diagnosis of megakaryocytic blast crisis of CML

Antigen	MoAb	Source
CD2	T11	Coulter
CD3	T3	Coulter
CD7	Leu-9	Becton Dickinson
CD10	J5	Coulter
CD11b	Leu-15	Becton Dickinson
CD13	My7	Coulter
CD14	My4	Coulter
CD15	BMA 0200	Behring Diagnostics
CD19	B4	Coulter
CD33	My9	Coulter
CD34	Anti-HPCA-1	Becton Dickinson; DAKO
CD41	Plt-1	Coulter
CD42a	GpIX	Immunotech
CD61	GpIII	DAKO
GpA	BMA 0160	Behring Diagnostics
HLA-DR	Anti-HLA-DR	Becton Dickinson
MPO	Myeloperoxidase	DAKO
FVIII	Factor VIII-related Ag	DAKO
CD162		DAKO

CD: Cluster of differentiation.

pulations (defined by forward and side-angle scatter characteristics)[9]. Positivity criteria for each MoAb were arbitrarily defined as ≥ 20% labeled cells against a negative control (second Ab only), except for CD34 antigen where this criterion was ≥ 10% positivity.

Immunohistochemistry was performed using bone marrow biopsy specimens (2/15 pts) or lymph node sections (1/15 pt). The 3 µm sections were stained for: CD34, CD61, MPO, fVIII, and CD162 (LSAB/LSAB+, DAKO, Denmark).

Cytogenetic Studies

The chromosome analyses were performed on metaphases from bone marrow aspirates. The chromosomes were prepared directly and also after 24 hour unstimulated short-term incubation. Harvesting and preparation were done according to our modified method[10]. An abnormal clone was ascertained by the finding of at least two mitoses with the same numerical aberration or structural chromosome rearrangement in three mitoses with the same missing chromosome[11].

Platelet Aggregation Studies

A standard aggregometry with adrenaline, ADP, collagen, ristocetine and arahidonic acid was performed[12]. The platelet aggregation-affecting medications were stopped at least 5 days before the assays were done.

RESULTS

Clinical Characteristics

There were 15 adult patients meeting morphologic and immunophenotypic criteria

Table 2. Clinical and hematological characteristics of patients with Mk BC of CML

Patient no	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age/sex	31/M	55/M	49/F	36/M	31/M	36/F	68/F	39/F	49/M	64/F	43/M	52/M	50/F	56/M	33/F
Hb (g/L)	92	77	95	86	77	92	90	108	88	70	87	91	95	98	72
WBC (x 10^9/L)	18	33	16	18	110	6	7	16	20	29	59	191	5	12	104
Plt (x 10^9/L)	544	130	62	112	560	431	385	930	166	10	1700	683	269	230	200
(Pbl %)	9	17	36	3	50	36	38	71	44	66	10	26	25	0	15
(BMbl %)	67	51	45	38	36	37	15	65	37	86	40	48	25	0	32
MPO	ND	-	-	-	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
SBB	ND	ND	ND	ND	-	-	-	-	-	-	-	-	-	ND	-
PAS	ND	+	+	-	+	ND	+	-	-	-	-	-	-	ND	-
DCP (months)	17	5	54	11	52	7	63	57	28	56	47	100	104	29	3
DAP (months)	2	5	20	1	1	2	4	3	4	4	7	4	2	12	2
AP (30-90)	188	62	285	265	200	132	98	99	240	215	142	176	110	192	136
LDH (90-320)	715	786	744	577	2900	1146	1737	547	1261	663	786	2270	356	820	1126

Abbreviations: Hb-hemoglobin, WBC-leukocyte count, Plt-platelet count, Pbl% - peripheral blast percent, BMbl%-bone marrow blast percent, AP-alkaline phosphatase, LDH-lactate dehydrogenase, DAP-duration of acute phase, DCP-duration of chronic phase, ND-not done.

for Mk BC in CML. Clinical and hematological characteristics at presentation are shown in Table 2. The median age was 46 years (range 35-68). There were eight males and seven females. Median duration of the chronic phase was 45 months (range 5-104). The BC lasted between 2-20 months (median 5.2 months). All patients had symptoms of fatigue, weight loss, weakness and two had fever without the signs of infection. Two patients had a simultaneous extramedullary disease in the lymph node (Table 2, patient No 14) and in the central nervous system and in the scull bones (Table 2, patient No 4). All of the patients had hepatomegaly and splenomegaly. Mean hemoglobin concentration was 88 g/L (range 70-108), leukocyte count 43 x 10^9/L (range 6-191) and platelet count 427.4 x 10^9/L (range, 10-1700). The median blast count in the peripheral blood ranged from 0% to 71% (median 29.7). All patients (except one) had elevated serum alkaline phosphatase (median 169.3 IU, range, 62-285) and lactate dehydrogenase (LDH) activities (median 1095.6 IU, range, 356-2900).

Morphological, Cytochemical and Histological Characteristics

Morphological and cytochemical characteristics of the bone marrow blast cell population are summarized in Table 2. The bone marrow aspirate disclosed a blast cell infiltration ranging from 0% to 86% (median 40.3). The blasts had a high nucleo-cytoplasmic ratio with a deep basophilic cytoplasm forming blebs or protrusions. The blasts were negative for MPO or SBB reaction in all patients. A few (< 1%) positive blasts for SBB reaction were detected in three patients. A diffuse pattern of PAS positivity was detected in four patients.

There was a marked fibrosis on the marrow histologic sections. Reticulin and collagen fibrosis was graded from II-IV. In 8/10 patients a grade IV marrow fibrosis was demonstrated. Osteosclerosis was diagnosed in one patient while another one had a nonspecific osteoporosis.

Immunological Phenotyping

Immunologic phenotype was analyzed on bone marrow or peripheral blood mononuclear cells by flow cytometry in 12 patients. The reactivity pattern of leukaemic blasts with MoAbs against lineage associated and/or differentiation antigens are shown in Table 3. The early and lineage unspecified antigens, CD34 and HLA-DR were expressed with a high frequency (12/12 and 8/11 patients respectively). The Mk nature of the blasts was confirmed in all patients, with significant expression of CD61 antigen (12/12 patients, median 49%, range 27-91%). We found CD41 and CD42a positivity in 2/8 and 3/5 patients, respectively. The CD13 and CD33 were significantly expressed in 9/12 and 10/12 patients, respectively. The erythroid and monocytic antigens showed negative reaction with anti-GpA and anti-CD14 MoAbs. The late myeloid differentiation antigen CD11b was expressed in 9/11 patients (median 50% range 5-77%). The CD15 antigen had a weak expression in 2/11 patients, median 11%, range 0-68%). The expression of lymphoid markers was excluded by negative reactions for T (CD3, CD2) and B (CD19, CD10) lineage associated antigens. A significant expression of CD7 was detected in 2/9 patients.

Immunohistochemistry on marrow biopsy sections and on lymph node sections was done in two patients (Table 3). On occasion fVIII, CD 61, CD42a was expressed on the surface of malignant cells.

Cytogenetics

Cytogenetic studies showed the evolving karyotype aberrations 9/15 patients (Table 4). All patients had t(9;22)(q34:q11) at diagnosis. Six patients had complex karyotype abnormalities with frequent numerical and structural chromosomal aberrations. One patient (No 5) had an additional monosomy (-17) while two patients had no additional aberrations. Among the additional chromosomal abnormalities the + t(9;22)(q34:q11) and + 8 were found.

Platelet Function

Aggregation studies showed an altered function of platelets in 4/5 patients studied. There was an absence of aggregation with collagen in 2/5 patients, diminished in 1/5 patient (patient No 5). Three patients had no platelet aggregation response with adrenaline and one had a diminished response. Two patients had absent aggregation with arachidonic acid.

DISCUSSION

The BC of CML has a poor prognosis due to treatment refractoriness[13]. The clinical outcome is uniformly fatal and the median survival is between 3-6 months[8,14]. The morphologic, immunologic and cytogenetic characteristics of BC of CML are quite heterogeneous[15,16]. They could be predominantly myeloid, lymphoid, erythroid, monoblastic, basophilic and megakaryoblastic[2]. This variability could not be confirmed by morphology[17]. The sub-classification of BC of CML into specific types akin to the FAB classification of acute leukaemias is difficult because of the frequent lineage infidelity which results from stem-cell origin of CML[18]. The Ph+ CML undergoes Mk BC in 5-31% of patients and a significant number of these patients have mixed myeloid phenotype which includes expression of CD14, CD15, CD61/CD41 and glycophorine A[2]. There are no reports presenting specific clinical and biological characteristics of “pure” Mk BC of CML. In our series of 15 patients the median duration of chronic phase and survival in BC were not significantly different from those previously reported[2,19]. A therapy with cytosine-arabinoside and doxorubicine was eventfully unsuccessful with the majority of patients. The single case reports showed significant advantage of low-dose etoposide or 6-mercaptopurine[23]. Immunophenotyping of blast cells population disclosed that the megakaryoblastic (CD61+) component of the blast cell population varied between 27% and 91%. Significant levels of

Table 3. Summary of immunophenotypic analysis (values presented in %)

Antigen	Pts no	1	2	3	4	5	6	7	8	9	10	11	12*	13*	14*	15
HLA-DR		22	19	20	3	39	62	81	40	87	ND	12	ND	ND	ND	47
CD34		34	17	24	34	35	62	86	70	82	57	31	ND	+	+	65
CD13		5	24	16	38	49	15	38	29	61	65	30	ND	ND	ND	85
CD33		11	64	0	84	52	41	23	23	64	72	51	ND	ND	ND	45
CD11b		5	64	ND	77	47	33	43	24	31	69	43	ND	ND	ND	13
CD15		5	68	ND	1	12	0	3	0	0	0	36	ND	ND	ND	0
CD61		80	32	28	42	46	61	68	37	91	28	27	+	+	ND	45
CD41		0	2	1	2	14	36	ND	ND	45	8	ND	ND	ND	ND	ND
CD42a		68	11	20	ND	ND	ND	ND	ND	82	16	ND	ND	ND	ND	ND
CD14		0	14	3	2	6	3	1	2	5	0	7	ND	ND	ND	5
GpA		ND	1	ND	6	7	4	3	1	2	ND	17	ND	ND	ND	3
CD19		0	0	0	4	5	7	6	11	0	0	3	ND	ND	ND	5
CD10		0	7	1	1	3	1	1	1	ND	2	8	ND	ND	ND	4
CD2		2	8	5	2	15	32	14	13	ND	8	17	ND	ND	ND	14
CD3		1	5	1	2	13	26	8	12	3	5	9	ND	ND	ND	11
CD7		1	4	ND	9	13	ND	7	11	ND	31	7	ND	ND	ND	46
MPO		ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	+	+	ND	ND
CD162		ND	ND	ND	ND	ND	ND	ND	ND	MD	ND	ND	ND	-	ND	ND
FVIII		ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	+	+	ND

* In these patients a diagnosis was made by immunohistochemistry on bone marrow or lymph node (Pts No 12, 13, 14)

Table 4. Cytogenetic analysis of bone marrow metaphases in 9/15 patients with Mk BC of CML (Patient no)

1.	46, XY, 1qh+ [1] / 46,XY, 1qh+, t(9;22)(q34:q11) [4] / 54 ~ 61, XY, +1, 1qh+, +2, +6, add(7)(q32), t(9;22)(q34:q11), +10, +10, +18, +19, +20, +21, +22, +der(22) t(9;22)(q34:q11), +mar, inc [10]
3.	46, XX, t(9;22)(q34:q11) [8] / 55, XX, t(9;22)(q34:q11), +1, +2, +4, +5, +8, +9 +der(22) t(9;22)(q34:q11), +10, +19, [4] / 80, XX [6] / 160,XX [2]
5.	45, XY, t(9;22)(q34:q11), -17 [13]
6.	46, XX [1] / 46, XX, t(9;22)(q34:q11) [18] / 59, XX, +1, +1, -2, +3, +3, +4, +6, +7, +8, +8, +9, +11, +12, +12, +14[2]
8.	46, XX, t(9;22)(q34:q11) [9] / 48, XX, +8, t(9;22)(q34:q11), +der(22) t(9;22)(q34:q11) [4]
9.	46, XY, t(9;22)(q34:q11) [2] / 46, XY, t(9;22)(q34:q11), add(21)(q22) [4] / 50, XY, (9;22)(q34:q11), +8, +10, +12, add(21)(q22), + add(21)(q22) [7]
12.	46, XY, t(9;22)(q34:q11), del (7)(q22) [15] / 46, XY, t(9;22)(q34:q11), del (7)(q22), add(17)(p12) [5]
13.	46, XX, t(9;22)(q34:q11) [16]
15.	46, XX, t(9;22)(q34:q11) [8]

expression of early and/or myeloid-associated Ags have defined immunologic profile of blast cells in Mk BC as (CD34, HLA-DR, CD61, CD13, CD33, CD11b)+. The CD61 Ag

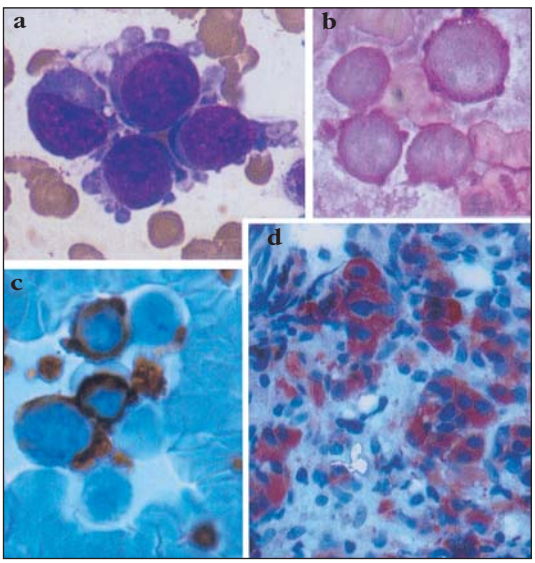

Figure 1a. Megakaryoblasts in bone marrow. Few of them are showing cytoplasmic budding. May-Grünwald-Giemsa, 1000. **1b.** PAS intense positivity of Megakaryoblasts, 1000x. **1c.** CD41 positivity of megakaryoblasts on smear, streptavidin biotin peroxydase, (ABC, Dacopaths 1000x). **1d.** CD41 positivity of megakaryoblasts in bone marrow trephine biopsy, Alkaline phosphatase, LSAB+, Dacopaths, 400x (Courtesy of Dr. AD Bogdanovic and Dr. V Cemerikic).

was more sensitive than CD41 and CD42a. High incidence of CD34 expression (14/14 or 100%) was found in our patient group. CD34 expression is known to predict shorter BC duration[21]. We have also found that our patients had very complex karyotype abnormalities, but none was typical for Mk BC. In a single patient who survived 20 months in BC, a hyperdiploid karyotype was detected. It is not clear why this particular patient had such a long survival compared to other patients. There are some reports about a correlation between blast cell phenotype and chromosomal abnormalities in the Mk BC of CML[25,22]. There was a marked reticulin fibrosis (grade IV) in 8/10 pts. The occurrence of a myelofibrosis is especially noted when platelet-derived growth factor (PDGF) is elevated in the serum and secreted from blasts in accelerated phase or BC of CML[23]. The expression of mRNA of PDGF in blasts was described in a patient with Mk BC of CML and a long survival[24]. Thrombocytosis is a well-known indicator of accelerated phase and a prognostic factor in BC of CML [8,25]. In BC patients with platelet count > 200 x 10^9/L have a relatively better prognosis than those who have < 200 x 10^9/L[14]. This feature could indicate a higher degree of marrow reserve at the time of diagnosis of blast cri-

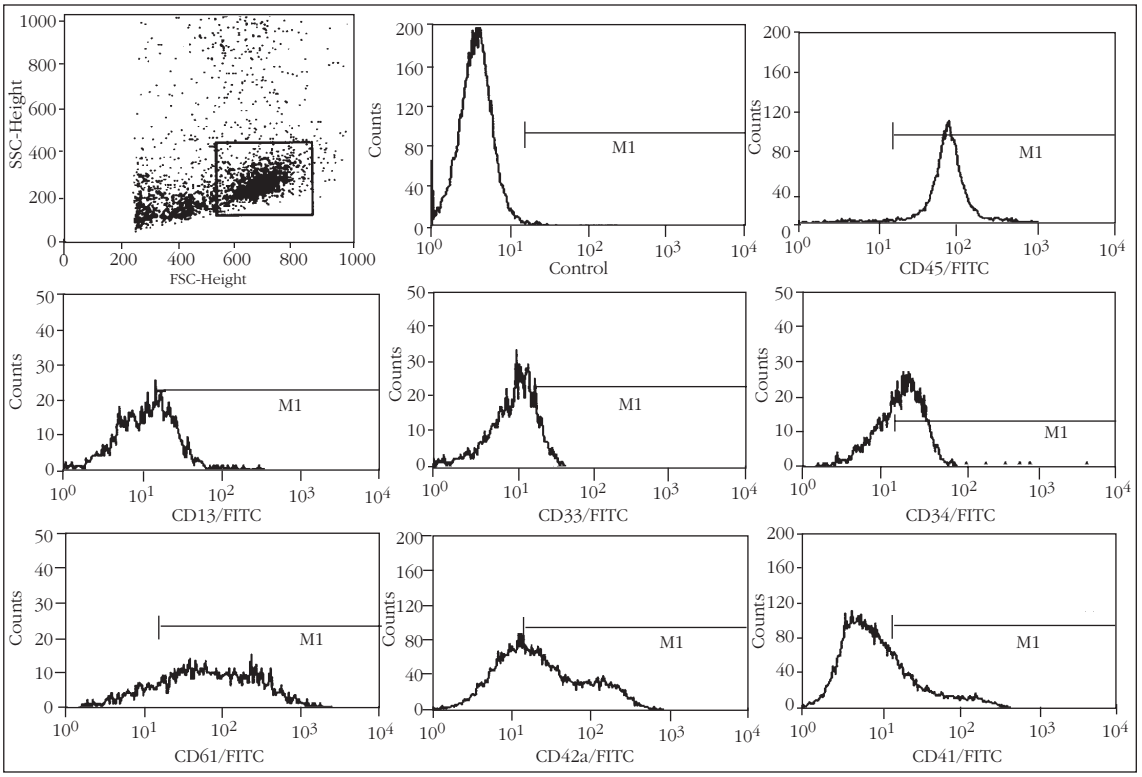

Figure 2. A light scatter characteristic of peripheral blood mononuclear cells of patient No 9 (A, FCS - forward light scatter vs. SSC - side light scatter) and representative individual histograms of CD antigens fluorescence intensity in a gated blast cell population (B-I, FITC - fluoresceinisothiocyanate fluorescence amplification vs. relative number of cells).

sis. Even though Mk BC is mentioned there was no data about platelet count in this particular subtype of blast crisis of CML and the number of patients was too small. The elevated platelet count at diagnosis of chronic phase of CML, which seems to be associated with c3-a2 type of BCR/ABL junction, is present in an entity different from typical CML and clinical characteristics similar to essential thrombocythaemia[26]. Unfortunately, the molecular mechanism of elevated platelet count in c3-a2 type of BCR/ABL is unknown, eventfully so in Mk BC. Noteworthy, 4/5 patients tested had dysfunctional platelets, a finding that is often encountered in patients in the chronic phase of CML. Despite an abnormal platelet function, none of these patients had bleeding events during BC. In two patients there was extramedullary disease (in one CNS chloroma, other in lymph node), which was not surprising in Mk BC.

In conclusion, a normal or high platelet count and a grade IV fibrosis, as well as prominent CD34 and CD61 antigen expression, were the most consistent clinical findings in Mk BC of CML in our patients.

This study was presented at EHA-5 in Birmingham 2000 in a poster session. Unfortunately, due to unintentional omission, coauthors were excluded. Therefore, I am using this occasion to express my regret and apologize for any inconvenience caused.

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