Diagnostic Significance of Detecting Dysgranulopoiesis in Chronic Myeloid Leukemia

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Key Words: Chronic myeloid leukemia; Accelerated phase; Blast crisis; Dysgranulopoiesis; Chromosome 17p

Abstract

We examined whether the detection of dysgranulopoiesis in blood or bone marrow would predict chronic myeloid leukemia (CML) in transformation in 31 cases that fulfilled World Health Organization criteria for disease transformation, including 14 in accelerated phase (AP), 10 in myeloid blast crisis (MBC), and 7 in lymphoid blast crisis (LBC). Dysgranulopoiesis was detected in 7 cases, 6 in AP and 1 in MBC, but not in LBC or chronic phase cases. In 3 AP cases, dysgranulopoiesis was identified 2 to 5 months before the morphologic diagnosis of transformation. Two AP cases showed no dysgranulopoiesis in previous blood or marrow smears. For 2 cases (1 AP and 1 MBC), no previous blood or marrow specimens were available. Cytogenetic information was available for 6 of 7 cases with and 22 of 24 cases without dysgranulopoiesis. All cases with dysgranulopoiesis had secondary chromosome abnormalities in addition to t(9;22). In 5 (83%) of 6 cases with dysgranulopoiesis, the secondary chromosome abnormalities included abnormalities of 17p. In contrast, none of the 22 cases of CML in AP or BC but without dysgranulopoiesis showed 17p abnormalities (P = .001). Our findings demonstrated that dysgranulopoiesis was associated strongly with chromosome 17p abnormalities and may indicate the onset of or impending disease transformation.

Chronic myeloid leukemia (CML) is a clonal disorder of hematopoietic stem cells that accounts for 15% to 20% of all leukemias in adults. The disease is characterized by bcr-abl gene fusion typically resulting from t(9;22)(q34;q11.2). The product of the translocation is a constitutively active bcr-abl tyrosine kinase that is believed to be the causative leukemogenic agent. CML usually progresses through 3 clinical stages: an indolent chronic phase lasting for 3 to 5 years followed by an accelerated phase (AP) and, ultimately, blast crisis (BC), a terminal, acute leukemic phase; the disease also can transform into BC without a detectable AP. Such clinical transformation of CML to AP or BC has been demonstrated to be associated with additional genetic changes.

The progressive clinical deterioration that occurs in patients with CML is accompanied by a number of morphologic changes in the blood and bone marrow. While dysgranulopoiesis is not a characteristic feature of the chronic phase of CML, it occasionally is observed in transformed CML. Dysgranulopoiesis originally was described in myelodysplastic syndrome (MDS) and in some cases of primary and secondary acute myeloid leukemia (AML). In MDS and AML, dysgranulopoiesis with pseudo–Pelger-Hüet anomaly has been reported to be associated with loss of chromosome 17p. Among recurring chromosomal abnormalities in CML in evolution, chromosome 17p anomalies are the third most common secondary abnormality following trisomy 8 and gain of an extra Philadelphia chromosome. An association between chromosome 17p abnormalities and dysgranulopoiesis in BC has been reported in a single study; to our knowledge, this association has not been confirmed by other studies. The purpose of the present study was to determine the frequency of dysgranulopoiesis in CML, its specificity...
with respect to disease progression and chromosome 17p abnormalities, and whether detecting dysgranulopoiesis in blood or bone marrow is of value in predicting CML transformation.

Materials and Methods

Cases

All cases of CML seen at the Fairview-University Medical Center, Minneapolis, MN, between July 1996 and July 2002, were retrieved from the hematopathology database.

Morphologic Examination

The Wright-Giemsa–stained peripheral blood and bone marrow aspirate smears were examined by light microscopy. Dysgranulopoiesis was defined as mature granulocytes with hyposegmented nuclei, hypogranular cytoplasm, or both. Dysgranulopoiesis was identified and confirmed independently by 2 hematopathologists (Y.X. and P.L.N.) without knowledge of the cytogenetic findings.

Cytogenetic Analysis

Conventional G-banded cytogenetic analysis was performed on bone marrow cells after 24- or 48-hour culture without mitogen stimulation. At least 20
metaphase cells were analyzed in each case, when possible. Karyotypes of all cases of transformed CML were reviewed by a cytogeneticist (M.M.D.). Fluorescence in situ hybridization was performed on cultured bone marrow cells after fixation in 3:1 methanol acetic acid (Vysis, Downers Grove, IL), according to the manufacturer’s instructions, using a probe to the \(p53\) locus mapped to 17p13.1.

Statistical Analysis

The categorical variables were analyzed with 2-tailed \(\chi^2\) tests by using SPSS statistical software (SPSS Science, Chicago, IL).

Results

Development of Dysgranulopoiesis in CML

Of 167 patients with CML identified, 136 (81.4%) were in chronic phase and 31 (18.6%) showed disease transformation according to the World Health Organization classification criteria,\(^{15}\) including 14 in AP, 10 in myeloid blast crisis (MBC), and 7 in lymphoid blast crisis (LBC). The marrow blast count in these cases ranged from 7% to 88% (median, 20%). In each case, there were at least 100 mature neutrophils available on blood or marrow smears for morphologic evaluation. Dysgranulopoiesis was identified in 7 cases; 4 exhibited pseudo–Pelger-Hüet nuclear features only, and 3 showed both nuclear hyposegmentation and cytoplasmic hypogranularity. Dysplastic neutrophils were seen in the peripheral blood smears in all 7 cases and constituted 11% to 63% of the total neutrophils \(\text{Table 1}\). The absolute neutrophil counts varied widely from 1,500 to 25,600/µL (1.5-25.6 × 10^9/L; \(\text{Table 1}\)). Six of 7 cases were in AP, and 1 was in MBC; dysgranulopoiesis was not identified in any cases of LBC (\(\text{Table 1}\)). Of 24 cases of transformed CML without dysgranulopoiesis, 8 were in AP, 9 in MBC, and 7 in LBC. Thus, compared with cases of CML in MBC, CML in AP was more likely to exhibit dysgranulopoiesis, although this difference did not reach statistical significance \((P = .087)\) \(\text{Table 2}\).

To determine the temporal relationship between dysgranulopoiesis and disease transformation, all available previous blood and bone marrow aspirate smears were examined for dysgranulopoiesis. Three patients exhibited dysgranulopoiesis 2 to 5 months before the transformation, and 2 exhibited dysgranulopoiesis at the time of transformation; for 2, no previous blood or marrow smears were available for review (\(\text{Table 1}\)). A review of treatment history of these patients revealed that 3 received chemotherapy (combined idarubicin and cytarabine in 1 and cyclophosphamide in 2) followed by bone marrow transplantation 3, 24, and 48 months before the onset of dysgranulopoiesis, respectively; 3 were treated with hydroxyurea; and 1 had no known history of drug exposure.

Correlation of Dysgranulopoiesis With Chromosome 17p Abnormalities

Cytogenetic results were available for 28 of 31 transformed CML cases, including 6 with and 22 without dysgranulopoiesis. Overall, 18 (64%) of these 28 cases showed secondary chromosome abnormalities in addition to t(9;22)(q34;q11.2) or its variant(s). All of the cases with dysgranulopoiesis had secondary chromosome abnormalities. Five of 6 cases with dysgranulopoiesis showed chromosome 17p abnormalities \(\text{Table 3}\). Three cases had loss of 17p as the sole secondary abnormality, and 2 cases exhibited complex cytogenetic abnormalities in addition to 17p abnormalities. One case with dysgranulopoiesis had no 17p abnormalities (see “Discordant Case”). In contrast, none of the 22 cases without dysgranulopoiesis had 17p abnormalities. Of these, 10 had no secondary cytogenetic abnormalities, and 12 showed cytogenetic evolution other than 17p abnormalities, including 7 cases with complex secondary cytogenetic abnormalities.

\(\text{Table 1}\)

Cases of Chronic Myeloid Leukemia With Dysgranulopoiesis

<table>
<thead>
<tr>
<th>Case No./Sex/Age (y)</th>
<th>Diagnosis</th>
<th>Cells With Dysgranulopoiesis (%)</th>
<th>Absolute Neutrophil Count, /µL (× 10^9/L)</th>
<th>Time of Onset of Dysgranulopoiesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/M/42</td>
<td>AP</td>
<td>63</td>
<td>17,100 (171)</td>
<td>3 mo before AP</td>
</tr>
<tr>
<td>2/M/62</td>
<td>AP</td>
<td>13</td>
<td>25,600 (25.6)</td>
<td>NA</td>
</tr>
<tr>
<td>3/M/45</td>
<td>AP</td>
<td>15</td>
<td>13,100 (13.1)</td>
<td>At the time of AP</td>
</tr>
<tr>
<td>4/F/62</td>
<td>MBC</td>
<td>21</td>
<td>2,900 (2.9)</td>
<td>NA</td>
</tr>
<tr>
<td>5/F/47</td>
<td>AP</td>
<td>11</td>
<td>6,300 (6.3)</td>
<td>At the time of AP</td>
</tr>
<tr>
<td>6/F/38</td>
<td>AP</td>
<td>49</td>
<td>1,500 (1.5)</td>
<td>5 mo before AP</td>
</tr>
<tr>
<td>7/F/56</td>
<td>AP</td>
<td>23</td>
<td>1,800 (1.8)</td>
<td>2 mo before AP</td>
</tr>
</tbody>
</table>

AP, accelerated phase; MBC, myeloid blast crisis; NA, no blood or marrow smears available for review.
Discordant Case

The 1 case of CML with dysgranulopoiesis but without an abnormality of 17p occurred in a 38-year-old woman who was diagnosed with CML in chronic phase in June 1999. She was treated with hydroxyurea for a couple of months with excellent response, followed by interferon alfa. However, her disease progressed to AP in April 2000, with cytogenetic evidence of clonal evolution

Table 4. At that time, no dysgranulopoiesis was found in the blood or marrow. After treatment with idarubicin and cytarabine and subsequent allogeneic bone marrow transplantation, she achieved hematologic remission. Although her bone marrow still showed a 9:22 translocation, the secondary clonal abnormalities present at the time of disease progression were absent (Table 4). In July 2000, rare dysplastic neutrophils with pseudo–Pelger-Hüet nuclei were observed in the blood smear. The dysgranulopoiesis became more prominent in the ensuing several months, and cytogenetic analysis performed in September 2002 demonstrated newly evolved clones (Table 4). Chromosome 17p deletion was not identified by either G-banded cytogenetic analysis or fluorescence in situ hybridization using a DNA probe to the p53 locus at 17p13. In November, the patient experienced hematologic relapse and died of blast crisis 1 month later.

Table 2

Transformed Chronic Myeloid Leukemia With Dysgranulopoiesis More Likely to Be Accelerated Phase Than Myeloid Blast Crisis

<table>
<thead>
<tr>
<th></th>
<th>Dysgranulopoiesis Present (n = 7)</th>
<th>Dysgranulopoiesis Absent (n = 17)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accelerated phase</td>
<td>6</td>
<td>8</td>
<td>.087</td>
</tr>
<tr>
<td>Myeloid blast crisis</td>
<td>1</td>
<td>9</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

Dysplasia in hematopoietic cells originally was described in MDS, a group of clonal hematopoietic stem cell diseases associated with ineffective hematopoiesis in one or more myeloid lineages.7,8 Dysgranulopoiesis is characterized primarily by abnormal nuclear configuration (hyposegmented, hypersegmented, or bizarrely segmented nuclei) and abnormal cytoplasmic granulation (hypogranularity or abnormal granules). Dysgranulopoiesis also may occur in a variety of myeloproliferative or myelodysplastic syndromes and in some cases of primary or secondary AML.9,16

Dysgranulopoiesis is not a characteristic feature of the chronic phase of CML. With disease progression, CML may exhibit a number of morphologic abnormalities, including

Table 3

Karyotypes of Patients With Chronic Myeloid Leukemia With Dysgranulopoiesis

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Karyotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Not available</td>
</tr>
<tr>
<td>2</td>
<td>46,XY,t(9;22)(q34;q11.2)[15]/46,idem,idic(17)(p11.1)[5]</td>
</tr>
<tr>
<td>3</td>
<td>46,XY,t(9;22;15)(q34;q11.1;q22)/17/46,idem,del(17)(q10)[2]/46,XY[1]</td>
</tr>
<tr>
<td>4</td>
<td>46,XX,t(9;22)(q34;q11.2)/17/46,idem,del(17)(p11.2)[8]</td>
</tr>
<tr>
<td>5</td>
<td>46,XX,t(9;22)(q34;q11.2),t(10;15;14)(q26.3;q24;q24),del(20)(q11.2q13.1)[12]/46,idem,t(X;20)(q13;q13.3)[13]/3][p12;p25, inv(17)(p13)[5]</td>
</tr>
<tr>
<td>6</td>
<td>46,XX,t(9;22)(q34;q11.2)[1]/46,idem,del(11)[p13p15][3]/46,idem,t(1;9)[q21;q34][6]/46,idem,t(X;9)[q21;p22][t(1;9)[q21;q34],[ t3;17;p23;p25],[del(5)[q22q33],[add(8)[p23][1]/46,XX[7]</td>
</tr>
<tr>
<td>7</td>
<td>46,XX,t(9;22)(q34;q11.2),i(17)[q10][13]/45,XX,t(9;22)[q34;q11.2],del(17)[p11.2],–22/11/45,XX,t(9;22)[q34;q11.2],del(19)[q13q34], del(17)[p11.2],–22[7]</td>
</tr>
</tbody>
</table>

Table 4

Discordant Chronic Myeloid Leukemia Case With Dysgranulopoiesis but Without Abnormalities of Chromosome 17p

<table>
<thead>
<tr>
<th>Date</th>
<th>Diagnosis</th>
<th>Morphologic Findings</th>
<th>Karyotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>June 1999</td>
<td>Chronic phase</td>
<td>No dysgranulopoiesis</td>
<td>46,XX,t(9;22)(q34;q11.2)</td>
</tr>
<tr>
<td>April September</td>
<td>Accelerated phase</td>
<td>No dysgranulopoiesis</td>
<td>46,XX,t(9;22)[q34;q11.2]/47,del(17)(p13p15)/46,idem,+8/48,idem,+8,+17</td>
</tr>
<tr>
<td></td>
<td>Hematologic remission</td>
<td>Rare dysgranulopoiesis</td>
<td>46,XX,t(9;22)[q34;q11.2]/46,XX</td>
</tr>
<tr>
<td>November</td>
<td>Hematologic relapse</td>
<td>Dysgranulopoiesis</td>
<td>46,XX,t(9;22)[q34;q11.2],del(17)[p13p15]/46,idem,t(1;9)[q21;q34][6]/46,idem,t(X;9)[q21;p22],[t(1;9)[q21;q34],[t3;17][p23;p25], del(5)[q22q33],[add(8)[p23][1]/46,XX</td>
</tr>
<tr>
<td>December</td>
<td>Died</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

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increased blast count, pronounced basophilia, myelofibrosis, dysgranulopoiesis, dyserythropoiesis, and marked monocytosis. Among those morphologic features, increased blast count (>10%) and profound basophilia (>20%) have been considered criteria for transformation. In contrast, because of scant evidence for a causal relationship, dysgranulopoiesis is not included among the independent diagnostic criteria for transformation.

In our study, using morphologic criteria that require hyposegmented nuclei, hypogranular cytoplasm, or both, we evaluated dysgranulopoiesis in CML. Previous investigators have reported that neutrophilic dysplasia may occur in reactive conditions (eg, due to infections, nutritional deficiencies, or drug exposure). The dysplastic changes in those conditions usually are transient, relatively mild, and often accompanied by other morphologic changes attributable to the causative agents. Detecting morphologically significant but unexplained dysgranulopoiesis in peripheral blood is alarming in clinical practice and likely would prompt examination of the bone marrow to rule out MDS or leukemia.

In patients with CML, we found that the development of dysgranulopoiesis was associated with disease transformation. Dysgranulopoiesis occurred in 7 (23%) of 31 cases of transformed CML in our series. All cases with dysgranulopoiesis exhibited pseudo–Pelger–Huet neutrophils and were associated with transformation of the myeloid lineage. The dysgranulopoiesis seen in these patients was absent in the chronic phase of CML but developed shortly before or during CML transformation. These features suggest that dysgranulopoiesis may serve as a surrogate phenotype of CML in progression.

Furthermore, we identified a significant association between dysgranulopoiesis and chromosome 17p abnormalities in cases of CML in transformation (P = .001). Chromosome 17p deletion has been reported to be associated with dysgranulopoiesis in MDS and AML. However, this association has not been well studied in CML. A single study by Sessarego and Ajmar examined pseudo–Pelger–Huet neutrophils in the peripheral blood of 83 patients with CML during blast crisis and found that 11 (13%) of these cases exhibited dysgranulopoiesis. All 11 of these cases showed karyotype evolution with abnormalities of the short arm of 1 chromosome 17. However, because the temporal relationship between dysgranulopoiesis in CML and disease transformation was not studied, a causal relationship between them was not established. Furthermore, Sessarego and Ajmar examined only cases that had progressed to blast crisis. The criteria for the diagnosis of blast crisis were not specified in their report, but they likely required 30% or more blasts in blood or marrow according to the French-American-British classification in use at that time. Thus, any case with a blast count between 10% and 29% might not have been included in the analysis. This could explain the discrepant frequency of dysgranulopoiesis in transformed CML between the study by Sessarego and Ajmar and the present one (13% vs 23%, respectively). Interestingly, we found a trend toward more frequent dysgranulopoiesis in AP than in BC, suggesting dysgranulopoiesis-positive CML could be detectable early in disease progression.

Abnormalities of 17p have been reported in de novo and therapy-associated MDS and AML. Of note, 4 of 5 patients with 17p abnormalities had exposure to chemotherapy, including hydroxyurea in 3 cases and interferon alfa in 1 case. We cannot exclude the possibility that chemotherapy might induce 17p abnormalities that, in turn, result in the manifestation of dysgranulopoiesis. Alternatively, the development of 17p abnormalities and dysgranulopoiesis may not be related to patient treatment, given the heterogeneity of the treatment histories in the 5 cases.

The presence of 17p abnormalities typically portends a worse prognosis. The impact of 17p abnormalities on the clinical course of CML remains controversial. Earlier studies suggested a shortened survival in CML with loss of chromosome 17p, while more recent studies found no significant difference in prognosis in cases with such abnormalities. Treatment of the patients in these studies varied considerably, however, including conventional single-agent chemotherapy, interferon alfa, multiple drug combinations, or allogeneic bone marrow transplantation. Because detailed information about the therapy regimes for those patients was not available, it is difficult to reconcile their conclusions. The small number of our cases precludes a meaningful analysis of survival in cases with and without 17p abnormalities.

In MDS and AML, a strong correlation between chromosome 17p deletion and t(15;17) mutation has been reported. Mutations of p53 are found in up to 30% of transformed CML and may have a significant role in disease progression. Indeed, acquired loss of p53 in hematopoietic cells expressing bcr-abl protein has been shown to induce blast transformation. A correlation between loss of one p53 allele and mutation of the other allele has been found in blast crisis of CML. However, another study failed to document a correlation of isochromosome 17q (resulting in loss of 1 copy of 17p) with mutation of the other p53 allele in a series of 21 cases of hematologic malignant neoplasms, including 8 cases of CML in BC.

In the present study, 1 case of CML with dysgranulopoiesis failed to show abnormalities of 17p or deletion of p53. This patient had a complex clinical history and developed dysgranulopoiesis following bone marrow transplantation. We cannot exclude the possibility that both the dysgranulopoiesis and the complex secondary cytogenetic
abnormalities might have resulted from previous therapy. We speculate that other cytogenetic and/or molecular genetic abnormalities may be responsible for the dysgranulopoiesis in this case.

Our study revealed that dysgranulopoiesis was uncommon in CML overall (7/167 [4.2%]), but that it occurred in a substantial number of cases of transformed CML (7/31 [23%]). Dysgranulopoiesis was associated strongly with abnormalities of 17p and disease progression, with a trend toward greater frequency in AP than MBC. Together, our findings suggest that detecting dysgranulopoiesis in blood or marrow is of value for diagnosing CML in transformation.

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References


