Concurrent Megakaryocytic and Erythroid Chronic Myelogenous Leukemia Blast Crisis

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- Chronic myelogenous leukemia with blast crisis is seen in 15% to 20% of patients with chronic myelogenous leukemia. Chronic myelogenous leukemia with either erythroid or megakaryocytic blast crisis is not uncommon in the clinical setting. The incidence ranges from 0% to 33% in accordance with literature reports. The diagnosis of erythroid or megakaryocytic blast phase is often challenging because the percentage of blasts in the blood or bone marrow required for diagnosis has not been firmly established. Also, some myeloblasts can have aberrant expression of either erythroid or megakaryocytic markers by flow cytometry during clonal evolution. Early recognition of this entity is crucial because either megakaryocytic or erythroid blast crisis predicts an aggressive clinical course. To our knowledge, the coexistence of megakaryocytic and erythroid blasts has not been reported. We report a unique case of chronic myelogenous leukemia with this rare bilineage blast crisis in the background of dysplasia and marked myelofibrosis. Related literature is also reviewed.

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Chronic myelogenous leukemia (CML) is a common myeloproliferative disease, accounting for about 15% to 20% of all cases of leukemia. It is a disorder of clonal expansion of bone marrow stem cells with the characteristic t(9;22)(q34;q11) cytogenetic abnormality that causes Philadelphia chromosome, del(22q). The natural history of the disease is progression from a chronic phase to an accelerated and/or blast phase within 2 to 5 years of the initial diagnosis. In approximately 70% of cases, the blast lineage is myeloid, including neutrophilic, eosinophilic, basophilic, monocytic, erythroid, or megakaryocytic blasts. Any combination of myeloid with erythroid, monocytic, or megakaryocytic blasts can occur. The remaining 20% to 30% of cases show lymphoblast proliferation. It is often difficult to determine the lineage of the primitive blast cells on the basis of morphology alone, and correlation with ancillary studies such as multiparametric flow cytometry, immunohistochemistry, and cytogenetics may be necessary.

Erythroid blast phase of CML is relatively rare and a literature review suggests that the incidence ranges from 0% to 10%. In comparison with the erythroid blast phase, cases of megakaryoblast phase of CML are more common with an incidence ranging from 4.8% to 33%. Either erythroid or megakaryocyte blast phase of CML has a poor prognosis, with a short mean survival of about 2 months postdiagnosis. To our knowledge, coexistence of both megakaryocytic and erythroid lineages in the blast phase of CML has not been reported in the literature. We report a 65-year-old man with a short known history of CML that rapidly progressed to blast crisis and death. This case is unique because it documents CML with myelofibrosis and transformation into concurrent megakaryocytic and erythroid blast crisis. Immunophenotypic characterization of the blasts is determined by multiparametric flow cytometry.

REPORT OF A CASE

A 65-year-old man presented to our hospital with fatigue, abdominal distension, and bilateral leg swelling. His medical history was insignificant for any hematopoietic malignancy including myelodysplastic syndromes, myeloproliferative disorders, leukemia, or lymphoma. Physical examination revealed significant hepatosplenomegaly. His complete blood count showed anemia (hemoglobin, 6.5 g/dL), thrombocytopenia (125 × 10^9/L), and leukocytosis (264.6 × 10^9/L) with 3% blasts, 10% promyelocytes, 19% myelocytes, 13% metamyelocytes, 37% neutrophils, 8% band neutrophils, 5% lymphocytes, 4% eosinophils, and 1% basophils. Bone marrow examination showed panmyeloplasia with myeloid predominance and moderate myelofibrosis. The diagnosis of CML was confirmed by detection of the BCR-ABL fusion product and translocation of chromosomes 9 to 22, or t(9;22), by fluorescence in situ hybridization, conventional karyotyping, and polymerase chain reaction.

Imatinib mesylate (500 mg daily) and hydroxyurea (1500 mg daily) were initiated immediately. His condition improved. Nevertheless, his anemia was persistent and profound despite multiple red blood cell transfusions. Three months post-imatinib mesylate treatment, he presented with nausea, vomiting, and diarrhea. At this time, his complete blood cell count showed leukocytosis (white cell count, 49.3 × 10^9/L), anemia (hemoglobin, 6.1 g/dL), and thrombocytopenia (platelets, 75 × 10^9/L). Flow cytometry performed on the peripheral blood supported a diagnosis of CML blast crisis with megakaryocytic and erythroid features. Despite all medical efforts, his condition deteriorated, culminating in severe acidosis, hypotension, and multiorgan failure, and he died.

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Figure 1. Morphologic changes seen in chronic myelogenous leukemia (CML) with blast crisis. A, Medium-power photomicrograph of bone marrow biopsy section showed an increase of atypical localization of immature precursors (ALIP) with vesicular nuclei, open chromatin, and visible nuclei (hematoxylin-eosin, original magnification ×500). B and C, Medium-power photomicrographs of bone marrow biopsy section with immunohistochemical stains demonstrating erythroid precursors positive for hemoglobin A (B) and megakaryoblasts dim positive for factor VIII (C) (original magnifications ×500). D, Medium-power photomicrograph of bone marrow biopsy section with immunohistochemical stain demonstrating blasts positive for CD34 (original magnification ×200). E, Medium-power photomicrograph of bone marrow biopsy section with immunohistochemical stain demonstrating immature erythroid precursors with intracytoplasmic periodic acid-Schiff–positive granules (original magnification ×500). F and G, Peripheral blood smear showed circulating blasts with a typical megakaryoblastic appearance (agranular, basophilic cytoplasm, distinct blebs, or pseudopod formation) and some erythroblasts with deep blue cytoplasm and vacuoles (Giemsa, original magnification ×1000). H, Peripheral blood smear showed circulating erythroblasts with deep blue cytoplasm, cytoplasmic vacuoles, and finely dispersed nuclear chromatin. A circulating, birefringent neutrophil and giant platelets are present (Giemsa, original magnification ×1000). I, Peripheral blood smear showed promyelocyte, myelocyte, basophil, and circulating nucleated red blood cells. Platelets were variable in size and some showed hypogranulation. Bluish cytoplasmic blebs are noted (Giemsa, original magnification ×1000).

PATHOLOGIC FINDINGS/RESULTS
Morphology and Laboratory Results

The initial peripheral blood smear revealed marked leukocytosis composed mainly of myelocytes, metamyelocytes, and some myeloblasts (3%). Occasional basophils were noted. Some polymorphonuclear cells showed pseudo–Pelger-Hüet nuclear anomaly changes, occasional ringed cells, and giant metamyelocytes. Circulating naked megakaryocytic nuclei and hypogranular giant platelets were also present.

The bone marrow biopsy with dry tap demonstrated a hematopoietic cellularity of 100%. Myeloid series showed hyperplasia with mild dysplastic features (eg, giant metamyelocytes, hypergranulated polymorphonuclear cells, pseudo–Pelger-Hüet changes). No significant increase of blasts (2%–3%) was present. The myeloid-erythroid ratio was approximately 10:1. Megakaryocytes were generally small in size and showed hyperplasia. Some of the megakaryocytes had moderate dysplasia with clustering and hypolobated and hyperchromatic forms. Eosinophils (5%) and basophils (5%) were increased. Erythroid maturation was normoblastic. There was an increase of atypical localization of immature precursors around sinusoidal areas (Figure 1, A). Immunohistochemical stains for hemoglobin A and factor VIII positivity verified that a subpopulation of these cells was megakaryocytic in origin and the remaining cells were erythroid in origin (Figure 1, B and C). Staining for myeloperoxidase proved a myeloid predominance in the background. CD34 and CD117 highlighted myeloblasts and an increase of megakaryoblasts (Figure 1, D). Periodic acid–Schiff stained purplish pink cytoplasmic inclusions in some immature or dysplastic erythroid cells (Figure 1, E). Reticulin stain showed 4+ reticulin fibrosis, and trichrome stain was negative for collagen fibrosis.

Peripheral blood reviewed 3 months after initial diag-
nosis showed a total leukocyte count of $49.3 \times 10^3/\mu L$ with 68% blasts, 3% metamyelocytes, 2% myelocytes, 9% neutrophils, 6% bands, 11% lymphocytes, 0% monocytes, 1% eosinophils, and 0% basophils. Twenty-three percent of the blasts showed the typical megakaryoblastic appearance of agranular, basophilic cytoplasm, distinct blebs, or pseudopod formations (Figure 1, F and G). Adjacent to megakaryoblasts, numerous macrothrombocytes to giant thrombocytes were noted, some demonstrating bizarre shapes and abnormal cytoplasmic contents. The second population of blasts (40%) was pronormoblasts with deep blue cytoplasm, cytoplasmic vacuoles, and finely dispersed nuclear chromatin (Figure 1, H). Multinucleated forms of pronormoblasts were seen. The remaining minor set of blasts was classified as myeloblasts (<5%) by morphology. In the background, there was a left shift of polymorphonuclear cells to the metamyelocyte and myelocyte levels (Figure 1, I). Dysplastic polymorphonuclear cells were also noted. Basophilia was present.

**Multiparametric Flow Cytometry**

Flow cytometry analysis was performed on the second peripheral blood specimen. CD45 gating (CD45 vs side scatter) demonstrated a blastic population (71%) (Figure 2, A) that was positive for CD71 and partially positive for CD13, CD34, CD41, and CD61. Using various back gating strategies, 2 distinct subsets of blasts were identified based on their expression patterns of CD45. One subset (about 65% of the blasts) expressed CD45 at low intensity but CD71 and cytoplasmic myeloperoxidase at high intensities, and only a subpopulation of these cells were partially positive for CD34 at low intensity and partial expression of CD13 (Figure 2, A through D, in red). The other subset (about 35% of the blasts) expressed CD45 at high intensity but CD71 and cytoplasmic myeloperoxidase at low intensities, with homogenous high-intensity expression of CD34 and partial expression of CD13 at low intensity (Figure 2, A through D, in blue). Both subsets of blasts expressed CD41 and CD61 in similar patterns (Figure 2, E and F), and both were negative for CD33 (Figure 2, G). Based on the immunophenotypic profiles of the blasts, 1 subset represented blasts with differentiation toward the megakaryoblastic lineage (in deep blue on the CD45 gating; Figure 2, A), whereas the other demonstrated more erythroid differentiation with loss of CD34, less expression of CD45, but increased expression of CD71 (in red on the CD45 gating; Figure 2, A). A transitional population of the 2 subsets was present and could be best appreciated on the CD13 versus CD34 and CD33 versus CD34 plots (Figure 2, D and G). The blasts were negative for CD3, CD7, CD10, CD19, CD20, glycophorin A, cytoplasmic CD79a, cytoplasmic μ heavy chain, and terminal deoxynucleotidyl transferase.

**Cytogenetics and Fluorescence In Situ Hybridization**

Cytogenetic analysis, using G-banding, revealed all 15 cells to be abnormal, with 3 related abnormal clones. All of the abnormal cells (100%) contained the translocation between chromosomes 9 and 22, which results in the Philadelphia chromosome. In addition to t(9;22), 2 evolved clones were identified. The largest clone (66% of cells), in addition to the t(9;22) translocation, had extra copies of chromosomes 8 and 19 and an inversion of the short arm of chromosome 7. The second sideline clone, in addition to the t(9;22) translocation, also showed trisomy 8. The analysis revealed the following karyotype: 46,XY,t(9;22)(q34;q11.2)[3]/48,ident, inv(7)(p13p21),+8,+19[10]/49,ident,inv(7)(p13p21),+8,+19[2]. A total of 200 cells were evaluated for the t(9;22)(BCR-ABL) translocation. One hundred twenty-six cells (63%) showed a fusion of the BCR-ABL genes.

**Quantitative Reverse Transcriptase–Polymerase Chain Reaction for BCR-ABL t(9;22)**

The initial blood sample showed translocation BCR-ABL gene rearrangement detected for major breakpoint cluster. Three months later, BCR-ABL gene rearrangement was detected with most cells showing major breakpoint cluster and a small subset of cells showing minor breakpoint cluster regions.

**COMMENT**

Chronic myelogenous leukemia evolves from a chronic phase into blast crisis, often associated with additional genetic and molecular secondary changes. Albeit the pathogenetic effects of most CML blast crises are poorly understood, ample evidence suggests that the phenotype of CML blast crisis cells depends on cooperation of BCR-ABL with gene(s) dysregulated during disease progression.10 There is obvious clonal evolution with the development of a minor breakpoint of BCR-ABL identified by polymerase chain reaction in the blast crisis phase in our patient. Although there is no further workup in its downstream genes, it is assumed that the additional minor BCR-ABL gene could have altered its downstream signaling transduction pathway to trigger a blast crisis. Certainly, additional cytogenetic abnormalities also play a critical role in disease progression. In view of cytogenetic abnormalities, there are no specific findings related to either erythroblast or megakaryocytic crisis of CML, although trisomy 8, isochromosomal 17q, and extra Philadelphia chromosome have been described in CML with accelerating phase and blast crisis.1,2,14 Gain of chromosome 19 (eg, trisomy 19) has been reported in CML with megakaryocytic blast crisis as well as in other myeloid disorders.9 In our case, extra copies of chromosome 19 have been identified in 66% of metaphase cells analyzed, which correlates with the morphologic observation and flow cytometry findings: existence of a population of megakaryoblasts. Chronic myelogenous leukemia blast crisis often shows bilineage differentiation, mainly myeloid with a subpopulation of megakaryocytic or other lineages, which is discussed later.

Chronic myelogenous leukemia with erythroid crisis is a rare entity with variable reported incidence rates because the percentage of the erythroid series in the bone marrow is seldom mentioned. Based on the World Health Organization classification, more than 50% of erythroid precursors in the entire nucleated cell population and 20% of myeloblasts from the nonerythroid component are required for a diagnosis of erythroleukemia, type II.1 Whether the same criteria should be applied in CML erythroid blast phase is poorly defined, with no currently established guidelines.7 Some studies have considered the percentage of normoblasts below 50% as criteria for erythroblast phase but not erythroleukemia.7 Although acute erythroid leukemia is far less common than CML erythroblast crisis, a few cases of Philadelphia-positive acute erythroid leukemia have been reported, which causes a diagnostic dilemma.10 The diagnosis of CML erythroid blast phase also proves challenging by flow cytometry. Only a few studies have shown that erythroblasts from...
Figure 2. Immunophenotypic analysis of blasts by multiparametric flow cytometry. A, The CD45 gating shows 2 subsets of blasts. One subset (in blue) represents about 35% of the total blasts expressing CD45 at higher intensity when compared with the other subset (in red, about 65% of the blasts). B, The red subset expresses CD71 at higher intensity when compared with the blue subset. C, Expression of cytoplasmic myeloperoxidase is higher in the red subset. D, The blue subset expresses CD34 homogeneously at higher intensity, whereas the red subset is only partially positive for CD34 at lower intensity in a subpopulation that is negative for CD13. This subpopulation of the red subset (negative for CD13 but partially positive for CD34 at lower intensity) has transitional features between the red and blue subsets. The remainder of the red subset is CD34 negative but mostly positive for CD13. E and F, Subpopulations of both subsets are positive for CD41 and CD61 with similar expression patterns. G, Both subsets are negative for CD33.

acute erythroid leukemia do not express CD45 but are positive for glycophorin and lack panmyeloid markers (CD33 and CD13), whereas normal erythroblasts are positive for CD45, CD34, CD38, and CD117.11 The staining pattern of either erythroleukemia or normal erythroblasts was not clearly revealed in our case because the erythroblasts from our patient were raised from the stem cells causing CML. In the CD71 versus glycophorin stains, the erythroblasts showed a wide spectrum (moderate to bright) expression for CD71 but were negative for glycophorin (Figure 2, B). Glycophorin A negativity and CD71 positivity are consistent with the proerythroblast stage of erythroid differentiation.11 In the leukemic phase of our patient’s peripheral blood, circulating pronormoblasts made up 40% of the differential by manual count with classical morphology. Bone marrow biopsy was not performed because of the patient’s death. In summary, erythroid lineage blast crisis was evident in our CML case by
The morphology of the circulating pronormoblasts and blasts with CD33+/CD71+/glycoporphin− by flow cytometry. Studies have also suggested that erythroid blast phase is not independent of CML chronic phase. A recent case report was able to demonstrate a BCR-ABL fusion product in the normoblasts of CML, which provides concrete evidence confirming erythroid leukemia rather than a hyperplastic process.7

The aforementioned diagnostic issues are particularly relevant to the megakaryocytic blast phase of CML. The percentage of blasts in megakaryocytic leukemia is defined as 20%.1 Megakaryoblast phase of CML and acute megakaryocytic leukemia are rare entities and both carry a poor prognosis. Some studies have reported that approximately 10% of CML cases in the blast phase show predominantly megakaryoblasts.12 Morphologically, experience is needed to identify megakaryoblasts in the blood or bone marrow because megakaryoblasts may be confused with undifferentiated myeloblasts. Some studies have reported that megakaryocytic blast crisis in CML can be identified by budding dysplastic platelets in the peripheral blood.13 Phenotypically, megakaryoblasts are detected by the expression of CD41 and CD61 by either flow cytometry (CD45 dim region) or factor VIII, CD61, and CD42b in immunohistochemical stains. In addition, mRNA expression of platelet factor 4, platelet peroxidase, and glycoprotein IIb could also be performed to identify the megakaryocytic lineage.8 In our patient, about 23% of megakaryoblasts were identified in the peripheral blood by manual count. Flow cytometry was unable to demonstrate a clear megakaryoblast population by CD41 or CD61 because of aberrant expression of megakaryocytic markers in myeloblasts and some erythroblasts (Figure 2, E and F). Marked increase of macrothrombocytes or giant thrombocytes in the case could also play a role in interfering with the identification of CD41 or CD61 populations by flow cytometry. A subset of both CD41+ and CD61+ blasts was also identified by the back gating technique of flow cytometry (Figure 2, E and F).

It has been established that bilineage crisis in CML is common because of disease heterogeneity.14,15 However, combined erythroid and megakaryocytic blast crisis in CML have rarely been reported. The cutoff for the percentage of erythroid blasts in CML could still be a problem for a diagnosis of both erythroid and megakaryocytic blast crisis. With an increase of megakaryoblasts up to 23% of the differential, a finding of 40% erythroblasts of total nucleated cells should be considered as involvement of an additional lineage, as demonstrated in our case.

Another unique feature of our case was a pronounced dysgranulopoiesis in the peripheral blood and bone marrow and dysmegakaryopoiesis and myelofibrosis in the bone marrow preceding blast crisis. These morphologic changes are commonly associated with the accelerated phase and with myelofibrosis.1 Because both dysplasia and myelofibrosis were noted in the initial bone marrow from our patient, it was not clear if the myelodysplasia preceded CML or if it was secondary to CML. Dysplasia was noted in the initial bone marrow from our patient. There was no bone marrow biopsy done before the diagnosis of CML was made in our patient, thus his myelodysplasia could have proceeded to CML or could have been related to myelofibrosis secondary to CML. It has been mentioned that increase of reticulin nesting results in the alteration of morphology in megakaryocytic and myeloid cell lines.1 Moreover, increased reticulin fibrosis is associated with an increased rate of blast crisis. Because megakaryocytic dysplasia is usually present in the background of myelofibrosis, and the latter is indicative of accelerating phase or evolving leukemia in a patient with CML, we concluded that megakaryocytic blast crisis did occur in our case. A case report by Wu et al16 showed that acute myelofibrosis developed in chronic myeloid leukemia and manifested during megakaryoblastic crisis. In our patient, myelofibrosis was detected 3 months before megakaryocytic blast crisis developed. The preexistence of myelofibrosis may have predicted or predisposed the patient to the subsequent development of leukemia.

Chronic myelogenous leukemia blast crisis is highly refractory to standard induction chemotherapy, with a response rate in myeloid blast crisis of less than 20% to 30%.17 There is no additional standard therapy for these patients. The treatment for either erythroid or megakaryoblast phase is similar to the general treatment of CML. Bilineage crisis with megakaryocytic and erythroid components is rare and more experience is needed to evaluate treatment options.

References