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 DD Abs cross-reacted 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 DD Abs failed to react with platelets in the presence of thiamazole. Analysis of DD Abs 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 DDA b 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 DD Abs, 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 DD Abs 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 B\(_{12}\) 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-separat ed (Nycomed Pharma, Maj orstu, Norway) bone marrow mononuclear cells by a guanine-isothiocyanate-phenol-chloroform method.\(^2\) cDNA was synthesized using 1.5 \(\mu\)g total RNA in a 30 \(\mu\)L 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 \(\mu\)L cDNA was subjected to 40 cycles of amplification in a 50 \(\mu\)L reaction mixture containing 0.225 mM dNTPs, 0.5 \(\mu\)M of each primer, 2.5 \(\mu\)L of a 10× PCR buffer (TaKaRa), 0.5 \(\mu\)L of both dNTPs, and 0.4 \(\mu\)l of Platinum Taq DNA Polymerase (Invitrogen). The initial denaturation was carried out at 94°C for 3 min, followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 45 s, and extension at 72°C for 45 s. The final extension was performed at 72°C for 7 min. The expected size of the PCR product was 359 bp. The amplification products were analyzed on a 2% agarose gel stained with ethidium bromide. The identity of the PCR products was confirmed by restriction digestion with EcoRI (data not shown). The vast majority of patients showed no amplification result, indicating the absence of BCR-ABL rearrangement in our study.

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>
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<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 2 y</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)(q32;q13), inv(11)(p12;q24).
Cytogenetics at conversion for cases 2-8 was unchanged.

primer, 1.0 U of Taq DNA polymerase (Roche Diagnostics, Mannheim, Germany), 1.5 mM MgCl2, 10 mM Tris-Cl (pH 8.3), and 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

The minimal level of the detection of the nested PCR was estimated adding to 5 × 10^6 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 10^5 BCR-ABL- HL60 cells.

In our group of 112 patients, 69 (61.6%) showed a platelet count lower than 1 × 10^11/L (range, 0.62 × 10^11 to 1 × 10^11) and 43 (38.3%) greater than 1 × 10^11/L (range, 1.05 × 10^11 to 2.7 × 10^11). 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 vitamin B12 level was in normal range in all patients. The bone marrow examination showed a more or less important megakaryocytic hyperplasia with dismegakaryocytosis in all cases; neither myelodysplastic features nor collagen fibrosis were detected in all cases, at diagnosis. Splenomegaly smaller than 3 centimeters palpable was observed in 13 patients. Thrombotic or hemorrhagic events, of variable gravity, were noted in 37 patients. At cytogenetic examination, no one showed the t(9;22) translocation, at diagnosis. For all patients, the median follow-up time was 39.70 months (range, 6 to 242); the 19 newly diagnosed patients were observed for a mean of 9 months (range, 8 to 11), whereas for 90 patients (80.35%) the median follow-up time was 62.79 months (range, 24 to 144) and for 3 patients was 193 months (range, 161 to 242). The patients followed for a mean of 108.11 months (range, 60 to 242) were 39.

The RT-PCR studies for BCR-ABL transcripts showed the chimeric product (b3a2 type) only in 1 patient (0.89%). The clinical and hematologic features remained unchanged in the majority of patients, with few exceptions; 8 patients showed a disease transformation (Table 1). Two patients died from brain crisis and two from cerebral stroke, from 6 to 10 years after diagnosis.

maintain the platelet count below 0.6 × 10^11/L, 73 patients underwent a cytoreductive therapy (hydroxyurea 55, uracil mustard 13, busulfan 4, and interferon 1).

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+ ET as well as the similarity of the chimeric transcripts. Blickstein et al1 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 small 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 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 thrombocytocenic 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 thrombocytemia.

**References**


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 dysmegalakrypoiesis, 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 and for 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. Erythropoietin has been shown to induce an increase in platelet count and has been employed in the preparation of anaemic patients for hip surgery and for 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 erythroid 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.

References

BCR-ABL rearrangement is not detectable in essential thrombocythemia

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