randomized in our study, patients who received HU alone did not seem to represent a population with particularly active disease, which could have been associated with a higher risk of evolution to AML. Indeed, our practice has been to treat most ET (<10% remained untreated in the present series) and, over the last 12 years, first-line treatment has almost always been HU. Only patients who did not achieve permanent normalization of platelet counts with HU (ie, presumably patients with more active disease) received additional drugs.

We believe that our findings confirm other reports that treatment with HU alone is associated with a certain risk of leukemic progression in ET and PV. The absence of progression reported by Tefferi with HU alone in two series rests on a relatively small number of cases and relatively small follow-up. The association between HU and leukemic progression in ET, especially with 17p deletion, does not prove that HU is directly a causative agent. Progression to AML, particularly with 17p deletion, is part of the natural evolution of another myeloproliferative disorder, ie, CML. Therefore, HU may not be leukemogenic in diseases other than MPD. On the other hand, caution over its use and close follow-up of patients treated for nonneoplastic disorders will certainly be required.

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REFERENCES


An Association Between the Common Hereditary Hemochromatosis Mutation and the Factor V Leiden Allele in a Population With Thrombosis

To the Editor:

It is now generally accepted that the occurrence of clinical thromboembolic events represent the pathological consequence of a combination of genetic and environmental prothrombotic factors. In the past 5 years, substantial progress has been made in identifying some of the more common genetic hypercoagulable traits, with the factor V Leiden (R506Q) and the prothrombin (G20210A) variants being associated with an increased relative risk of venous thrombosis of sevenfold1 and threefold,2 respectively. Furthermore, recent subgroup analysis indicates that these genetic variants may also enhance the relative risk of arterial thrombosis in some populations.3,4 In addition, there are likely many as yet undefined genetic influences that contribute small but significant effects to the prothrombotic state and determine why the factor V Leiden and prothrombin variant traits show such marked variability of clinical expression.

In this preliminary report, we describe the results of a genetic study that may have identified a trait that contributes to the hypercoagulability associated with the factor V Leiden variant. We have documented the prevalence of the recently described common hereditary hemochromatosis (HH) mutation of the Hfe gene (C282Y)5 in a population of unscreened patients with thrombosis referred for molecular genetic studies of hypercoagulability. Eighty-seven of these patients (all unrelated) were heterozygous for the factor V Leiden mutation. In this population, the carrier frequency for the Hfe C282Y mutation was 18.7% (Table 1). In contrast, in 105 unrelated patients who were negative for the factor V Leiden mutation, the frequency of Hfe gene C282Y heterozygotes was only 3%. The results obtained for these two groups were compared with each other and with normal control populations in which the frequency of C282Y heterozygotes is approximately 6%.5,6 It was found that the frequency of C282Y heterozygotes was significantly higher in the factor V Leiden carriers ($\chi^2$, $P < .001$) than that observed in either the factor V Leiden-negative patients or in control populations (Table 1). In contrast, the frequency of C282Y heterozygotes in the factor V Leiden-negative patients was not significantly different from that observed in control populations.

This small preliminary study has documented a highly significant and biologically interesting association between the heterozygous states for the factor V Leiden and the Hfe gene C282Y mutations in an unscreened population of patients referred for hypercoagulable testing. Is there a biologically plausible association between the thrombotic events in these patients and the doubly heterozygous state for factor V Leiden and HH? This is unclear at present and, indeed, neither the heterozygous nor homozygous state for HH has been reported to be complicated by an increase in thrombotic events.7 However, a preliminary association between the heterozygous state for HH and total cardiovascular death has recently been reported,8 and an interesting association of thrombosis and secondary hemochromatosis has been documented in a mouse model of hereditary spherocytosis.9 We speculate that perhaps even minor dysregulation of iron metabolism may, under certain circumstances, such as when the heterozygous state for factor V Leiden is also present, contribute to the enhanced risk of a clinical thrombotic event. In contrast, the thrombotic population testing negative for both factor V Leiden and HH may have an increased thrombotic risk that is the result of distinct and independent metabolic defects.

We propose that this preliminary observation deserves further evaluation in larger and more stringently defined populations.

Table 1. Hfe Gene C282Y Carrier Status in Factor V Leiden-Negative and -Positive Thrombotic Patients

<table>
<thead>
<tr>
<th>Hfe Genotype</th>
<th>Normal/C282Y</th>
<th>Normal/Normal</th>
<th>$\chi^2$ Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal/Normal</td>
<td>3</td>
<td>102</td>
<td>NS</td>
</tr>
<tr>
<td>Normal/Leiden</td>
<td>16</td>
<td>71</td>
<td>$P &lt; .001$</td>
</tr>
</tbody>
</table>

*As compared with a carrier frequency of C282Y in the normal population of 6%.
To The Editor:

In a recently published letter by O’Brien and Goldman in Blood, the investigators addressed clinical data that point to the benefits of using busulfan (BU) alone as a cytoreductive regimen in the treatment of chronic myelogenous leukemia (CML) before autologous bone marrow transplantation (BMT). They argued that conditioning with BU alone leads to maximal cytoreduction with minimal treatment-related toxicity. This conclusion has a bearing on adding cyclophosphamide (CY) to BU treatment, because such a combination is often considered the conditioning regimen of choice for diseases such as CML may be similarly unaffected by the addition of CY if the malignant cells share the same chemoresistance to the normal primitive stem cell counterpart that produces long-term chimerism. It remains questionable whether this agent has any advantages when applied in the autologous BMT setting.

In this communication, we wish to support the arguments of O’Brien and Goldman by presenting data on mice that were transplanted with syngeneic or allogeneic bone marrow cells after BU conditioning with or without CY. These murine BMT models use a difference in donor and host glucose-6-phosphate isomerase (Gpi-1) as a means of determining the extent of short- and long-term erythroid chimerism. Previous studies in our laboratories have established that, although donor-type engraftment in the short-term is dependent on the depletion of committed cycling progenitor populations in the recipient bone marrow, it is the ability of conditioning therapy to ablate quiescent primitive stem cells (of high self-renewal) that largely determines the level of blood chimerism beyond 3 months after transplant. The data given in Fig 1A show that a fractionated dose of BU is able to induce high levels of donor marrow engraftment of about 90% in the absence of an immunological barrier, whereas CY alone had minimal effects. These in vivo results are reflected in the amount of primitive stem cell killing by these agents as measured using the cobblestone area forming cell (CAFC) assay (CAFC day 35 subset survival of <0.001% and 81% for BU and CY, respectively). The addition of CY to BU treatment did not improve on the level of syngeneic engraftment. Indeed, there was a tendency for this combination to result in decreased chimerism levels and this difference was significant at all times after 4 weeks when BU was administered as a single dose of 50 mg/kg (data not shown) via mechanisms that are as yet unclear.

Very different results emerged from the use of allogeneic BMT, in which the immunological disparity conferred a rapid rejection of the donor cells and complete host marrow repopulation by 1 month after BU treatment alone (Fig 1B). In this case, the addition of CY produced dramatic results where it appeared to prevent the acute rejection and produce levels of allogeneic chimerism that were comparable with the syngeneic situation. The clear advantage of using CY with BU is that our mice were not leukemic. However, it may be expected that diseases such as CML may be similarly unaffected by the addition of CY if the malignant cells share the same chemoresistance to the normal primitive stem cell counterpart that produces long-term chimerism. Therefore, these results are consistent with the hypothesis by O’Brien and Goldman in that the addition of CY in autologous BMT may not be beneficial in terms of clinical outcome and may carry the burden of unnecessary enhanced toxicities.
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