marrow preparations were directly labeled by a mixture of anti-CD34-phycocerythrin (PE) (clone 8G12; Becton Dickinson, San Jose, CA) and anti-CD7-fluorescein isothiocyanate (FITC) (clone 3A1E-12H7; Coulter Inc., Hialeah, FL), or other pairs of antibodies, along with anti-CD45-peridin chlorophyll α protein (PerCP) (Becton Dickinson). After labeling and lysing, nucleated cells were analyzed on FACS Calibur (Becton Dickinson) with an argon-ion laser tuned at 488 nm. On a CD45 versus sideward light scatter (SSC) dot plot, blast cells occupy a unique blast region characterized by intermediate CD45 intensity and low SSC. Cells labeled with mouse IgG2b-FITC and IgG1-PE were used as control to define the nonlabeled population. Cells were defined to be positive when 10% or more blasts were labeled with the specific monoclonal antibodies. We found that CD7 expression was detected in 80.6% of myeloid BC and 67.7% of undifferentiated BC cases but in neither cases of lymphoid BC, all of which unequivocally revealed B-lineage or mixed-lineage phenotypes (Table). On the other hand, CD34 was expressed in 75% of lymphoid and 96.4% of myeloid BC (Table). Analysis of the relationship between CD7 and CD34 expression on blasts of myeloid crisis showed their dual expression in 24 of 27 cases analyzed.

Similar to our results, Urbano-Ispizua et al observed CD7 expression in 7 of 26 cases (27%) of myeloid BC, and Martin-Henao et al reported that CD7 was expressed in 4 of 5 cases. On the contrary, Nair et al did not point out its expression in myeloid BC. There are some variations in the level of CD7 expression among the reports. These variations would reflect the differences in detection methods. Both Urbano-Ispizua et al and Nair et al used indirect immunohistochemical methods, while Martin-Henao et al performed flowcytometry using a conventional gating strategy. In this respect, the method we applied makes it possible to accurately estimate the immunophenotype of the blasts even when they represent the minor population. Our results suggest that myeloid BC coexpressing CD7 and CD34 may represent the maturation arrest of immature myeloid progenitor cells when CD7 is transiently expressed.

To the editor:

Effects of GP IIb/IIIa receptor antagonists on the activated clotting time of heparinized blood

The activated clotting time (ACT) is a functional measure of the intrinsic coagulation pathway that has become a standard monitoring device of heparin. In the Evaluation of c7E3 Fab in the Prevention of Ischemic Complications (EPIC) study, ACT has been measured during percutaneous transluminal coronary angioplasty (PTCA) to minimize thrombotic events and hemorrhagic complications. To investigate whether a GP IIb/IIIa receptor antagonist influences ACT, Moliterno et al reviewed data from the EPIC trial and demonstrated that ACT was more prolonged in heparinized patients treated with c7E3 Fab (abciximab-Reopro) than in those treated with placebo. Subsequently, it was shown in vitro that the addition of c7E3 Fab significantly prolonged ACT of blood anticoagulated with heparin, hirudin, and D-phenylalanine-L-propyl-L-arginyl chloromethyl ketone (PPACK), suggesting that in vivo c7E3 Fab acts not only as an antiplatelet but also as an anticoagulant agent. The ACT of in vitro heparinized blood obtained from healthy donors or obtained from patients during cardiopulmonary bypass was prolonged when a platelet inhibitor (prostacyclin or carbacyclin, respectively) was added. Since platelet activation leads to generation of thrombin, it is not surprising that a coagulation time measured in whole blood might be dependent on platelet function. Consequently, the presence of platelet inhibitors can influence ACT.

In current practice, 2 automated devices, HemoTec (Medtronic Inc, Parker, CO) and Hemochron (ITC Technidyne Corp, Edison, NJ) are commonly used for measuring ACT, each one using a different technique to determine clot formation in the presence of diatomaceous earth or kaolin as contact activator. As shown in patients undergoing coronary angioplasty, HemoTec and Hemochron ACT measurements cannot be used interchangeably after heparin administration because Hemochron values are about 28% higher than the HemoTec values.

Here we report that when ACT is determined by the Hemochron device (Figure 1A, B), the time elapsed between blood sampling in

<table>
<thead>
<tr>
<th>Type of crisis</th>
<th>No of cases</th>
<th>CD7</th>
<th>CD34</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myeloid</td>
<td>39</td>
<td>29/36 (80.6)</td>
<td>27/28 (96.4)</td>
</tr>
<tr>
<td>Lymphoid</td>
<td>10</td>
<td>0/9 (0)</td>
<td>3/4 (75.0)</td>
</tr>
<tr>
<td>Undifferentiated</td>
<td>3</td>
<td>2/3 (67.7)</td>
<td>0/0</td>
</tr>
<tr>
<td>Total</td>
<td>52</td>
<td>31/48 (64.6)</td>
<td>30/32 (93.8)</td>
</tr>
</tbody>
</table>

Positive numbers per analyzed sample numbers are indicated. Percentages are shown in parentheses.
heparin and the actual measurement plays a crucial role: the longer the delay time, the shorter the ACT. In the presence of a GP IIb/IIIa antagonist such as c7E3 Fab or the nonpeptide antagonist lamifiban\(^1\), in the heparinized blood, a clear prolongation of ACT was observed as compared to the heparinized blood without GP IIb/IIIa antagonist, and in addition the ACT values became more constant over time. In contrast, the ACT of heparinized blood determined by the HemoTec device (Figure 1C, D) was stable for at least 40 minutes. In the presence of the GPIIb/IIIa antagonist no additional prolongation of ACT was measured.

The differences in the ACT observed between the Hemochron and the HemoTec devices can be explained by in vitro platelet activation occurring when the Hemochron FTK-ACT cartridges are used, because vigorous shaking of the tube is required to disperse the activator (kaolin) in the blood (instructions of the manufacturer). Under these conditions the continuous decrease of the ACT of the heparinized blood over time might be indicative of increased thrombin generation due to the platelet activation. In the presence of an antiplatelet agent such as c7E3 Fab or lamifiban, the thrombin generation is inhibited,\(^10\) resulting in a prolongation of ACT that maintains constant ACT values over 40 minutes. In contrast, when the HemoTec High Range (HR)-ACT cartridges (activator: kaolin) are used, no manual agitation is required to activate the coagulation process (instructions of the manufacturer). Because of this softer handling of the blood samples, less or no platelet activation occurs, which explains why the ACT of heparinized blood remains stable over 40 minutes and is independent of the presence of a GP IIb/IIIa antagonist.

Which of the two ACT devices is more exact for measuring the in vivo anticoagulation is difficult to assess. Use of the Hemochron device means that the platelets contribute to the coagulation time, and therefore, GPIIb/IIIa antagonists are prolonging the ACT. One could argue that this device mimics the situation at a site of injury where platelets become activated and contributes to the coagulation process. In contrast, in the HemoTec system the platelet function appears to play a minor role because GPIIb/IIIa antagonists have no influence on the ACT.

The analysis of the EPIC trial pointed to the importance of ACT measurements for patients undergoing concomitant heparin and GPIIb/IIIa antagonist therapy.\(^3\) Based on our findings, we conclude that the optimal adjustment of the anticoagulation in patients treated with heparin and a GPIIb/IIIa antagonist depends on the ACT device used. Thus, there is a need for a standardized method for ACT measurements in the case of this combination therapy.

References


Figure 1. Effect of c7E3 Fab (ReoPro) and lamifiban on the activated clotting time of heparinized blood. Native fresh blood from healthy donors was collected by vein puncture directly in syringes containing 2.5 µg/mL heparin (0.375 U/mL) alone, and 2.5 µg/mL heparin + 20 µg/mL of c7E3 Fab, or 2.5 µg/mL heparin + 300 nM lamifiban. Two blood samples were collected from each donor, the first on heparin and the second on heparin plus c7E3 Fab or lamifiban. The ACT was determined following the instruction of the manufacturer either in a Hemochron device (A and B) or in a HemoTec device (C and D) using FTK-ACT and HR-ACT cartridges, respectively, containing in both cases kaolin as contact activator. The ACT of each blood sample was measured within 1 minute and then over 45 minutes. Individual data obtained from 4 donors are pooled for each graph.
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