To the editor:

**CFU-EC: how they were originally defined**

We read with interest the article “Redefining endothelial progenitor cells via clonal analysis and hematopoietic stem/progenitor cell principles” by Yoder et al.\(^1\) The authors compared 2 types of colony assays that have been shown to identify endothelial progenitor cells (EPCs)\(^2,3\) and studied the function of the cells derived from these assay systems. They found that a commercially available kit, which is widely used to quantify EPCs, actually detects cells of the monocytic lineage, whereas an assay developed by the authors unequivocally measures the colony-forming potential of true EPCs. Although we agree with the authors that it is time to clearly define the term EPC and the results presented are consistent with our own experience, we have some comments regarding the nomenclature of the endothelial colonies.

In the May 15, 2000 issue of Blood, we reported the identification of human endothelial progenitor cells expressing CD133 (previously known as AC133).\(^4\) We described a suspension culture system that induces differentiation of CD133-positive (+) cells into functional endothelial cells and a methylcellulose-based colony assay for EPCs. In this assay, CD133\(^+\) progenitor cells give rise to colonies with a unique morphology, which is different from CD133-derived hematopoietic colonies grown in methylcellulose. We showed that these colonies are composed of small-sized cells that express the endothelial cell antigen von Willebrand factor. Because our colony assay for EPCs was developed in analogy to the standard colony assay for hematopoietic stem and progenitor cells, we called the EPC-derived colonies “colony-forming unit endothelial cells (CFU-EC)” and introduced this term to scientific readership for the first time.

Meanwhile, numerous studies have been published claiming that they investigated the clonogenic potential of EPCs. We are aware that some of these studies adopted the term CFU-EC, although they used different culture systems.\(^5\) However, we would like to stress that the colonies presented by Hill et al have not been referred to as CFU-EC by the authors themselves.\(^3\) With regard to the already existing confusion about the definition of EPC and their progeny, we propose to refer to the myeloid colonies occurring in the Hill assay as “CFU-Hill” according to nomenclature used by the manufacturer of this assay. The term CFU-EC should be reserved for colonies derived from CD133\(^+\) EPC as described in our study.\(^4\)

In addition, we would like to note that the study by Reyes et al, cited as reference 11 in the article by Yoder and colleagues, did not use colony assays.\(^6\)

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**References**


To the editor:

**Associations between type of product and inhibitors in previously untreated patients (PUPs) with severe hemophilia: switches and particular products can disturb analysis**

Genetic and environmental risk factors for inhibitor development in severe hemophilia A have been reported in various observational studies. The CANAL (Concerted Action on Neutralizing Antibodies in severe hemophilia A) historical cohort studied the effect of product type by searching for differences between 1) recombinant factor VIII (rFVIII) and plasma-derived factor VIII (pdFVIII) and 2) depending on the concentration of von Willebrand factor (VWF).\(^1\) Despite a large number of patients (316 previously untreated patients [PUPs]) and a multivariate survival analysis, no difference was found. Regarding the first objective, Gouw et al observed an adjusted relative risk (aRR) rFVIII/pdFVIII of 1.4 (confidence interval [CI], 0.9 to 2.5)\(^4\) while we found an aRR of 2.4 (CI, 1.0 to 5.8),\(^2\) and a recent English study reported an adjusted odds ratio of 1.83 (CI, 0.9 to 3.72).\(^3\) The confidence intervals overlap, but 2 differences could account for the RR gradient observed. First of all, Gouw et al considered product type as a time-dependent covariate. The definition of switches was not clearly specified, but at least 54 patients, including 49 (36%) of the patients initially treated with pdFVIII, changed product early (after a median of 5 cumulative exposure days [CEDs]), probably for rFVIII in most cases.\(^1\) These patients were kept in the analysis until 50 CEDs and were included in the estimation of inhibitor incidence with rFVIII. This analysis option is based on the hypothesis that product-associated risk is only determined by the product received
at the last injection. However, according to Dasgupta et al, VWF protects FVIII from endocytosis by human dendritic cells and subsequent presentation to FVIII-specific T cells.\(^4\) If VWF provides protection during the initiation of treatment, this analysis option underestimates the rFVIII/pdFVIII RR and, by the same mechanism, could hinder study of the effect of VWF concentration. Chalmers et al took into account the appearance of inhibitors within the first 50 CEDs and studied the effects of the initial FVIII treatment.\(^3\) If patients changed product before 50 CEDs (which is probable), the difference between the treatments could also have been underestimated. Using a nonexperimental approach, we think that the best strategy is to consider product type as a fixed cofactor and to not take into account follow-up after the first switch.

Secondly, in the CANAL study, the patients received 23 different pdFVIIIIs, including 1201 CEDs to Beriate,\(^1\) which represents 38% of the CEDs to pdFVIII “containing considerable quantities of VWF.”\(^1\) However, this product has very low VWF concentration (0.09 IU/IU FVIII), and it appears that the immunoprotective effect of VWF is concentration dependent.\(^4\) Furthermore, in FVIII knockout mice, this product was as immunogenic as 2 first-generation rFVIII products.\(^5\) Thus, this pdFVIII could be associated with a particular immunogenicity, and it would be interesting to perform a sensitivity analysis excluding patients having received it in the rFVIII/pdFVIII comparison and to include testing of the classification of Beriate with pdFVIII products with low VWF content to study the effect of VWF concentration. The possibility that certain pdFVIII products could be less immunogenic and, most importantly, identification of the physiopathological mechanisms of these possible differences remain major issues for the development of new FVIIIIs.

Response:

Plasma-derived or recombinant factor VIII products and inhibitors in previously untreated patients with severe hemophilia

We appreciate the letter by Calvez et al and we agree that analytic decisions affect the findings from studies. Calvez et al propose 2 explanations for the observed differences between the findings of the study by Goudemand et al,\(^1\) the study by Chalmers et al,\(^2\) and our analyses of the Concerted Action on Neutralizing Antibodies in severe hemophilia A (CANAL) study.\(^3\) It was suggested that we found a smaller effect from recombinant products as opposed to plasma-derived products because we misclassified the exposure days of patients who received Beriate. Calvez et al proposed that Beriate should not have been included in the high–von Willebrand factor group. To examine this possibility, we repeated our analyses after excluding all 32 patients who had been treated with Beriate. In accordance with our previous findings, we found that patients on recombinant factor VIII products have the same risk as the patients on plasma-derived products with high–von Willebrand factor content (Table 1). Thus, Beriate did not explain the difference between our findings and the ones from Goudemand et al.\(^1\)

The other explanation concerned the fact that we had considered the factor VIII product as a time-varying variable, implying that the patients on plasma-derived products who switched to recombinant products during follow-up contribute their early exposure days to the plasma-derived group, and, immediately after the switch, they contribute exposure days to the recombinant product group. To evaluate this possibility, we excluded all postswitch exposure days and again repeated our analyses. The findings confirmed that the risk of inhibitors is not clearly increased in patients who received recombinant products as opposed to plasma-derived products with high–von Willebrand factor content (Table 1). In the table, we also present the findings of the CANAL study in the subgroup of patients who did not receive Beriate and whose exposure days are censored after switching from one product to another.

We have shown that the proposed explanations for differences between our study and the study by Goudemand et al do not hold. Two other explanations could be the subject of future research:

**Table 1. Relative rate of developing inhibitor antibodies against factor VIII in severe hemophilia A patients receiving recombinant factor VIII products as compared to plasma-derived factor VIII products**

<table>
<thead>
<tr>
<th>Source</th>
<th>RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients(^*)</td>
<td>N=322</td>
</tr>
<tr>
<td>Crude relative rate</td>
<td>1.0 (0.5-1.7)</td>
</tr>
<tr>
<td>Adjusted relative rate</td>
<td>1.2 (0.7-2.1)</td>
</tr>
<tr>
<td>Exposure days on Beriate excluded</td>
<td></td>
</tr>
<tr>
<td>Crude relative rate</td>
<td>0.9 (0.5-1.6)</td>
</tr>
<tr>
<td>Adjusted relative rate</td>
<td>1.1 (0.6-2.0)</td>
</tr>
<tr>
<td>Exposure days after switch of product excluded</td>
<td></td>
</tr>
<tr>
<td>Crude relative rate</td>
<td>1.3 (0.7-2.4)</td>
</tr>
<tr>
<td>Adjusted relative rate</td>
<td>1.5 (0.9-3.0)</td>
</tr>
<tr>
<td>Beriate and postswitch exposure days excluded</td>
<td></td>
</tr>
<tr>
<td>Crude relative rate</td>
<td>1.3 (0.6-2.7)</td>
</tr>
<tr>
<td>Adjusted relative rate</td>
<td>1.4 (0.6-2.9)</td>
</tr>
</tbody>
</table>

\(^*\)For recombinant F VIII compared with plasma-derived products with high VWF content.

High von Willebrand factor concentration was defined as more than 0.01 IU VWF antigen per IU factor VIII antigen. RR indicates relative rate; CI, confidence interval; and VWF, von Willebrand factor.

References


Associations between type of product and inhibitors in previously untreated patients (PUPs) with severe hemophilia: switches and particular products can disturb analysis

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