tion can be suspected. Rosendaal et al. have recently demonstrated the relation between 20210 GA heterozygosity and MI. They studied young women with MI and reported an odds ratio of 4.0 in comparison with healthy age-matched women. Another study from Austria also suggests an association with coronary heart diseases. The possible relation with arterial thrombotic events in the Mediterranean area, however, has not been well established. This mutation was not associated with cerebral ischemia in a retrospective Italian case-controlled study nor in another Spanish prospective one. This latter study was not able to show an effect in coronary heart disease either, because the result (4% versus 2%) did not reach statistical significance. No homozygous patients (20210 AA) were found in any of these series.

We report the case of a 65-year-old man who developed an extensive femoro-iliac venous thrombosis the seventh day after a transurethral resection for benign prostatic hyperplasia. He was heparinized with standard LMWH doses but, eight days afterwards, during coumarin introduction, he developed a new thrombosis in the same territory (left limb). In reviewing his previous clinical history two further episodes were brought out: transient global amnias suggesting transient ischemic attacks of thrombotic origin when the patient was 63 years old. The electroencephalographic record was normal. The electrocardiographic and echocardiographic studies ruled out an embolicigenic heart disease. The supra-aortic echo-Doppler ruled out vascular stenosis and the CT scan showed multiple hypodense ischemic areas. He was diagnosed as having cerebrovascular disease involving small vessels. He was analyzed for DNA mutations in factor V and factor II by PCR, a homozygous state for the FII: G20210A (FII 20210A) being found. The amplified DNA digested by HindIII showed a single 322-bp band in electrophoresis versus a 345-bp band found in normal (20210 GG) subjects and both bands (345 and 322-bp) in heterozygotes. One month after the acute event, oral anticoagulant therapy was stopped and a subtherapeutic dose of nadroparine was started to manage the patient's molecular status. Sixteen patients who consented a low mortality rate, has been proposed as an alternative approach to maintenance chemotherapy in acute promyelocytic leukemia (APL) in first complete remission irrespective of the patients’ molecular status. Sixteen patients with acute APL in complete remission were submitted to ABMT and were analyzed for the presence of the

Correspondence
Angel J. Gonzalez Ordoñez, M.D., Servicio de Hematología, Hospital de S. Agustin, 33400 Avilés, Spain. Fax: international +34.985.123012 • E-mail: jagonzalez@medinet.es

References

Role of autologous bone marrow transplantation as consolidation chemotherapy in acute promyelocytic leukemia patients in complete remission
Emanuela Ottaviani, Giovanni Martinelli, Nicoletta Testoni, Giuseppe Visani, Monica Tani, Sante Turra
Institute of Hematology and Medical Oncology “Seràgnoli”, University of Bologna, Italy

Autologous bone marrow transplantation (ABMT), which consents a low mortality rate, has been proposed as an alternative approach to maintenance chemotherapy in patients with acute promyelocytic leukemia (APL) in first complete remission irrespective of the patients’ molecular status. Sixteen patients with acute APL in complete remission were submitted to ABMT and were analyzed for the presence of the

Key words
Prothrombin gene, 3'-UTR, factor II, homozygous, thrombosis
PML-RARα fusion gene by reverse transcription-polymerase chain reaction (RT-PCR). Our study demonstrated that continued positivity of PCR analysis before ABMT could predict subsequent relapse in patients who undergo un-purged ABMT procedures.

Acute promyelocytic leukemia (APL) is a particular type of acute myeloid leukemia with a favorable outcome and a low rate of clinical relapse. Nevertheless, a subgroup of patients relapse, particularly when persistently PCR positive for the PML-RARα transcript after induction chemotherapy. While the induction and initial consolidation treatment in APL patients is well defined, different protocols of maintenance chemotherapy have been proposed, although the role of these protocols is still debated. Autologous bone marrow transplantation (ABMT), has been proposed as an alternative approach to maintenance chemotherapy in first and second complete remission (CR).

We have consecutively analyzed a series of 16 APL patients in complete remission (14 in 1st CR, 1 in 2nd CR and 1 in 3rd CR) submitted all but one to unpurged autologous bone marrow transplantation (ABMT). The purged ABMT was treated as reported elsewhere.

All the patients were studied by cytogenetic analysis and for the presence of the PML-RARα fusion gene by reverse transcription-polymerase chain reaction (RT-PCR) at diagnosis, during consolidation chemotherapy, before ABMT and at different times during their post-transplant follow-up. Cytogenetic studies were performed on short-term cultures without stimulation, as previously described.

At diagnosis, fifteen patients had a positive karyotypic examination on bone marrow aspirates, showing the presence of a typical t(15;17) aberration in their blasts (Table 1).

Table 1. Clinical characteristics, chemotherapy, transplant procedures and clinical outcome of APL patients.

<table>
<thead>
<tr>
<th>ID</th>
<th>Pt.</th>
<th>Sex/age</th>
<th>Status at ABMT</th>
<th>Induction CT</th>
<th>Consolidation CT</th>
<th>Type of BMT</th>
<th>Maintenance CT</th>
<th>Cytogenetic and molecular status at BMT</th>
<th>ABMT conditioning</th>
<th>1st CR duration (months)</th>
<th>2nd CR duration (months)</th>
<th>Actual disease status/survival (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>RA</td>
<td>F/31</td>
<td>3rd CR</td>
<td>Dauno</td>
<td>AUTO</td>
<td>No</td>
<td>-/-</td>
<td>BUS+CY</td>
<td>7</td>
<td>22</td>
<td>III CR/124</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>SGF</td>
<td>M/20</td>
<td>1st CR</td>
<td>IDA</td>
<td>AUTO</td>
<td>MTX+6-MP</td>
<td>-/-</td>
<td>BUS+CY</td>
<td>71</td>
<td>I CR/77</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>CML</td>
<td>F/29</td>
<td>1st CR</td>
<td>IDA+AraC</td>
<td>AUTO</td>
<td>Dauno</td>
<td>ATRA</td>
<td>-/+ (purging)</td>
<td>BUS+CY</td>
<td>69</td>
<td>I CR/71</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>DMA</td>
<td>F/26</td>
<td>1st CR</td>
<td>IDA+AraC</td>
<td>AUTO</td>
<td>Dauno</td>
<td>ATRA</td>
<td>-/+ (purging)</td>
<td>BUS+CY</td>
<td>29</td>
<td>Died/41</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>MC</td>
<td>M/37</td>
<td>1st CR</td>
<td>IDA+AraC</td>
<td>AUTO</td>
<td>Mitox+VP16</td>
<td>-/-</td>
<td>BUS+CY</td>
<td>16</td>
<td>10</td>
<td>III CR/70</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>TL</td>
<td>F/42</td>
<td>1st CR</td>
<td>IDA+AraC</td>
<td>AUTO</td>
<td>Mitox+VP16</td>
<td>ATRA</td>
<td>-/+ (purging)</td>
<td>BUS+CY</td>
<td>63</td>
<td>I CR/64</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>FP</td>
<td>M/32</td>
<td>1st CR</td>
<td>IDA+AraC</td>
<td>AUTO</td>
<td>Mitox+VP16</td>
<td>ATRA</td>
<td>-/+ (purging)</td>
<td>BUS+CY</td>
<td>62</td>
<td>I CR/64</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>BI</td>
<td>M/50</td>
<td>1st CR</td>
<td>ATRA+Dauno</td>
<td>AUTO</td>
<td>Dauno+6TG</td>
<td>Dauno+AraC</td>
<td></td>
<td>BUS+CY</td>
<td>11</td>
<td>Died/15</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>FE</td>
<td>F/17</td>
<td>2nd CR</td>
<td>ATRA+Dauno</td>
<td>AUTO</td>
<td>Dauno+3A7</td>
<td>Dauno+6TG</td>
<td></td>
<td>BUS+CY</td>
<td>3</td>
<td>30</td>
<td>III CR/61</td>
</tr>
<tr>
<td>10</td>
<td>DP</td>
<td>F/25</td>
<td>1st CR</td>
<td>ATRA+Dauno</td>
<td>AUTO</td>
<td>Dauno+6TG</td>
<td>Dauno+3A7</td>
<td></td>
<td>BUS+CY</td>
<td>60</td>
<td>I CR/61</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>SA</td>
<td>M/28</td>
<td>1st CR</td>
<td>ATRA+IDA</td>
<td>AUTO</td>
<td>Dauno+6TG</td>
<td>Dauno+3A7</td>
<td></td>
<td>BUS+CY</td>
<td>57</td>
<td>I CR/58</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>SD</td>
<td>F/14</td>
<td>1st CR</td>
<td>ATRA+Dauno</td>
<td>AUTO</td>
<td>Dauno+6TG</td>
<td>Dauno+3A7</td>
<td></td>
<td>BUS+CY</td>
<td>55</td>
<td>I CR/56</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>BV</td>
<td>M/43</td>
<td>1st CR</td>
<td>ATRA+Dauno</td>
<td>AUTO</td>
<td>Dauno+6TG</td>
<td>Dauno+3A7</td>
<td></td>
<td>BUS+CY</td>
<td>49</td>
<td>I CR/52</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>CF</td>
<td>M/51</td>
<td>1st CR</td>
<td>ATRA+Dauno</td>
<td>AUTO</td>
<td>Dauno+6TG</td>
<td>Dauno+3A7</td>
<td></td>
<td>BUS+CY</td>
<td>49</td>
<td>I CR/51</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>RS</td>
<td>M/16</td>
<td>1st CR</td>
<td>ATRA+Dauno</td>
<td>AUTO</td>
<td>Dauno+6TG</td>
<td>Dauno+3A7</td>
<td></td>
<td>BUS+CY</td>
<td>47</td>
<td>I CR/48</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>CM</td>
<td>F/41</td>
<td>1st CR</td>
<td>ATRA+Dauno</td>
<td>AUTO</td>
<td>Dauno+6TG</td>
<td>Dauno+3A7</td>
<td></td>
<td>BUS+CY</td>
<td>7</td>
<td>3</td>
<td>Died/22</td>
</tr>
</tbody>
</table>
Figure 1. RT-PCR results of all tested APL patients. Filled and open dots represent molecular analysis by RT-PCR positive and negative for PML-RARα transcript, respectively.Filled and open boxes represent cytogenetic analysis, positive and negative for t(15;17), respectively. Vertical arrows indicate ABMT and relapse; crosses indicate a death. The time of follow-up is denoted in months.
In all cases, bone marrow cells collected at diagnosis and cryopreserved in GITC (guanidium-isothiocyanate solution) were available for total RNA extraction. RT-PCR was performed as described.\(^1\)

Table 1 gives the clinical and biological data of the APL patients entered in this study. The results obtained from each sample are reported in Figure 1.

All patients were studied just prior to the conditioning regimen. Twelve patients were found to be cytogenetically (Cy) and molecularly (PCR) negative for PML-RAR\(_x\) transcript expression in bone marrow samples collected one month prior to ABMT. In this group, all but two patients remained in prolonged CR after ABMT (median survival 59.5 months, range 41-124 months). The two patients (FE and MC in Table 1) relapsed, respectively, 11 and 24 months after ABMT. One of these (MC in Table 1) had molecular evidence of leukemia three months before relapse, while the other was not studied for eight months before relapse. Both were further re-induced by chemotherapy and reached 2\(^{nd}\) and 3\(^{rd}\) CR, respectively.

Of the remaining three patients who were not in molecular remission before transplantation, two (BI and CM in Table 1) remained persistently PCR positive: two relapsed early post-transplant, and died of leukemia progression; one patient (CML), whose bone marrow was purged because she was found to be PCR positive before ABMT, became persistently PML-RAR\(_x\) negative after ABMT and remained in long-lasting 1\(^{st}\) molecular CR after 71 months. The remaining patient (DMA) transplanted during 1st CR developed a MDS 33 months after diagnosis and died of pulmonary infection 2 months after an allogeneic bone marrow transplantation from a partially matched HLA donor.

Several recent studies indicate that molecular monitoring of PML-RAR\(_x\) fusion transcripts in APL patients may be useful for predicting relapse and identifying patients who need further antileukemic therapy.\(^2,4,7\) While the induction and initial consolidation treatment of choice in APL is well defined, there is considerable debate regarding the subsequent decision in patients who have achieved CR as to whether to perform further consolidation with ABMT (in particular in molecularly positive patients) or initiate maintenance therapy. When APL patients have achieved first CR, molecular monitoring by PCR becomes particularly important for further therapeutic decisions. Although the vast majority of APL patients become persistently RT-PCR negative after consolidation chemotherapy,\(^4\) those patients who have persistently positive RT-PCR results or who convert to RT-PCR positivity after a negative result are at very high risk of relapse. Thus, after initial consolidation therapy, it is possible to distinguish, at a molecular level, two broad groups of APL patients.

The first group of persistently PCR negative APL patients have a low risk of relapse when further consolidated with ABMT. PCR monitoring studies have shown that APL patients with prolonged disease-free survival (the majority of whom have received BMT or ABMT) do not have RT-PCR detectable residual PML-RAR\(_x\) rearrangement in their BM cells and can be considered cured.\(^2,8,10\) This suggests that: 1) PCR negativity must be considered the therapeutic goal in APL patients; 2) intensification of consolidation chemotherapy by ABMT (instead of prolonged maintenance therapy) could eliminate APL cells.

In our series, the 11 patients who reached 1st molecular CR and subsequently received ABMT maintained molecular negativity and could thus be considered cured. Ten out of 12 patients, who remained in CR after ABMT, were in first CR at the time of ABMT. Furthermore, the median time of CR prior to ABMT was 7 months and one may argue that this added no benefit since patients might had been cured by the previous chemotherapy.

The second group of APL patients, who expressed persistent PCR positivity before ABMT despite their clinical status, have a high risk of relapse. Allogeneic BMT has been shown to produce persistent PCR negativity; so, it is possible that the APL has been cured. In contrast, if an HLA identical donor is not available, ABMT has to be considered.\(^6\) Some authors\(^3\) showed in a few cases that APL patients PCR positive before ABMT become PCR negative early after transplantation. Consequently, they suggest that the preparative regimen effectively suppressed the malignant clone below the limit of detection of their PCR assay and they conclude that transplantation is capable of curing APL mainly through the antileukemic action of the conditioning regimen.

This was not our experience. Of the 3 patients who were PCR positive before ABMT, two who received unpurged bone marrow collections rapidly relapsed, whereas the one who received a purged collection achieved persistent PCR negativity. The role of purging in ABMT in APL patients has been little studied but our isolated experience of this single patient is encouraging. The role of ABMT in clinically refractory APL\(^3\) seems to suggest that this procedure should be avoided and purging procedures should be strongly considered.

In conclusion, we think that utilization of ABMT in APL patients in molecular CR could be a valid alternative to prolonged maintenance chemotherapy, and that purging could be effective in PCR positive APL patients.

**Acknowledgments**

This work was supported by “AIL 30 ore per la vita”, by MURST 40%-60% target projects and by Italian Association of Cancer Research (A.I.R.C.), by Italian C.N.R. A.C.R.O. no. 94.012222.PF39-952206 CNR target projects. The authors are grateful to Mr. Robin M.T. Cooke for editorial assistance.
**Key words**
PML-RARs, acute promyelocytic leukemia, BMT

**Correspondence**
Giovanni Martinelli MD, Institute of Hematology and Medical Oncology “Seràgnoli”, Policlinico S. Orsola, via Masantorelli 9, 40138 Bologna, Italy. Phone: international +39-051-6363680 • Fax: international +39-051-398973.

**References**

**Circulating thrombopoietin and interleukin-6 concentrations were investigated in two different settings of thrombocytopenia. Twenty patients with autoimmune thrombocytopenic purpura (ATP), 12 patients with aplastic anemia (AA), and 15 healthy subjects were studied. Thrombopoietin was significantly increased in AA and deficient in ATP. Interleukin-6 was significantly increased in ATP, compared to both other groups.**

Assaying thrombopoietin (TPO) and interleukin-6 (IL-6) levels in autoimmune thrombocytopenic purpura (ATP) and aplastic anemia (AA) may provide important clues for understanding the regulation of plasma TPO levels and pathobiology of thrombocytopenia in these disorders. In this study, plasma concentrations of these molecules were measured in newly diagnosed patients with ATP and AA before any therapies, including immunosuppressive drugs and transfusions, were initiated.

The study groups consisted of 20 patients with ATP (12 females, 8 males; mean age, 30±3 years), 12 patients with AA (5 females, 7 males; 36±3 years); the control group was formed of 15 healthy adult subjects (10 females, 5 males; 28±2 years). Plasma TPO and IL-6 concentrations were assayed by sandwich type ELISAs (TPO Quantikine™, R&D Systems, Minneapolis, USA and Human IL-6 ELISA Kit, Serotec Ltd, Oxford, UK).

TPO was significantly increased in AA, and deficient in ATP. IL-6 levels were significantly increased in ATP, compared to both other groups. IL-6 concentrations in AA patients were not statistically different from those in the control subjects (Table 1).

The proliferation and maturation steps of megakaryocytopoiesis are regulated by many lineage nonspecific megakaryocytopoietic cytokines, including IL-6, and the lineage-specific cytokine, TPO.1 Lineage

**Table 1. Median (range) thrombopoietin and interleukin-6 concentrations in the control group and in the patients with autoimmune thrombocytopenic purpura and aplastic anemia.***

<table>
<thead>
<tr>
<th></th>
<th>ATP (n=20)</th>
<th>AA (n=12)</th>
<th>Control group (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombopoietin (pg/mL)</td>
<td>0&lt;sup&gt;c&lt;/sup&gt; (0-28)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>20.91&lt;sup&gt;c&lt;/sup&gt; (6-125)</td>
<td>15.73 (4-45)</td>
</tr>
<tr>
<td>Interleukin-6 (pg/mL)</td>
<td>22.4&lt;sup&gt;c&lt;/sup&gt; (6-76)</td>
<td>10.6 (4-32)</td>
<td>7.6 (1-24)</td>
</tr>
<tr>
<td>Platelet count (mm&lt;sup&gt;3&lt;/sup&gt;)</td>
<td>17400 (2,000-45,000)</td>
<td>18000 (10,000-35,000)</td>
<td>229000 (145,000-420,000)</td>
</tr>
</tbody>
</table>

Abbreviations: ATP = autoimmune thrombocytopenic purpura; AA = aplastic anemia.

*The Mann-Whitney U test was used for statistical comparisons (p values <0.05 are significant); *Not detectable; **p<0.0001 vs. aplastic anemia and control group; ***p<0.001 vs. control group; ****below the detection limit (15 pg/mL) in 13 patients; *****<0.01 vs. aplastic anemia and control group.

**Circulating thrombopoietin and interleukin-6 concentrations were investigated in two different settings of thrombocytopenia. Twenty patients with autoimmune thrombocytopenic purpura (ATP), 12 patients with aplastic anemia (AA), and 15 healthy subjects were studied. Thrombopoietin was significantly increased in AA and deficient in ATP. Interleukin-6 was significantly increased in ATP, compared to both other groups.**

Assaying thrombopoietin (TPO) and interleukin-6 (IL-6) levels in autoimmune thrombocytopenic purpura (ATP) and aplastic anemia (AA) may provide important clues for understanding the regulation of plasma TPO levels and pathobiology of thrombocytopenia in these disorders. In this study, plasma concentrations of these molecules were measured in newly diagnosed patients with ATP and AA before any therapies, including immunosuppressive drugs and transfusions, were initiated.

The study groups consisted of 20 patients with ATP (12 females, 8 males; mean age, 30±3 years), 12 patients with AA (5 females, 7 males; 36±3 years); the control group was formed of 15 healthy adult subjects (10 females, 5 males; 28±2 years). Plasma TPO and IL-6 concentrations were assayed by sandwich type ELISAs (TPO Quantikine™, R&D Systems, Minneapolis, USA and Human IL-6 ELISA Kit, Serotec Ltd, Oxford, UK).

TPO was significantly increased in AA, and deficient in ATP. IL-6 levels were significantly increased in ATP, compared to both other groups. IL-6 concentrations in AA patients were not statistically different from those in the control subjects (Table 1).

The proliferation and maturation steps of megakaryocytopoiesis are regulated by many lineage nonspecific megakaryocytopoietic cytokines, including IL-6, and the lineage-specific cytokine, TPO.1 Lineage

**Table 1. Median (range) thrombopoietin and interleukin-6 concentrations in the control group and in the patients with autoimmune thrombocytopenic purpura and aplastic anemia.***

<table>
<thead>
<tr>
<th></th>
<th>ATP (n=20)</th>
<th>AA (n=12)</th>
<th>Control group (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombopoietin (pg/mL)</td>
<td>0&lt;sup&gt;c&lt;/sup&gt; (0-28)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>20.91&lt;sup&gt;c&lt;/sup&gt; (6-125)</td>
<td>15.73 (4-45)</td>
</tr>
<tr>
<td>Interleukin-6 (pg/mL)</td>
<td>22.4&lt;sup&gt;c&lt;/sup&gt; (6-76)</td>
<td>10.6 (4-32)</td>
<td>7.6 (1-24)</td>
</tr>
<tr>
<td>Platelet count (mm&lt;sup&gt;3&lt;/sup&gt;)</td>
<td>17400 (2,000-45,000)</td>
<td>18000 (10,000-35,000)</td>
<td>229000 (145,000-420,000)</td>
</tr>
</tbody>
</table>

Abbreviations: ATP = autoimmune thrombocytopenic purpura; AA = aplastic anemia.

*The Mann-Whitney U test was used for statistical comparisons (p values <0.05 are significant); *Not detectable; **p<0.0001 vs. aplastic anemia and control group; ***p<0.001 vs. control group; ****below the detection limit (15 pg/mL) in 13 patients; *****<0.01 vs. aplastic anemia and control group.

**Circulating thrombopoietin and interleukin-6 concentrations were investigated in two different settings of thrombocytopenia. Twenty patients with autoimmune thrombocytopenic purpura (ATP), 12 patients with aplastic anemia (AA), and 15 healthy subjects were studied. Thrombopoietin was significantly increased in AA and deficient in ATP. Interleukin-6 was significantly increased in ATP, compared to both other groups.**