endothelial cell adhesion molecule-1 (PECAM-1, CD31). Because the chemical analog of carbimazole, thiamazole (3-methyl-2-thioimidazole), is widely used for the treatment of hyperthyroidism in the US, we asked the question whether carbimazole DDAb cross-reacts with thiamazole. Sera from 4 patients of our initial study (patients 2-5; patient 1 died and no further serum was available) were analyzed by enzyme immunoassay with intact platelets in the presence and in the absence of 1 mg/mL carbimazole or thiamazole, as previously described.¹ As shown in Figure 1, all carbimazole DDAb failed to react with platelets in the presence of thiamazole. Analysis of DDAb in the glycoprotein-specific immunoassay (MAIPA) revealed positive reactions with PECAM-1 in the presence of carbimazole but not with thiamazole (data not shown). Specific interaction between carbimazole and the platelet membrane has to be assumed because only a minor chemical modification of carbimazole led to destruction of the carbimazole DDAb reactivity. This has already been suggested by our finding that the second extracellular loop of PECAM-1 was crucial for epitope formation.¹ In addition, the carbethoxy group of carbimazole at position C-1 seems to be important for the immune response and subsequent thrombocytopenia in the patient after drug treatment. Carbimazole is a prodrug that is rapidly and totally converted to thiamazole in the body by cleavage of the carbethoxy group.² This phenomenon could contribute to the clinical presentation of mild thrombocytopenia in our patients. Due to the high specificity of DDAb, binding and platelet destruction could only occur during the phase immediately after carbimazole administration. After metabolism to thiamazole, no further platelet destruction takes place. This phenomenon might represent an interesting counterpart of the situation observed in patients in whom metabolite-specific DDAb induce a prolonged effect in relation to drug excretion.³

We conclude that both the chemical structure and the metabolism of the drug may have a major influence on the clinical presentation of DITP, particularly on the degree of thrombocytopenia. Further observations will be required to define whether thiamazole itself is able to raise a drug-dependent immune response against platelets.

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References


To the editor:

BCR-ABL rearrangement is not detectable in essential thrombocythemia

Essential thrombocythemia (ET) is a chronic myeloproliferative disorder (MPD) characterized by an elevated thrombocytosis, an increased number of megakaryocytes with dismegakaryopoiesis in the bone marrow, and no identifiable underlying primary causes. The disease can evolve into myelofibrosis and, rarely, into acute leukemia. According to the current diagnostic criteria of the Polycythemia Vera Study Group (PVSG), ET is lacking in features diagnostic for other MPDs, including Philadelphia chromosome (Ph). Karyotypic anomalies are rare and not specific, and the clonality is controversial; so this disorder remains a diagnosis of exclusion, and the identification of subgroups of patients at risk for progression to leukemia is quite difficult. Recently, some authors reported a BCR-ABL transcript positivity in about a half of 25 Ph⁻ ET cases.¹

To verify the hypothesis of a new ET variant with possible clinical implications, we investigated the presence of the molecular counterpart of the Ph chromosome in a larger series of ET patients with a longer follow-up. We investigated 112 white patients (44 males, 68 females; median age, 56 years, range 23 to 98 years) diagnosed with ET following the criteria of the PVSG. The patients were from 3 different institutions of the same region (Po Valley, northern Italy). At admission, routine laboratory investigations, including complete blood film, leukocyte alkaline phosphatase (LAP) score, and serum vitamin B₁₂ levels, were carried out. Bone marrow aspiration and biopsy were performed for histological, cytogenetic, and molecular studies. Bone marrow examinations were repeated at least once, in the majority of cases. Cytogenetic analyses were performed, at diagnosis and prior to any treatment, in all patients using conventional banding methods. Molecular studies for the detection of chimeric messengers BCR-ABL, coding for p190 and p210 proteins, were performed by “nested” reverse transcriptase–polymerase chain reaction (RT-PCR) on total RNAs extracted from Lymphoprep-separated (Nycomed Pharma, Majorstua, Norway) bone marrow mononuclear cells by a guanidine-isothiocyanate-phenol-chloroform method.² cDNA was synthesized using 1.5 μg total DNA in a 30 μL reaction mixture as described elsewhere,¹ using an antisense primer specific for the exon a3 of the ABL gene. Nested PCR was performed as follows: 25 μL cDNA was subjected to 40 cycles of amplification in a 50 μL reaction mixture containing 0.225 mM dNTPS, 0.5 μM of each

Figure 1. Carbimazole-dependent antibodies in enzyme immunoassay. Binding of carbimazole-dependent antibodies in sera from 4 patients in the presence of carbimazole (1 mg/mL), thiamazole (1 mg/mL), and without either drug. O.D., optical density.
at 94°C, 30 seconds at 60°C, and 30 seconds at 72°C with 2 and 50 mM KCl. The conditions of amplification were: 30 seconds

patients, with few exceptions; 8 patients showed a disease transfor-

242) were 39.

242). The patients followed for a mean of 108.11 months (range, 60
to 242) and for 3 patients was 193 months (range, 161 to

months (range, 6 to 242); the 19 newly diagnosed patients were

diagnosis. For all patients, the median follow-up time was 39.70

observed for a mean of 9 months (range, 8 to 11), whereas for 90

events, of variable gravity, were noted in 37 patients. At cytoge-

cases, at diagnosis. Splenomegaly smaller than 3 centimeters

cytic hyperplasia with dismegakaryocytosis in all cases; neither

primer, 1.0 U of Taq DNA polymerase (Roche Diagnostics,

Mannheim, Germany), 1.5 mM MgCl2, 10 mM Tris-HCl (pH 8.3),

50 mM KCl. The conditions of amplification were: 30 seconds

at 94°C, 30 seconds at 60°C, and 30 seconds at 72°C with 2

minutes of an initial denaturation step at 94°C and 10 minutes of a

final extension step at 72°C. 2.5 μL of the first round of amplification

were subjected to a second round with 2 internal primers at the same conditions of amplification. As control, 5 μL of

the same retrotranscription reactions were subjected to 35 cycles of

PCR using as primers two oligonucleotides specific for the ABL

gene. The sequence of the primers used in PCR reactions was

reported elsewhere.4,5

The minimal level of the detection of the nested PCR was

estimated adding to 5 × 107 HL60 BCR-ABL− cells a progressive

lower amount of K562 cells expressing the b3a2 form of the

BCR-ABL chimeric mRNA. After RNA extraction and nested PCR

as reported above, the detection level of the method was 1

BCR-ABL− K562 cell in 105 BCR-ABL− HL60 cells.

In our group of 112 patients, 69 (61.6%) showed a platelet count

lower than 1 × 107/μL (range, 0.62 × 107 to 1 × 107) and 43

(38.3%) greater than 1 × 107/μL (range, 1.05 × 107 to 2.7 × 107).

The white blood cell count showed a mean count of 8 300/μL, with

more than 3% basophils only in 3 patients and the absence of

immature cells, in the peripheral blood. The hematocrit was less

than 40% in all patients. The LAP score was increased in 54

patients, normal in 23, and not done in the remaining 35. Serum

variant forms with thrombocythemia.

whether an MPD with marked thrombocythemia and expressing

the BCR-ABL transcripts might be considered a variant form of ET

or of CML has raised controversies for several years. Many,

including the PVSG, agreed with the latter option because of the

high incidence of leukemic transformation of the Ph+ ET6,7 as well

as the similarity of the chimeric transcripts.8 Recently, Blickstein et

al9 reported an incidence of 48% of the BCR-ABL transcript in 25

ET patients, with neither clinical nor laboratory differences com-

pared with BCR-ABL− patients, suggesting, with others, the

possibility of a new ET variant. Subsequently, Singer et al9 detected

the molecular rearrangement in 63% of their 16 patients. Neverthe-

theless, these observations were not confirmed in a further

smaller series of ET patients, recently reported.10 Most recently,

some investigators reported the absence of BCR-ABL rearrange-

ment in all 41 of their ET patients, studied by fluorescence in situ

hybridization (FISH).11

The results of our observations on a larger series of patients with

ET showed the absence of the BCR-ABL rearrangements in this
disease. The only BCR-ABL+ patient of our series (0.89%), on

which we already reported,10 is probably an unusual case of CML

at thrombocytthemic onset and long survival, a case that finally

progressed to acute leukemia. The longer follow-up of our patients

(median 62.79 months for the 80.35% of our series of patients,

compared with medians 22.5 and 37 months in Blickstein et al1 and

Singer et al9, respectively) allowed us to document a disease course

more consistent with the natural history of ET than that of CML.

The discrepancies between the 2 groups of observations, rather

than between technical procedures (methodologies and sensitivities

appear equivalent) might be due to inaccuracies in ET diagnosis or,

at least, to racial differences. Our suggestion is that true ET does

not carry the Ph anomaly that, instead, might characterize the CML

variant forms with thrombocythemia.

Table 1. Disease transformation

<table>
<thead>
<tr>
<th>Case</th>
<th>Disease conversion</th>
<th>Time to conversion (y)</th>
<th>BCR-ABL–</th>
<th>Outcome after conversion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AML</td>
<td>12</td>
<td>yes</td>
<td>Death in 3 mo</td>
</tr>
<tr>
<td>2</td>
<td>AML</td>
<td>6</td>
<td>no</td>
<td>Alive at 3 y</td>
</tr>
<tr>
<td>3</td>
<td>IM</td>
<td>6</td>
<td>no</td>
<td>Alive at 2 y</td>
</tr>
<tr>
<td>4</td>
<td>IM</td>
<td>10</td>
<td>no</td>
<td>Alive at 2 y</td>
</tr>
<tr>
<td>5</td>
<td>IM</td>
<td>4</td>
<td>no</td>
<td>Alive at 2 y</td>
</tr>
<tr>
<td>6</td>
<td>MDS</td>
<td>9</td>
<td>no</td>
<td>Alive at 6 mo</td>
</tr>
<tr>
<td>7</td>
<td>MG</td>
<td>5</td>
<td>no</td>
<td>Alive at 2 y</td>
</tr>
<tr>
<td>8</td>
<td>MG</td>
<td>7</td>
<td>no</td>
<td>Alive at 2 y</td>
</tr>
</tbody>
</table>

Cytogenetics at conversion for case 1: t(7;13)(q33;q13), inv(11)(p12;q24).

Cytogenetics at conversion for cases 2-8 was unchanged.

AML, acute myeloid leukemia; IM, idiopathic myelofibrosis; MDS, myelodisplastic syndrome; MG, monoclonal gammopathy.

Whether an MPD with marked thrombocythemia and expressing the BCR-ABL transcripts might be considered a variant form of ET or of CML has raised controversies for several years. Many, including the PVSG, agreed with the latter option because of the high incidence of leukemic transformation of the Ph+ ET6,7 as well as the similarity of the chimeric transcripts.8 Recently, Blickstein et al9 reported an incidence of 48% of the BCR-ABL transcript in 25 ET patients, with neither clinical nor laboratory differences compared with BCR-ABL− patients, suggesting, with others, the possibility of a new ET variant. Subsequently, Singer et al9 detected the molecular rearrangement in 63% of their 16 patients. Nevertheless, these observations were not confirmed in a further smaller series of ET patients, recently reported.10 Most recently, some investigators reported the absence of BCR-ABL rearrangement in all 41 of their ET patients, studied by fluorescence in situ hybridization (FISH).11

The results of our observations on a larger series of patients with ET showed the absence of the BCR-ABL rearrangements in this disease. The only BCR-ABL+ patient of our series (0.89%), on which we already reported,10 is probably an unusual case of CML at thrombocytthemic onset and long survival, a case that finally progressed to acute leukemia. The longer follow-up of our patients (median 62.79 months for the 80.35% of our series of patients, compared with medians 22.5 and 37 months in Blickstein et al1 and Singer et al9, respectively) allowed us to document a disease course more consistent with the natural history of ET than that of CML.

The discrepancies between the 2 groups of observations, rather than between technical procedures (methodologies and sensitivities appear equivalent) might be due to inaccuracies in ET diagnosis or, at least, to racial differences. Our suggestion is that true ET does not carry the Ph anomaly that, instead, might characterize the CML variant forms with thrombocythemia.

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References


To the editor:

Increase in platelet count in response to rHuEpo in a patient with thrombocytopenia and absent radii syndrome

The thrombocytopenia and absent radii (TAR) syndrome is a rare congenital defect characterized by the association of skeletal malformations with hematologic disturbances. Additional manifestations are absence or hypoplasia of other bones of the extremities, short stature, dislocation of hip, and various other abnormalities. In an investigation on 5 unrelated children with TAR syndrome, Ballmaier et al found that the thrombocytopenia was caused by a defective megakaryopoiesis and thrombocytopoiesis, which was due to a lack of response to thrombopoietin, despite normal expression of the thrombopoietin receptor on the megakaryocytes.

Letestu et al showed evidence of dysmegakaryopoiesis, with a blockage of cellular differentiation at an early stage. In these experiments, megakaryopoiesis in cell culture was not responsive to stimulation with mixtures of cytokines, including erythropoietin.

We now report the case of a 49-year-old female with TAR syndrome who was referred to our clinic for investigation of bleeding risk for elective hip surgery. The patient had suffered from severe coxarthrosis for several years. Absence of radii was confirmed by roentgenograms. The patient is of short stature, with a body height of 148 cm. Platelet count was in the range of 50 × 10^9/L to 60 × 10^9/L both in ethylenediaminetetraacetic acid (EDTA) anticoagulated blood, as well as in citrated whole blood. Red blood cells were within the normal range, whereas the patient showed elevated leukocyte counts throughout the observation period. Expression of platelet receptors CD41, CD61, CD42a, and CD42b was found to be normal in flow cytometric analysis. Erythropoietin levels were within the normal range.

Erythropoietin has been shown to induce an increase in platelet count and has been employed in the preparation of anaemic patients for hip surgery. In prevention of anaemia and thrombocytopenia in cancer patients receiving radiotherapy, treatment with rHuEpo leads to elevated numbers of megakaryocytes in the bone marrow of patients with renal anemia and an increase in platelet counts in animal experiments.

We treated the patient with 2 courses of erythropoietin. Upon the first instance, she received 1000 IU/d of recombinant erythropoietin (rHuEpo) (Neo-Recormon; Roche, Mannheim, Germany) (16 IU/kg body weight) for 3 days. The second time, we administered 2000 IU/d (32 IU/kg body weight) for 4 days. During the first series, platelet counts increased from 48 × 10^9/L to 84 × 10^9/L on day 5. In the second series, platelet count increased from 50 × 10^9/L to 80 × 10^9/L on day 6. Erythrocyte count, as well as leukocyte counts, remained unchanged during HuEpo treatment. (See Table 1 for a summary.) The results indicate that the thrombocytopenia of patients with TAR syndrome may be responsive to rHuEpo treatment, resulting in a therapeutically relevant increase in platelet count. Treatment with rHuEpo may be useful in patients scheduled for surgical procedures, in order to reduce the amount of heterologous platelet concentrates needed for maintaining a sufficient hemostatic capacity. Additional experiences are needed in order to determine the required dosage of rHuEpo. In animal experiments, large chronic doses of rHuEpo have been shown to cause thrombocytopenia, caused by competition between precursor cells of the erythrocytic and megakaryocytic cell lines. In view of these limitations, treatment with rHuEpo in patients with TAR syndrome should presumably be limited to short-term applications.

Table 1. Platelet count, erythrocyte count, leucocyte count, and rHuEpo dosage administered

<table>
<thead>
<tr>
<th>Date</th>
<th>Platelets (×10^9/L)</th>
<th>Erythrocytes (×10^12/L)</th>
<th>Leukocytes (×10^9/L)</th>
<th>rHuEpo dose</th>
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<tbody>
<tr>
<td>7/24/99</td>
<td>57</td>
<td>5.20</td>
<td>15.1</td>
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<td>4.43</td>
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<tr>
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<td>4.10</td>
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</tr>
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<td>ND</td>
<td>ND</td>
<td>1000</td>
</tr>
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</tr>
<tr>
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<td>84</td>
<td>4.64</td>
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<td>4/27/00</td>
<td>49</td>
<td>4.80</td>
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<tr>
<td>5/4/00</td>
<td>50</td>
<td>4.97</td>
<td>16.4</td>
<td>2000</td>
</tr>
<tr>
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<td>54</td>
<td>5.20</td>
<td>12.9</td>
<td>NA</td>
</tr>
</tbody>
</table>

ND, not determined; NA, not applicable.

References

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