Isolated hyperferritinemia with normal transferrin saturation and dysmetabolism, in the absence of the two known mutations in the HFE gene of hereditary hemochromatosis

Sir,

We report on a patient who had isolated hyperferritinemia with normal transferrin saturation, dysmetabolism, and mild hepatic iron overload. He was negative for the two known mutations in the HFE gene of hereditary hemochromatosis. Other factors that could induce hyperferritinemia with normal transferrin saturation were excluded. A brother with normal iron parameters was heterozygous for the Cys282Tyr mutation in the HFE gene. The physiopathologic mechanism of the association of unexplained hyperferritinemia with dysmetabolism, described by others as a syndrome, is still unknown. This disorder should be considered in the differential diagnosis of hyperferritinemic states.

The association of elevated serum ferritin levels with high serum iron level and increased transferrin saturation is highly suggestive of hereditary hemochromatosis (HH). Elevated serum ferritin values without high transferrin saturation should be considered with caution, and hepatocellular necrosis, chronic inflammatory disorders, or malignancies should be investigated. In addition, two hyperferritinemic conditions have recently been reported: 1) the “hereditary hyperferritinemia-cataract syndrome” (HHCS) caused by a mutation in the iron-responsive element (IRE) of the ferritin L-subunit gene. This autosomal dominant disorder patients have normal iron status and early-onset congenital cataracts;1-4 and 2) a non-HLA-linked syndrome described by Moirand et al. in 1997,5 in which an isolated hyperferritinemia is associated with mild to moderate iron overload and common metabolic disorders, such as diabetes, hyperlipidemia, obesity, and hypertension.

We report on a patient who presented with isolated hyperferritinemia, normal transferrin saturation, mild iron overload, and common metabolic disorders. He was negative for the known mutations of HH. One of the two proband’s brothers was heterozygous for the Cys282Tyr mutation in the HFE gene (Figure 1).

The proband (arrowed in Figure 1), a 55-year-old man of Italian origin, was referred to our institution because of monoclonal IgG gammopathy and hyperferritinemia. Both abnormalities had been found incidentally one year earlier (IgGκ 1.2 g/dL; serum ferritin 1,727 µg/L). At admission, the patient was overweight (92 kg). He had hypertension (150/90 mm Hg) and was on treatment with oral antidiabetic therapy because of abnormal glucose metabolism (fasting serum glucose 145 mg/dL). Alcohol abuse was excluded. At physical examination, liver enlargement was present (2 cm below the costal margin). Serum ferritin value was markedly increased (2,650 µg/L), while serum iron (115 µg/dL) and transferrin saturation (43%) were normal. Erythrocyte sedimentation rate and acute phase reactants were normal. Blood counts were within the normal range (RBC 4,900 x 10^12/µL, Hb 15.9 g/dL, MCV 86.9 fL, WBC 6.06 x10^9/µL with a normal differential count, PLT 164 x 10^3/µL). The monoclonal IgGκ was stable (1.16 g/dL) and bone marrow biopsy showed 8% of plasma cells; no Gaucher’s cells were detected on bone marrow aspirate. Serology for hepatitis B and C was negative. Liver enzyme activities (transaminases, γGT, alkaline phosphatase, LDH) were normal. Serum acid phosphatase activity was normal (3.2 mU/mL), and serologic markers for neoplasia (α-fetoprotein, carcinoembryonic antigen, CA 19-9, CA 125, and PSA) were within the normal ranges. No clotting factor abnormalities were present. Thoracic and skeletal X-rays showed normal findings, consistent with the patient’s age. At abdominal ultrasonography, only a moderate hepatomegaly was observed. Liver biopsy showed very mild and focal staining for iron with steatosis and mild portal fibrosis; iron was detectable only within hepatocytes. The liver iron concentration (LIC) was 30 µmol/g and the hepatic iron index was 0.54. No abnormalities related to cataracts were found at slit-lamp lens examination.

Molecular analysis was performed on genomic DNA from peripheral blood samples. The two known mutations in the HFE gene of HH (Cys282Tyr and His63Asp) were investigated by PCR amplification and RsaI and MboI digestion, respectively.6 No mutations were detected. The entire IRE sequence of the ferritin L-subunit gene was amplified by PCR and purified DNA fragments were automatically sequenced as previously described.7 The IRE sequence was wild-type.

Figure 1. Clinical and molecular characteristics of the three brothers investigated. (wt: wild-type).

<table>
<thead>
<tr>
<th>Age, yrs</th>
<th>56</th>
<th>55</th>
<th>53</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum iron, µg/dL</td>
<td>130</td>
<td>115</td>
<td>120</td>
</tr>
<tr>
<td>Serum ferritin, µg/L</td>
<td>159</td>
<td>2,650</td>
<td>119</td>
</tr>
<tr>
<td>Transferrin saturation</td>
<td>43</td>
<td>43</td>
<td>42</td>
</tr>
<tr>
<td>Cys282Tyr</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
</tr>
<tr>
<td>His63Asp</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
</tr>
<tr>
<td>IRE sequencing</td>
<td>wt</td>
<td>wt</td>
<td>wt</td>
</tr>
<tr>
<td>Diabetes</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hypertension</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Up to now, the patient has not received any treatment, and is being closely followed-up with monitoring of iron indices and gammopathy.
The patient’s two brothers, aged 56 years and 53 years, were investigated. Both were hypertensive and had mild hypercholesterolemia; one was on therapy with low-dose steroids for rheumatoid arthritis and had an increased fasting serum glucose level (211 mg/dL). Serum ferritin level (119 µg/L and 159 µg/L), serum iron level (120 µg/dL and 130 µg/dL) and transferrin saturation (42% and 43%) were normal in both cases. Lens abnormalities were excluded. At DNA analysis, one of them resulted to be heterozygous for the Cys282Tyr mutation in the HFE gene.

The proband does not meet the clinical diagnostic criteria for HH, and he does not carry the two known HH mutations in the HFE gene. Despite the genetic heterogeneity of HH in Italy,1,2 the hypothesis that non-HFE related hemochromatosis could account for the isolated hyperferritinemia in this patient seems remote, given that the Cys282Tyr mutation was detectable in a proband’s brother.

The recently described HHCS can also be ruled out in our case, as the IRE gene showed a normal sequence and no lens abnormalities were observed. Other factors that could induce hyperferritinemia with normal transferrin saturation3 were reasonably excluded. The patient denied alcohol intake and biochemical markers related to alcohol abuse were absent. No neoplasia was detectable; in this respect, it should be noted that hyperferritinemia was already present one year before our observation and that the patient is still in good clinical condition after 18 months of follow up. Homozygosity for Gaucher’s disease (type 1) seems to be excluded on clinical grounds. Because of the absence of clinical or biochemical stigmata of the disease, specific molecular and enzymatic investigations were not performed. Ceruloplasmin deficiency is highly improbable.

This patient with unexplained hyperferritinemia, normal transferrin saturation, mild iron overload and metabolic abnormalities resembles patients described by Moirand et al.,4 although in Moirand’s cases the mean serum ferritin value was lower and the LIC values were higher than in the present case. The French authors later reported that two thirds of patients with dysmorphic iron overload syndrome were heterozygous for one HFE mutation.10 As the physopathology of the association of iron overload with dysmetabolism is still unknown, the clinical features corresponding to this disorder are not clearly stated. Nonetheless, this disorder should be taken into account in the differential diagnosis of hyperferritinemic patients. The clinical evolution of this condition and the need for treatment with phlebotomy or iron-chelating agents are still to be defined.

**Key words**

Hyperferritinemia, metabolic disorders

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**References**


The dysmorphic iron overload syndrome is clinically and genetically distinct from HFE-related genetic hemochromatosis

Sir,

We describe two monozygotic twins who developed non-insulin-dependent diabetes mellitus and hyperferritinemia. They have no molecular lesions diagnostic of HLA-related genetic hemochromatosis or hyperferritinemia/cataract syndrome. The condition found in our patients closely resembles the dysmorphic iron overload syndrome. The fact that these monozygotic twins have a combination of abnormal glucose metabolism and hyperferritinaemia suggests a genetic basis for their disease.
for this dysmetabolic syndrome. In 1997 Moirand et al. described 65 patients with a non-HLA-linked iron overload syndrome characterized by normal transferrin saturation and elevated serum ferritin. These individuals were significantly older and had significantly less hepatic iron overload than individuals with HFE-related genetic hemochromatosis. Almost all patients had concomitant metabolic disorder (obesity, hyperlipidemia, abnormal glucose metabolism, or hypertension). The French authors later studied the prevalence of HFE mutations in these patients. They found that two-thirds of these individuals had at least one HFE mutation (C282Y and/or H63D, mainly the latter) and concluded that heterozygosity for one of these mutations is likely to be responsible for the expression of this dysmetabolic iron overload syndrome.

We studied two monozygotic twins referred to us because of hyperferritinemia. These HLA-identical 54-years-old men had had non-insulin-dependent diabetes mellitus since the age of 49. Their serum ferritin was found to be elevated during routine investigations (652 and 780 µg/L) and subsequent checks showed stable values in a range from 500 to 800 µg/L. Serum iron and transferrin saturation have always been normal. Since hyperferritinemia with normal to low serum iron is a typical pattern of inflammation, studies for evaluation of acute phase reactants were performed. ESR, reactive protein C, α2 globulins and fibrinogen were completely normal. Furthermore, the two patients had no evidence of congenital iron loading anemia.

Some of the authors have recently described the so-called hereditary hyperferritinemia/cataract syndrome, a new genetic disorder inherited as an autosomal dominant trait and characterized by elevated serum ferritin not related to iron overload and congenital nuclear cataracts. Several point mutations in the iron regulatory element (IRE) of ferritin light-chain mRNA have been found in the families described so far. These mutations have been shown to variably prevent binding of an inhibitory iron regulatory protein, thus leading to excessive L-ferritin synthesis. Although our twins had no evidence of cataract, we sequenced the 5' untranslated region of ferritin light-chain mRNA as previously described. We found no mutation either in the IRE or in the surrounding regions (5' of the IRE to the end of the transcript and 50 nucleotides 3'), thus ruling out hereditary hyperferritinemia/cataract syndrome at the molecular level.

We then studies C282Y and H63D HFE mutations using a PCR-RFLP detection method. The absence of any mutation excluded HFE-related genetic hemochromatosis. Liver function tests were completely normal and alcohol consumption was <50 g/day in both cases. Both twins refused a liver biopsy. The condition found in our patients closely resembled that described by Moirand et al. Although we could not evaluate liver iron concentration, we ruled out a genetic dysregulation in ferritin synthesis, so that the hyperferritineemia probably reflects increased iron stores in these individuals. The fact that these monozygotic twins have a combination of abnormal glucose metabolism and hyperferritinemia suggests a genetic basis for this dysmetabolic syndrome. The absence of HFE mutations indicates that this condition is genetically distinct from HLA-related genetic hemochromatosis. These observations may help to overcome our ignorance about ferritin metabolism.

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Key words
HFE-related hemochromatosis, iron overload syndrome

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References
Variation in the phenotypic expression of C282Y hemochromatosis in an Italian family

Sir,

Although genetic hemochromatosis (GH) is genetically homogeneous, its phenotypic expression is variable, depending on both environmental and genetic factors. Studying 3 members of the same family carrying C282Y with different clinical presentations, we speculate that the HLA-A3 genetic constitution is associated with a more severe disease and that modifier genes are close to, or identifiable with, HLA-A3.

GH is an autosomal recessive disorder leading to iron overload. Although GH is caused by a prevalent mutation (C282Y) in the HFE gene, its clinical expression is variable. The effect of age, sex, dietary iron, blood losses and blood donations on iron accumulation is well known. Iron burden may also be modulated by genetic determinants, particularly by the ancestral haplotype, the ancient chromosomal background on which the C282Y mutation occurred in the HFE gene. This haplotype is defined by HLA-A3, D6S105 allele-8 and D6S265 allele-1. We describe an Italian family in which all members are affected by GH (Figure 1). The proband, II-1, presented with weakness, arthritis and an altered glucose tolerance test, when aged 29. The parents were both symptomless and diagnosed because of family screening. I-2 was a 53-year-old woman with slight hypertransaminasemia without apparent cause. I-1 was a symptomless 60-year-old man, who had been a blood donor since the age of 35. Clinical data and iron status are summarized in Table 1. Liver biopsy was not performed in any of these patients because of the absence of significant alterations of liver function tests. Iron depletion (ferritin <50 µg/L) was achieved by weekly phlebotomies; the amount of iron removed is shown in Table 1. All patients had the same HFE genotype (C282Y/C282Y). HLA A3 was present in the heterozygous state in I-1 and I-2 and in the homozygous state in II-1. I-2 had a typical ancestral haplotype, whereas the HLA-A3-associated haplotype in I-1 was not of the ancestral type (D6S265 allele-3 and D6S105 allele-6) (Figure 1).

The expression of the disease in I-1 was unusually mild. However, blood donations could have had a protective effect (calculated iron removal = 18 g), resulting in a slower iron accumulation. The expression of the disease in I-2 was that expected in a postmenopausal woman. By contrast, II-1 was the first family member in whom the disease was diagnosed. Her age at diagnosis correlates with the severity of the iron burden. Confirming the more severe disorder, II-1 showed the greatest IR/age value in the family. IR/age is a good estimate of iron overload in the absence of liver biopsy. The severity of the disorder in II-1 suggests the effect of modifier genes. II-1 has the same HFE genotype as her parents and carries a single copy of the ancestral haplotype, as does I-2. She is, however, A3 homozygous at the HLA-A locus. It has been hypothesized that modifier genes are associated with the ancestral haplotype, but the markers which characterize the haplotype are spread over 3 megabases on 6p. Since II-1 is homozygous for HLA-A3, but is heterozygous for the other markers, this haplotype constitution allows a dissection of the different genetic components, suggesting a major contribution – as a modifier – of the region close to HLA-A3. We cannot at present define whether this is due to a direct role played by HLA-A, as suggested by others, or indicates the effect of other nearby genes.

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Key words
Hemochromatosis, HFE mutation, ancestral haplotype, phenotype

Table 1. Clinical, hematologic and biochemical data of family members.

<table>
<thead>
<tr>
<th></th>
<th>I-2</th>
<th>I-1</th>
<th>II-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age  (yr)</td>
<td>53</td>
<td>60</td>
<td>29</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>13</td>
<td>14.7</td>
<td>12.9</td>
</tr>
<tr>
<td>Alanine transaminase (IU/L)</td>
<td>40</td>
<td>22</td>
<td>6</td>
</tr>
<tr>
<td>Oral glucose tolerance test (OGTT)</td>
<td>N</td>
<td>N</td>
<td>A</td>
</tr>
<tr>
<td>Transferrin saturation (%)</td>
<td>83.3</td>
<td>55</td>
<td>82.3</td>
</tr>
<tr>
<td>Ferritin (µg/L)</td>
<td>626</td>
<td>827</td>
<td>213</td>
</tr>
<tr>
<td>Iron removal (IR) (g)</td>
<td>3.150</td>
<td>2.625</td>
<td>2.100</td>
</tr>
<tr>
<td>IR/age</td>
<td>0.06</td>
<td>0.043</td>
<td>0.073</td>
</tr>
</tbody>
</table>

N = normal; A = OGTT of diabetic type.
Pilot study of combined therapy with interferon-\(\alpha\), arabinosyl cytosine and all-trans retinoic acid in patients with chronic myeloid leukemia in the chronic phase

Sir,

The beneficial effect of IFN-\(\alpha\) on survival of Ph+ CML patients is known to be associated with the achievement of cytogenetic remission.\(^1\)\(^2\) Low-dose arabinosyl cytosine (LDAC)\(^7\) and all-trans retinoic acid (ATRA)\(^4\)\(^5\)\(^8\)\(^9\) can increase the response rate to IFN-\(\alpha\). This study was designed to evaluate the feasibility of treatment with IFN-\(\alpha\), LDAC and ATRA in patients with Ph+ CML in the chronic phase with special attention focused on dose adjustment and side effects. Our observations suggest that if one gives and maintains IFN-\(\alpha\) at 9 MU/daily or at the maximum tolerated dose, LDAC and ATRA cannot be given at the dose and schedule that were tested in this study.

Eleven consecutive patients received IFN-\(\alpha\) at a dose of 9 MU/day s.c. and in addition, monthly courses of LDAC (40 mg/day s.c., for 10 days every month, from day 1 to day 10) and ATRA (80 mg/sqm/day p.o., for 10 days every month, from day 20 to day 30) (Table 1). Treatment adjustment was decided every 30 days with the purpose of maintaining IFN-\(\alpha\) at the maximum tolerated dose. When WBC was 3-3.9 x 10\(^9\)/L or PLT 75-99 x 10\(^9\) (grade I toxicity), IFN-\(\alpha\) and ATRA were continued at full dose while the next course of LDAC was purposely omitted; if WBC was 2-2.9 x 10\(^9\)/L or PLT 50-74 x 10\(^9\) (grade II toxicity), IFN-\(\alpha\) was reduced to 3 MU/day for the next 30 days, ATRA was kept at full dose and the next course of LDAC was skipped; in case of WBC < 2 x 10\(^9\) or PLT < 50-10\(^9\) (grade III toxicity), IFN-\(\alpha\), LDAC and ATRA were discontinued for the next 30 days, and if the recovery did not take place within 90 days LDAC was stopped permanently. Treatment adjustment for non hematologic toxicity was based on IFN-\(\alpha\), LDAC and ATRA related side-effects which were graded according to the WHO scale. In case of grade II toxicity, the dose was reduced by 50% for the next month, after which the full dose was restored. In case of grade III toxicity, the drug was discontinued for the next month and then, if complete recovery occurred, 50% of the dose was given. In case of grade IV toxicity or refusal of the drug was discontinued permanently.

During the first 12 months of therapy we observed that: i) LDAC had to be discontinued in the majority of patients (72%) because of persistent leukopenia and/or thrombocytopenia (grade III); ii) ATRA had to be discontinued in 45% of patients, mainly due to headaches (WHO grade III-IV); iii) IFN-\(\alpha\) was never discontinued and was maintained at a dose of 9 MU/day in 60% of patients. By the 3rd month, all of the drugs had to be reduced in 511 patients (45%) (Table 2). None of the patients experienced bleeding or infectious episodes, or required blood transfusions (the lowest hemoglobin level was 8.2 g/dL). As for the hematologic effects, the majority of patients (82%) achieved and maintained a complete hematologic response\(^1\)\(^2\) and five (45%) obtained a cytogenetic response (Table 3). Two out of the 4 patients who displayed a major cytogenetic response (Ph-neg > 66%) where those who received the combined therapy for a prolonged period of time.

These observations suggest that this combination could be potentially effective in the treatment of Ph+ CML. If one gives and maintains IFN-\(\alpha\) at 9 MU/daily or at the maximum tolerated dose, LDAC and ATRA cannot be given at the dose and schedule that were tested in this study. To administer this drug combination for a longer time a reduction of either IFN-\(\alpha\) or LDAC and ATRA is required.

References

Table 1. Clinical and hematologic features of the 11 Ph+ CML patients in chronic phase at diagnosis and before starting the combined therapy with IFNα + LDAC + ATRA.

<table>
<thead>
<tr>
<th>Case pts.</th>
<th>Sex/ WBC MB (x10^9/L) (%)</th>
<th>PLT (x10^9/L)</th>
<th>Hb (g/dL)</th>
<th>Spleen (cm)</th>
<th>Ph+ Sokal risk</th>
<th>Previous therapy</th>
<th>Months from diagnosis</th>
<th>WBC MB PLT Hb Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 NC</td>
<td>M/47</td>
<td>208</td>
<td>6</td>
<td>285</td>
<td>9.9</td>
<td>7</td>
<td>100</td>
<td>1.343</td>
</tr>
<tr>
<td>2 ZC</td>
<td>M/45</td>
<td>141</td>
<td>1</td>
<td>225</td>
<td>13.4</td>
<td>0</td>
<td>100</td>
<td>0.654</td>
</tr>
<tr>
<td>3 DMA</td>
<td>M/28</td>
<td>146</td>
<td>2</td>
<td>164</td>
<td>13.0</td>
<td>3</td>
<td>100</td>
<td>0.622</td>
</tr>
<tr>
<td>4 ZE</td>
<td>M/35</td>
<td>239</td>
<td>0</td>
<td>822</td>
<td>9.9</td>
<td>0</td>
<td>100</td>
<td>0.864</td>
</tr>
<tr>
<td>5 SR</td>
<td>M/35</td>
<td>72</td>
<td>1</td>
<td>127</td>
<td>12.5</td>
<td>3</td>
<td>100</td>
<td>0.596</td>
</tr>
<tr>
<td>6 SA</td>
<td>F/47</td>
<td>164</td>
<td>2</td>
<td>500</td>
<td>9.9</td>
<td>4</td>
<td>100</td>
<td>0.998</td>
</tr>
<tr>
<td>7 TA</td>
<td>F/47</td>
<td>177</td>
<td>2</td>
<td>272</td>
<td>10.0</td>
<td>2</td>
<td>100</td>
<td>0.768</td>
</tr>
<tr>
<td>8 DL</td>
<td>M/52</td>
<td>33</td>
<td>3</td>
<td>206</td>
<td>14.0</td>
<td>2</td>
<td>100</td>
<td>0.860</td>
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<tr>
<td>9 FA</td>
<td>M/25</td>
<td>150</td>
<td>1</td>
<td>452</td>
<td>11.7</td>
<td>7</td>
<td>100</td>
<td>0.885</td>
</tr>
<tr>
<td>10 PV</td>
<td>M/35</td>
<td>280</td>
<td>6</td>
<td>570</td>
<td>10.9</td>
<td>16</td>
<td>100</td>
<td>1.100</td>
</tr>
<tr>
<td>11 CG</td>
<td>F/39</td>
<td>280</td>
<td>3</td>
<td>940</td>
<td>11.8</td>
<td>9</td>
<td>100</td>
<td>1.380</td>
</tr>
</tbody>
</table>

*Median daily dose.

Table 2. Hematologic and non-hematologic drug-related toxicity in the 11 Ph+ CML patients treated with IFNα + LDAC + ATRA.

<table>
<thead>
<tr>
<th>Months from start of combined therapy</th>
<th>Cases treated with the combined therapy</th>
<th>Drug discontinuation</th>
<th>Dose reduction and drug-related side-effects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>drug No. cases Causes (no. of pts)</td>
<td>Drug No. cases (%)</td>
<td>Median dose administered</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3rd 11/11 (100%)</td>
<td>IFNα 5/11 (45)</td>
<td>56%</td>
<td>Leukopenia, Thrombocytopenia (II)</td>
</tr>
<tr>
<td></td>
<td>LDAC 7/11 (64)</td>
<td>33%</td>
<td>Leukopenia, Thrombocytopenia (I-II)</td>
</tr>
<tr>
<td></td>
<td>ATRA 6/11 (54)</td>
<td>58%</td>
<td>Leukopenia, Thrombocytopenia (I-II)</td>
</tr>
<tr>
<td>6th 6/11 (54%)</td>
<td>IFNα 5/11 (45)</td>
<td>66%</td>
<td>Leukopenia, Thrombocytopenia (II)</td>
</tr>
<tr>
<td></td>
<td>LDAC 3/7 (43)</td>
<td>33%</td>
<td>Leukopenia, Thrombocytopenia (I-II)</td>
</tr>
<tr>
<td></td>
<td>ATRA 1/6 (17)</td>
<td>28%</td>
<td>Headache (II)</td>
</tr>
<tr>
<td>9th 2/11 (18%)</td>
<td>IFNα 4/11 (36)</td>
<td>33%</td>
<td>Leukopenia, Thrombocytopenia (II)</td>
</tr>
<tr>
<td></td>
<td>LDAC 1/3 (33)</td>
<td>33%</td>
<td>Leukopenia, Thrombocytopenia (II)</td>
</tr>
<tr>
<td></td>
<td>ATRA 3/6 (50)</td>
<td>50%</td>
<td>Headache, Dry Skin (I)</td>
</tr>
<tr>
<td>12th 2/11 (18%)</td>
<td>IFNα 5/11 (45)</td>
<td>33%</td>
<td>Leukopenia, Thrombocytopenia (II)</td>
</tr>
<tr>
<td></td>
<td>LDAC 1/3 (33)</td>
<td>33%</td>
<td>Leukopenia, Thrombocytopenia (I)</td>
</tr>
<tr>
<td></td>
<td>ATRA 2/6 (33)</td>
<td>40%</td>
<td>Headache (II)</td>
</tr>
</tbody>
</table>
Table 3. Hematologic and karyotypic response in the 11 Ph+ CML patients treated with IFNα+LDAC+ATRA.

<table>
<thead>
<tr>
<th>Months from start</th>
<th>Complete hematologic response (CHR)</th>
<th>Karyotypic response</th>
<th>Progression to accelerated blastic phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>3\textsuperscript{rd}</td>
<td>9/11 (81%) //</td>
<td>/</td>
<td>//</td>
</tr>
<tr>
<td>6\textsuperscript{th}</td>
<td>10/11 (91%)</td>
<td>2/11 (18%)</td>
<td>case 2 (50% Ph- neg.)</td>
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<td>case 7 (82% Ph- neg.)</td>
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<td>case 3 (63% Ph- neg.)</td>
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<td>case 2 (78% Ph- neg.)</td>
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<td>case 8 (25% Ph- neg.)</td>
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<td>case 10 (72% Ph- neg.)</td>
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Key words
CM L, IFNα, arabinosyl cytosine, ATRA

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References

Reconstitution of alveolar macrophages from donor marrow in allogeneic BMT: a study of variable number tandem repeat regions by PCR analysis of broncho-alveolar lavage specimens

Sir,

In this study, PCR amplification of VNTR regions was used in order to determine the origin of pulmonary alveolar macrophages (PAM) in five BMT patients. Our results show that this technique is feasible allowing the pattern of reconstitution of this cell population to be determined regardless of whether the donor and recipient were sex-matched or not.

The high incidence and severity of pulmonary complications after allogeneic bone marrow transplantation (BMT) led us to carry out systematic bronchoalveolar lavages (BAL), as a guide to pre-emptive therapy.1 This allowed us to obtain pulmonary alveolar macrophages (PAM) during the first 100 days after BMT. Tissue macrophages derive from terminal differentiation of blood monocytes originating in the bone marrow. This is supported by the demonstration that within 3 months following BMT, host tissue macrophages differ from 25% PH-neg.

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1. The high incidence and severity of pulmonary complications after allogeneic bone marrow transplantation (BMT) led us to carry out systematic bronchoalveolar lavages (BAL), as a guide to pre-emptive therapy.1 This allowed us to obtain pulmonary alveolar macrophages (PAM) during the first 100 days after BMT. Tissue macrophages derive from terminal differentiation of blood monocytes originating in the bone marrow. This is supported by the demonstration that within 3 months following BMT, host tissue macrophages differ from donor origin. However, there is some evidence to support the existence of a local lung stem cell population able to differentiate and maintain the number of PAM.4 Different conditioning schemes may damage these two populations to be determined regardless of whether the donor and recipient were sex-matched or not.
Polymerase chain reaction (PCR) of variable number tandem repeats (VNTR) has been used to determine chimerism status of marrow or peripheral leukocytes post BMT, circumventing the need of sex mismatch. In the present study, PCR was used to determine the reconstitution pattern of post-BMT PAM.

PAM were obtained by BAL from five patients (2 CML, 1 ALL, 2 AML) performed between days +30 and +100 after the BMT. All of the patients had received BUCY2 as a conditioning regimen. PAM were isolated by adhesion to the plastic bottom of a culture flask after 1-2 h incubation at 37ºC. The supernatant cells were removed with RPMI 1640 medium and adherent cells rinsed twice. The purified macrophages were lysed following conventional methods and the lysates were used directly for PCR analysis. DNA was extracted from whole blood samples or monocyte cells according to classical methods. Chimerism studies by PCR of VNTR analysis were performed as described above.10

One BAL sample for each patient was analyzed. Blood monocytes were simultaneously isolated in two patients. Chimerism results are depicted in Table 1.

Our results showed that PCR of VNTR is a feasible method for detecting donor reconstitution of PAM after BMT. As far as we know, our study is the first to report a bone marrow origin of PAM confirmed by PCR of VNTR regions. In our series a pure donor PAM pattern was only detected in the patient with the latest BAL (day +108), a pure recipient pattern was detected in the earliest BAL, and a mixed pattern was present in the other three cases. A donor pattern of peripheral blood leukocytes was detected earlier than PAM. Alveolar macrophage reconstitution seems to be a time dependent phenomenon. As reported by Thomas et al., host macrophages are largely replaced by donor derived macrophages in less than 100 days, and have an estimated life span of 81 days. Our results are on keeping with theirs. Our patients received chemotherapy (BUCY) as a conditioning regimen but the pattern of PAM reconstitution appears to be similar to that reported by Thomas et al. whose patients received radiotherapy (CYTBI). To sum up, our results show that PCR detection of VNTR polymorphisms is a feasible method for ascertaining the donor origin of PAM. The pattern of reconstitution after BUCY seems to be similar to that observed after irradiation-based regimens, although the small number of patients included in our study precludes a firm conclusion on this issue.

Key words
Bone marrow transplantation, pulmonary alveolar macrophages, VNTR, chimerism, bronchoalveolar lavage

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References
A phase-II study with idarubicin, etoposide and prednisone (IVPP), in patients with refractory or early relapsed intermediate or high grade non-Hodgkin’s lymphoma

Sir,

Patients with non-Hodgkin’s lymphoma (NHL) refractory to primary chemotherapy (CT) or relapse have an unfavorable prognosis. A variety of salvage protocols for such patients are available, and, overall, approximately 60% of all patients with relapsed or refractory disease can achieve complete remission (CR) or partial remission (PR) following reinduction.

Idarubicin is a new anthracycline derivative which when given as monotherapy or in combination with other agents, to either relapsed or refractory patients or previously untreated patients with NHL, has shown a considerable efficacy.1-5

The object of this prospective study was to determine whether a moderately intensive regime comprising idarubicin, etoposide and prednisone (IVPP) is effective in relapsed or refractory patients with intermediate or high grade NHL.

Between March 1994 and September 1996, 18 consecutive patients with intermediate or high grade NHL were diagnosed and treated in our units. Patients were eligible for the study if they had not reached CR or relapsed after front line treatment for their lymphoma. All patients had been previously treated according to the protocol of a randomized study with either CEOP (cyclophosphamide, epirubicin, vincristine, prednisone) or CNOP (novantrone instead of epirubicin). For various reasons, mainly age related, the patients included were not eligible for megatherapy. After first line treatment the disease remained resistant in 14 (78%) patients. In the other four patients, the disease recurred within 2-10 months after the induced CR has been achieved.

The treatment regimen under study consisted of idarubicin 10 mg/m² days 1-3 IV, etoposide 100 mg/m² days 1-3 IV, and prednisone 100 mg p.o. days 1-7 (IVPP regimen). A CR was defined as the clinical and X-ray disappearance of all detectable disease for a minimum of two months, without the appearance of any new lesion. A PR was defined as a 50% or greater reduction of the measurable lesions for at least one month. Responding patients (CR or PR) received six courses of treatment. Patients who developed progressive disease after one course or who failed to achieve at least a PR after two, were regarded as treatment failures and taken out of the study. All patients starting therapy were considered evaluable.

The characteristics of the patients participating in this study are summarized in Table 1.

Of the 18 patients, 4 received only one cycle of CT due to disease progression. Eight patients received 2-4 cycles and 6 patients 5-6 cycles. All courses of the IVPP regimen were given as in-patient therapy. Six patients (33%) responded: four achieved CR and two PR. Complete remission lasted 3 months in one patient and 16 months in another. One CR has now lasted 30 months and another 37 months so far. Of the four CR patients one had been resistant to front line treatment and 3 had relapsed early. Of the two patients who exhibited PR one was resistant and the other had relapsed early.

As far as concerns toxicity, all patients developed alopecia and 5 of them oral mucositis. Hematologic toxicity according to the WHO scale was as follows: anemia was observed in 10/18 patients (7 grade 1-2 and 3 grade 3-4), neutropenia in 13/18 patients (5 grade 2 and 8 grade 3-4) and thrombocytopenia in

Table 1. Characteristics of the 18 patients with resistant or relapsed intermediate or high grade NHL.
8/18 patients (4 grade 1-2 and 4 grade 3-4). Supportive treatment with G-CSF was given to 12 patients. Blood transfusions were given to patients with grade 3-4 anemia, while platelet transfusion was used in only one case with bone marrow involvement.

The prognosis of patients with NHL who have relapsed or were resistant to front line treatment remains poor. Several conventional salvage protocols are available for such patients.1-4,7,8 Overall response rates (CR+PR) of up to 60% have been reported, depending on the characteristics of the patients included i.e. age, primary resistance, early or late relapse. The prognosis is particularly dismal for elderly patients or those with primary refractory disease.

Idarubicin is an interesting agent whose use in the treatment of NHL is worth investigation.9 Idarubicin was initially tested as monotherapy in patients with relapsed or refractory disease9 to confirm its activity. Next it was combined with other agents with known activity either in relapsed or refractory patients or as a front line treatment, often used in place of doxorubicin.10 In relapsed or refractory disease the response rates to idarubicin-containing regimens are up to 60%.2,3

This study included 18 patients with unfavorable prognostic according to the international index3 and the majority of them (14/18) exhibited resistance to front line treatment. The response rate was 33% which is lower than that obtained in other studies.3,4 The lower remission rate in the present study can be attributed to the unfavorable clinical characteristics of the patients.

Hematotoxicity, mainly neutropenia, was common with this regimen, but no toxic death occurred. Supportive treatment with G-CSF was used in the majority of patients.

In summary the IVPP regimen was effective in some relapsed or refractory patients with intermediate or high grade NHL and unfavorable clinical characteristics. It may be an alternative treatment for elderly or other patients not eligible for intensive regimens.

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References

Genetic polymorphism of methylenetetrahydrofolate reductase and venous thromboembolism: a case-control study

Sir,
Moderately high total plasma homocysteine (Hcy) levels have been demonstrated to be an independent risk factor for arterial and venous diseases.1,2 This situation can result from genetic defects and folate, B6, and high dose cytarabine as salvage therapy for refractor non-Hodgkin’s lymphoma. Cancer 1989; 64:1388-92.

We assessed the MTHFR polymorphism in a non-matched case-control study: 107 consecutive thromboembolic patients (deep venous thrombosis and pulmonary embolism), aged 54 years (range 18 to 80)
and 200 healthy donors (42 years, range 25 to 54). Venous blood samples in EDTA were obtained for DNA analysis by PCR. The amplified fragments were cut with TaqI which recognized the C→T substitution. Additionally, we measured plasma homocysteine levels in fasting conditions by EIA in all the patients.

The overall frequencies of the three MTHFR genotypes were similar among patients and control subjects. The thermolabile variant (Val/Val equivalent to T/T homozygosity in 677 position) was found in 13/107 (12.1%) patients and in 20/200 (10%) controls (NSD) with an odds ratio of 1.24 (CI95%=0.6-2.6). The heterozygous variant (C/T Ala/Val) frequency was 44%, both in the patients group (47/107) and in the control group (88/200), and the normal homozygous variant (C/C or Ala/Ala) occurred in 44% of the patients and 46% (92/200) of the controls. After adjustment for FV R506Q and FII G20210A mutations, the estimated risk of venous thrombosis among T/T carriers increased up to 1.33 (CI95%=0.6-2.9), but it did not reach statistical significance (p=0.50).

Those with abnormal genotype (T/T), have higher total homocysteine levels (13.3±4.6 µmol/L) than the others (10.1±6.6 µmol/L in C/T and 9.2±5.3 µmol/L in C/C) however, without statistical significance (p=0.19).

In conclusion, our data show that homozygosity for the C677T mutation in the MTHFR is not associated with increased risk of venous thromboembolism or, at least, suggest that a big multicenter study would be necessary to obtain a definitive answer.

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Recombinant human tissue plasminogen activator without heparin is effective in the treatment of hepatic veno-occlusive disease

Sir,

We report on 6 patients with veno-occlusive disease (VOD) who have been treated with recombinant human tissue plasminogen activator (rh-tPA) alone, without heparin, at a dose of 50 mg daily for 4 days. Four of them responded. Major bleeding was not observed in any of the patients.

The most effective therapy for VOD after bone marrow transplantation (BMT) has not yet been established. Rh-tPA has been employed both alone and in combination with heparin, but its efficacy remains unclear, and hemorrhagic complications are often described.1-4 We describe our successful experience in the treatment of VOD with rh-tPA alone.

Our series consisted of 6 patients, (4 males, 2 females), who developed VOD after autologous BMT (5 cases) or syngeneic BMT (1 case). Five patients had multiple myeloma, and one had T-lymphoblastic lymphoma. Median age at BMT was 42 years (range 20-54). Conditioning regimens for transplantation were: Bus 16 mg/kg and Cy 200 mg/kg for 3 patients, Bus 16 mg/kg and melphalan 100 mg/m² for 2 patients and melphalan 140 mg/m² plus single dose TBI (1000 cGy) for 1 patient. VOD was defined according to the Seattle criteria.5 The clinical diagnosis of VOD was made at a median of 21 days after BMT (range 15-30). Rh-tPA was administered by 3-4 hours intravenous infusion at a dose of 50 mg daily for 4 days. No patient received heparin in addition to rh-tPA. No patient had renal or pulmonary failure or encephalopathy. The median total serum bilirubin at diagnosis of VOD was 3.14 mg/dL (range 0.87-5.51), while the median serum levels of ALT and AST were 671 U/L and 588 U/L, respectively.

Four patients (66%) responded to rh-tPA, with a complete resolution of painful hepatomegaly or ascites and normalization of hepatic function. Improvement of clinical symptoms was fast, starting 48 h after the beginning of rh-tPA. Recovery of normal laboratory hepatic values was observed within two weeks. Three patients are long-term survivors, in CR. One responder patient died of interstitial pneumonia.
35 days after rh-tPA therapy. The two non-responder patients died of multiorgan failure 32 and 40 days after the start of rh-tPA. Life-threatening bleeding did not occur in any patient. Three patients experienced minor bleeding episodes: two in the gastrointestinal tract, and one at CVC cutaneous insertion.

Hepatic VOD is a dramatic toxic complication of hematopoietic progenitor cell transplantation. Once the disease is clinically apparent, few therapeutic approaches are available and the mortality rate is very high. It has been suggested that thrombolytic agents such as rh-tPA, could be therapeutically effective. Rh-tPA regimens have been 50 mg/kg/daily by 3-hour i.v. infusion for 4 days, or 0.2 mg/kg/h by continuous i.v. infusion for 5 days. In both cases, rh-tPA has often been associated with low-dose heparin. The risk of major bleeding is the principle concern associated with rh-tPA therapy. The largest published series reports that 10/42 (28%) patients receiving both rh-tPA and heparin experienced major bleeding, which was directly fatal in 3 cases and contributed to death in a further 3 patients. It is striking that 9 out of 10 of these patients were on heparin at the time they began to bleed. Our findings suggest that the risk of major bleeding in patients on rh-tPA is substantially reduced in patients receiving rh-tPA alone, with adequate supportive therapy, including massive transfusions of platelets and plasma.

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Key words
Recombinant human tissue plasminogen activator, hepatic veno-occlusive disease, bone marrow transplant, bleeding

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References