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 DDAbs 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. As shown in Figure 1, all carbimazole DDAbs failed to react with platelets in the presence of thiamazole. Analysis of DDAbs 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. 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. This phenomenon could contribute to the clinical presentation of mild thrombocytopenia in our patients. Due to the high specificity of DDAbs, 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 DDAbs induce a prolonged effect in relation to drug excretion. 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.

Hartmut Kroll, Astrid Giptner, and Sentot Santoso
Institute for Clinical Immunology and Transfusion Medicine,
Justus Liebig University Giessen
Giessen, Germany

References

To the editor:

BCR-ABL rearrangement is not detectable in essential thrombocytopenia

Essential thrombocytopenia (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. 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. cDNA was synthesized using 1.5 µg total RNA in a 30 µL reaction mixture as described elsewhere, 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 primer, and 0.025 µL AmpliTaq Gold polymerase (Applied Biosystems, Foster City, CA). Conditions were: 94°C for 2 minutes followed by 30 cycles of 94°C for 30 seconds, 54°C for 30 seconds, and 72°C for 2 minutes, followed by a final elongation step of 72°C for 7 minutes. The presence of cDNA was confirmed by agarose gel electrophoresis and ethidium bromide staining. Primers were designed to amplify a 110 bp fragment encoding the junction of the ABL1 and the 5’ end of BCR. PCR products were analyzed on 1.5% agarose gels, visualized with ethidium bromide, and photographed as described elsewhere.1

The sensitivity of amplification was determined using serial concentrations of cDNA, ranging from 0.01 to 5000 copies, and found to be 1 copy per 50 µL reaction mixture. Specific amplification was confirmed by sequencing. Results were compared with the data from the disease control group, where no patients carried a BCR-ABL transcript. All 112 investigated patients were BCR-ABL negative, indicating that BCR-ABL rearrangement is not detectable in essential thrombocytopenia.
primer, 1.0 U of Taq DNA polymerase (Roche Diagnostics, Mannheim, Germany), 1.5 mM MgCl₂, 10 mM Tris-HCl (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

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 3 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)(p12q24). Cytogenetics at conversion for cases 2-8 was unchanged.

AML, acute myeloid leukemia; IM, idiopathic myelofibrosis; MDS, myelodysplastic syndrome; MG, monocytic malignancy.

maintain the platelet count below \(0.6 \times 10^7/\mu\text{L}\). 73 patients underwent a cytoreductive therapy (hydroxiurea 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 \(\Phi^+\) ET\(^6,7\) as well as the similarity of the chimeric transcripts. Recently, Blickstein et al\(^8\) 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 al\(^9\) 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 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 thrombocytopenic 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 al\(^1\) and Singer et al,\(^9\) 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 \(\Phi\) anomaly that, instead, might characterize the CML variant forms with thrombocytopenia.

Giovanni Emilia, Roberto Marasca, Patrizia Zucchi, Paola Temperani, Mario Luppi, and Giuseppe Torelli
Department of Medical Sciences
Section of Hematology and Internal Medicine
University of Modena
Modena, Italy

Francesco Lanza, Cristiano De Angelis, Domenica Gandini, and GianLuigi Castoldi
Department of Biomedical Sciences
Section of Hematology
University of Ferrara
Ferrara, Italy

Daniele Vallisa and Luigi Cavanna
Division of Internal Medicine
AUSL Piacenza
Piacenza, Italy

Laura del Senno
Department of Biochemistry and Molecular Biology
University of Ferrara
Ferrara, Italy

Supported by AIRC (M.E.), AIRC (G.E., R.M., P.T., M.L., and G.T.), by FIRC (C.D.A.), and by AIRC (M.L.).

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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. 1 Additional manifes-
tations 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
Erythropoietin levels were within the normal range.
CD42b was found to be normal in flow cytometric analysis.
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
count2 and has been employed in the preparation of anaemic
patients for hip surgery3 and for prevention of anaemia and
thrombocytopenia in cancer patients receiving radiotherapy.4 Treat-
ment with rHuEpo leads to elevated numbers of megakaryocytes in
the bone marrow of patients with renal anemia5 and an increase in
platelet counts in animal experiments.5,6

We treated the patient with 2 courses of erythropoietin. Upon
the first instance, she received 1000 IU/d of recombinant erythropoi-
etin (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 rHuEpo 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 heterolo-
gous 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 thrombo-
cytopenia, caused by competition between precursor cells of the
erthrocytic and megakaryocytic cell lines.7,8 In view of these
limitations, treatment with rHuEpo in patients with TAR syndrome
should presumably be limited to short-term applications.

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2. Letestu et al showed evidence of dysmegakaryopoiesis, with a
blockage of cellular differentiation at an early stage.9 In these
experiments, megakaryopoiesis in cell culture was not responsive
to stimulation with mixtures of cytokines, including erythropoietin.

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

<table>
<thead>
<tr>
<th>Date</th>
<th>Platelets (×10^12/L)</th>
<th>Erythrocytes (×10^12/L)</th>
<th>Leukocytes (×10^9/L)</th>
<th>rHuEpo dose</th>
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<tr>
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<td>5.20</td>
<td>15.1</td>
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<tr>
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<tr>
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<td>48</td>
<td>4.10</td>
<td>14.7</td>
<td>1000</td>
</tr>
<tr>
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<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>1000</td>
</tr>
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<td>4.97</td>
<td>16.4</td>
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<tr>
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<td>4.76</td>
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<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.
BCR-ABL rearrangement is not detectable in essential thrombocythemia

Giovanni Emilia, Roberto Marasca, Patrizia Zucchini, Paola Temperani, Mario Luppi, Giuseppe Torelli, Francesco Lanza, Cristiano De Angelis, Domenica Gandini, GianLuigi Castoldi, Daniele Vallisa, Luigi Giovanni Emilia, Roberto Marasca, Patrizia Zucchini, Paola Temperani, Mario Luppi, Giuseppe Torelli, Francesco Lanza, Cristiano De Angelis, Domenica Gandini, GianLuigi Castoldi, Daniele Vallisa, Luigi Cavanna and Laura del Senno

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