To the editor:

**ABO blood group also influences the von Willebrand factor (VWF) antigen level in heterozygous carriers of VWF null alleles, type 2N mutation Arg854Gln, and the missense mutation Cys2362Phe**

The levels of von Willebrand factor (VWF) in plasma are influenced by several variables, such as age, blood group, pregnancy, hormones, and smoking. The VWF levels may vary greatly on repeated laboratory testing in the same subject. About 60% of the variation in plasma VWF is caused by genetic factors, with ABO blood group accounting for only 30% of this. Indeed, in the normal population VWF levels are 25%–35% lower in individuals with blood group O than in individuals with non-O blood groups. Based on the results of epidemiologic studies, blood group–specific reference ranges have been suggested for diagnosis in von Willebrand disease (VWD). This biologic variation in VWF concentration may cause pitfalls when diagnosing mild cases of VWD.

There are no reports on the effect of ABO blood group in subjects heterozygous for VWF null alleles or heterozygous for some specific missense mutations. The influence of the ABO blood group in these individuals would be worthy of reporting since in several of these cases bleeding is rare despite the presence of clearly reduced VWF levels in plasma.

We have compared the VWF antigen (VWF:Ag) levels, according to blood group, of the heterozygous subjects from our families with recessive VWD. These individuals are heterozygous for VWF null alleles (splice site mutation, stop codon, frameshift, gene deletion), the type 2N mutation Arg854Gln, or the recessive missense mutation 7085G→C (Cys2362Phe) in exon 42 of the VWF gene (and unpublished data, 1999). Cys2362Phe is a true recessive mutation since it causes bleeding in compound heterozygous or homozygous states only.

All heterozygous subjects carrying blood group O have significantly lower VWF:Ag levels regardless of the type of mutation (Table 1). Interestingly, subjects heterozygous for Cys2362Phe showed VWF:Ag values almost indistinguishable from those observed in carriers of a null allele. Subjects with the type 2N mutation Arg854Gln seem to have values roughly similar to those of healthy individuals. The same analysis was carried out also for factor VIII procoagulant activity (FVIII:C), but no significant differences in FVIII:C levels between subjects with O and non-O blood groups, according to the different types of mutations, were observed (data not shown).

Zhang et al. reported phenotypic data in 3 groups of subjects heterozygous for null alleles (2430delC, Arg1659Xaa, and Arg1853Xaa). Subjects with Arg1853Xaa showed significantly lower VWF:Ag levels than did subjects heterozygous for 2430delC. But an excess of subjects with a non-O blood group (21, versus 9 with blood group O) was present in the group of subjects with 2430delC, compared to the group of subjects with Arg1853Xaa (1, versus 5 with blood group O). Thus the discrepant VWF:Ag levels between the 2 groups could be simply due to the different prevalence of blood groups. We have calculated VWF:Ag levels of subjects heterozygous for null alleles reported by Zhang et al. The mean VWF:Ag value for blood group O subjects (n = 17) was 32.3 ± 17.3 U/dL, in comparison with 48.8 ± 25.9 U/dL in non-O blood group subjects (n = 25; P = .05), thus confirming our results. As a further confirmation of the present findings, also in the study of Zhang et al., no differences were observed as to FVIII:C.

It has been recently suggested that VWF levels in carriers of blood group O are presumably lower due to increased clearance of VWF. The present results show that blood group–associated VWF difference still exists despite the fact that individuals with null alleles (and possibly Cys2362Phe) have a clear defect in synthesis. This defect causes a 50% reduction of VWF which is larger than the blood group–dependent variation. The fact that in addition to the synthetic defect of the null allele, the VWF synthesized by the second normal allele still shows variation due to blood group may suggest that the effect of blood group is independent of the synthesis and occurs after VWF is secreted into bloodstream. This hypothesis is supported also by the demonstration that platelet VWF content, which reflects endothelial synthesis and storage of VWF, is similar in subjects with O and non-O blood groups.

In conclusion, blood group significantly influences VWF:Ag level not only in normal subjects but also in heterozygous subjects with a null allele, Arg854Gln, or Cys2362Phe missense mutation. The contribution of blood group may explain in itself, at least in part, the heterogeneity of plasma phenotypes observed in heterozygous carriers for type 3 VWD. A puzzling, still unanswered question is why subjects with absolute reduction of VWF in plasma associated with heterozygosity for null alleles or, for instance, Cys2362Phe mutation are almost asymptomatic.

Table 1. Von Willebrand factor antigen levels in subjects heterozygous for the Cys2362Phe mutation, null alleles, or Arg854Gln (type 2N) according to blood group

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Mean VWF:Ag ± SD, U/dL</th>
<th>Range, U/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cys2362Phe</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O blood group</td>
<td>35.2 ± 16.2</td>
<td>25–75</td>
</tr>
<tr>
<td>Non-O blood group</td>
<td>61.5 ± 26.6</td>
<td>30–140</td>
</tr>
<tr>
<td>Null allele†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O blood group</td>
<td>43.2 ± 10.8</td>
<td>30–66</td>
</tr>
<tr>
<td>Non-O blood group</td>
<td>61.3 ± 23.6</td>
<td>25–95</td>
</tr>
<tr>
<td>Arg854Gln‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O blood group</td>
<td>75.4 ± 31.4</td>
<td>50–142</td>
</tr>
<tr>
<td>Non-O blood group</td>
<td>105.3 ± 24.3</td>
<td>74–153</td>
</tr>
</tbody>
</table>

P Values determined by Mann-Whitney test and indicate the differences between blood groups.

*P = .01.
†P = .03.
‡P = .003.

Giancarlo Castaman and Jeroen C. J. Elkenboom

Correspondence: G. Castaman, Department of Hematology, San Bortolo Hospital, I-36100 Vicenza, Italy; e-mail: castaman@hemato.ven.it
To the editor:

Therapy-related MDS and AML in acute promyelocytic leukemia

We read with great interest the report of Latagliata et al.1 on therapy-related myelodysplastic syndrome (t-MDS) and acute myelogenous leukemia (t-AML) following treatment of acute promyelocytic leukemia (APL). The authors observed 5 out of 77 patients with APL, who after intensive chemotherapy including all-trans retinoic acid (ATRA) relapsed with MDS or AML while in molecular remission of APL. Before relapse progressive cytopenia was observed in all 5 patients and at relapse, a phase of MDS was verified in 4. Three of the patients presented new, cytogenetically unrelated clones. The authors conclude that all 5 cases are induced by idarubicin, mitoxantrone, and etoposide used as induction therapy or by alkylating agents used before autologous stem cell transplantation in 2 patients.

The chemotherapy regimen administered as frontline therapy for APL included 3 courses of idarubicin with a total cumulative dose of 80 mg/m² and one single course of mitoxantrone and etoposide, all topoisomerase II inhibitors, plus cytosine- arabinoside and ATRA. Maintenance treatment was methotrexate plus 6-mercaptopurine, and in some instances ATRA. This regimen is not suspected to be extremely leukemogenic. Cases of relapsing APL were reinduced with mitoxantrone and cytosine-arabinoside, were conditioned with alkylating agents, and received an autologous stem cell transplantation. Following such a procedure, the risk of t-MDS and t-AML has been shown mainly to depend on the cumulative dose of alkylating agents and topoisomerase II inhibitors administered as primary therapy before transplantation.2 The 2 patients with t-MDS or t-AML receiving autologous stem cell transplantation for APL were apparently not heavily pretreated.

Table 1. Type of chromosome aberration and type of previously administered chemotherapy in 49 consecutive cases of t-MDS and t-AML from the Copenhagen series3

| Deletion or loss of 5q or 7q, monosomy 5 or monosomy 7 | 20/24 | 12/25 | .02 |
| Balanced translocations to chromosome band 11q23 or 21q22 | 0/24 | 12/25 | .0001 |

Previously, close associations have been observed between therapy with alkylating agents and development of t-MDS with deletions or loss of 5q or 7q; and between therapy with topoisomerase II inhibitors and development of t-AML with the recurrent balanced translocations, most often to chromosome bands 11q23 and 21q22. Results from our studies of 49 consecutive cases of t-MDS or t-AML diagnosed in 7 closely followed cohorts of patients receiving intensive chemotherapy3 are shown in Table 1.

In the study by Latagliata et al.1 none of the 5 relapsing patients presented any of the recurrent balanced chromosome abnormalities characteristic of t-AML following therapy with topoisomerase II inhibitors. Furthermore, the patient relapsing with monosomy 7 had not received alkylating agents. This raises doubt about the origin of the relapses of MDS or AML, at least in 4 of the 5 patients discussed, and questions the postulated extremely high risk of t-MDS and t-AML after therapy of APL.

Relapse of leukemia, for instance AML, with a cytogenetically unrelated clone is a well-described phenomenon.4,5 Previously, authors have been very cautious to classify such cases as therapy-induced. Rather, a shift of karyotype with the same underlying stem cell disease has been considered. This possibility was emphasized in one of the reports on MDS following successful therapy of APL.6 A similar phenomenon with development of unrelated clones, sometimes also with loss of 5q or 7q, has been observed following therapy of CML with interferon alpha7 or imatinib mesylate8 without any previous chemotherapy. Gene-based therapy such as ATRA or imatinib mesylate may facilitate a complete shift of karyotype in myeloid malignancies with a common underlying stem cell abnormality.

In the future, it is recommended that cases of MDS and AML, as described by Latagliata et al, are considered as therapy-induced, only if molecular studies have ascertained an independent origin.

Mette Klarskov Andersen and Jens Pedersen-Bjergaard

Correspondence: Mette Klarskov Andersen, Section of Hematology, Cytogenetic Laboratory, Dept of Clinical Genetics, Sct 4052, Rigshospitalet, Blegdamsvej 9, DK 2100 Ø, Denmark; e-mail: mka@rh.dk

References