Measurement of Hemostatic Factors in EDTA Plasma

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Key Words: Factor VII; Factor VIII; von Willebrand factor; EDTA; Citrate

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Abstract

This study determined whether immunoassays of factors VII (FVII) and VIII (FVIII) and von Willebrand factor (vWF) in EDTA-anticoagulated plasma samples are comparable to bioassays and immunoassays of these factors in citrate-anticoagulated plasma. Blood from 40 healthy volunteers was collected in EDTA- and citrate-anticoagulant tubes and assayed using immunoassays (EDTA and citrate) and clotting assays (citrate). Linear regression analyses were performed and Pearson correlation coefficients recorded. The correlation coefficients (95% confidence intervals [CIs]) between levels in EDTA- and citrate-anticoagulated plasma samples were 0.893 (0.806-0.943) for FVII antigen (ag), 0.930 (0.870-0.962) for FVIIIag, 0.990 (0.981-0.995) for vWFag, and 0.949 (0.906-0.973) for vWF activity. Coefficients (CIs) were 0.811 (0.668-0.896) for FVII coagulant activity (c) in citrate and FVIIIag in EDTA and 0.608 (0.366-0.774) for FVIIIc in citrate and FVIIIag in EDTA. Measurements of FVII, FVIII, and vWF antigens in EDTA-anticoagulated plasma samples give values comparable to similar measurements in citrate-anticoagulated samples. Clotting activity, especially of FVIII, is less well correlated. Although antigen assays using EDTA are not recommended for patients with coagulopathies, they may be suitable for population-based studies.

Hemostatic factors are usually measured in citrated plasma by bioassays. However, most other clinical laboratory tests are performed using blood collected in EDTA. Situations occasionally arise where a clinician requires clotting tests on a patient who has had only EDTA-anticoagulated blood drawn, or the blood collected in a citrate tube has clotted. If the patient is still available and agrees, another blood sample may be collected and placed in a citrate-containing tube, but if a fresh sample cannot be obtained, the tests are usually omitted. To avoid this outcome, we examined whether immunoassays of 3 hemostatic factors (factors VII [FVII] and VIII [FVIII] and von Willebrand factor [vWF]) using blood collected in EDTA would provide results comparable to bioassays and immunoassays for these factors using citrate blood.

Materials and Methods

Blood samples were obtained from 40 healthy volunteers, comprising an equal number of men and women aged 23 to 53 years. Excluded were persons with self-reported anemia; people taking medications for high blood pressure, heart disease, or diabetes; and women with self-reported pregnancy or taking oral contraceptives or estrogens. The study was approved by the Northwestern University Institutional Review Board (Chicago, IL), and all volunteers provided written informed consent.

For the study, 30 mL of blood was collected from each volunteer and divided into aliquots for 2 EDTA tubes and 2 citrate Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ). The blood was mixed with the anticoagulant and immediately centrifuged at 1,500g at room temperature for 15 minutes.
and then recentrifuged for 10 minutes to prepare platelet-poor plasma. The plasma was rapidly frozen and stored in aliquots at −70°C until assayed. Frozen samples were thawed at 37°C and tested in batches of 20. All samples were assayed in duplicate.

FVII coagulant activity (FVIIc) was assayed in citrate plasma using a 1-stage method based on the prothrombin time.

The assay was performed on the STA Compact (Diagnostica Stago, Asnières, France) using Neoplastine CL Prothrombin Time reagent and calibrator plasma and system controls from Diagnostica Stago, Parsippany, NJ. FVIIc-deficient plasma was obtained from George King Biomedical, Overland Park, KS. The coefficient of variation (CV) of the assay was 6.9%.

FVIII coagulant activity (FVIIIc) was assayed in citrate plasma using a 2-stage method based on a modified thromboplastin generation test. The assay was calibrated using the Unicalibrator from Diagnostica Stago and standardized using World Health Organization standards, and all samples were tested at 2 dilutions. The CV of the assay was 5.2%.

FVII antigen (FVIIag) was measured in EDTA-anticoagulated plasma using the Asserachrom VII-Ag reagents and enzyme-linked immunosorbent assay (ELISA; Diagnostica Stago). The CV of the assay was 4.6%. FVII antigen (FVIIag) in EDTA-anticoagulated plasma was assayed with the VisuLize FVII Antigen Kit (Affinity Biologicals, Ancaster, Canada), which uses a double-antibody ELISA. The CV was 4.7%.

vWF was measured in citrate- and EDTA-anticoagulated plasma using 2 assay methods. One is an immunoassay that recognizes an epitope on the vWF factor involved in its interaction with GPIb-α² (Imubind vWF Activity ELISA, American Diagnostica, Stamford, CT). The CV of the assay was 6.5%, and we have used the designation vWFac to differentiate this activity antigen from the antigen measured by the immunoturbidimetric method (vWFag, Liatest vWF Antigen Activity, American Diagnostica Stago). The CV of that assay was 3.3%.

Statistical Methods

Based on data in the literature, it was estimated that measurements of hemostatic factors in 40 subjects would provide a better than 97% chance of correctly concluding that the correlation coefficient for clotting and immunologic assays using citrate or EDTA anticoagulants would be greater than 0.85. The correlation coefficients and the corresponding 95% confidence intervals (CIs) between various measurements in citrate and EDTA were determined for the 4 antigen measurements: FVIIag, FVIIIag, vWFag, and vWFac. In addition, the correlation coefficients between FVIIc in citrate and FVIIag in EDTA and between FVIIIc in citrate and FVIIIag in EDTA were calculated. Simple linear regression analyses were used to examine the relationships between the various measurements of the hemostatic factors in citrate and EDTA.

Results

The mean and SD of the various measurements in the 40 participants are summarized in Table 1. The mean values for the antigens measured when EDTA was the anticoagulant were generally higher than when citrate was the anticoagulant, probably reflecting a small degree of dilution owing to the liquid citrate (EDTA is a powder). The mean and range of values for FVII clotting activity measured in citrate (0.985 [0.66-1.46]) were very close to the antigen values, whether measured in citrate (0.995 [0.63-1.72]) or in EDTA (0.997 [0.60-1.39]). The mean and range of values for FVIII clotting activity measured in citrate (1.19 [0.68-2.10]) or in EDTA (1.147 [0.75-1.89]) were slightly higher than the antigen values measured in citrate (1.109 [0.75-2.20]) or in EDTA (1.147 [0.75-1.89]).

Figure 1 includes 4 scatter plots for each antigen (FVIIag, FVIIc, vWFag, and vWFac) in citrate vs their counterparts in EDTA. The fitted regression lines were plotted to describe the relationship between the antigen measurements in citrate vs those in EDTA. The Pearson correlation coefficients (95% CIs) were 0.893 (0.806-0.943) for FVIIag, 0.930 (0.870-0.962) for FVIIIag, 0.990 (0.981-0.995) for vWFag, and 0.949 (0.906-0.973) for vWFac. Least square analysis yielded the fitted lines EDTA = 0.518 + 1.177 × citrate for vWFag, EDTA = 12.98 + 0.969 × citrate for vWFac, and vWFac − citrate = 1.131 (0.311) − 0.63-1.95

Table 1

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Mean (SD) EDTA</th>
<th>EDTA (Range)</th>
<th>Mean (SD) Citrate</th>
<th>Citrate (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVIIag</td>
<td>0.997 (0.199)</td>
<td>0.60-1.39</td>
<td>0.995 (0.222)</td>
<td>0.63-1.72</td>
</tr>
<tr>
<td>FVIIc</td>
<td>—</td>
<td>—</td>
<td>0.995 (0.261)</td>
<td>0.66-1.46</td>
</tr>
<tr>
<td>FVIIag</td>
<td>1.147 (0.319)</td>
<td>0.75-1.89</td>
<td>1.109 (0.342)</td>
<td>0.75-2.20</td>
</tr>
<tr>
<td>FVIIc</td>
<td>—</td>
<td>—</td>
<td>1.196 (0.393)</td>
<td>0.68-2.10</td>
</tr>
<tr>
<td>vWFag</td>
<td>1.441 (0.396)</td>
<td>0.92-2.28</td>
<td>1.221 (0.333)</td>
<td>0.75-2.17</td>
</tr>
<tr>
<td>vWFac</td>
<td>1.225 (0.318)</td>
<td>0.63-1.95</td>
<td>1.131 (0.311)</td>
<td>0.61-1.80</td>
</tr>
</tbody>
</table>

FVIIag, factor VII antigen; FVIIc, factor VII coagulant activity; FVIIIag, factor VIII antigen; FVIIIc, factor VIII coagulant activity; vWFag, von Willebrand factor activity antigen; vWFag, von Willebrand factor activity antigen.

* Values are given in IU/mL.
= 23.32 + 0.768 × citrate for FVIIag, and EDTA = 18.37 + 0.868 × citrate for FVIIIag. Figure 2 shows 2 scatter plots: FVIIc in citrate vs FVIIag in EDTA and FVIIIc in citrate vs FVIIIag in EDTA. The Pearson correlation coefficients (95% CIs) were 0.811 (0.668-0.896) between FVIIc in citrate and FVIIag in EDTA and 0.608 (0.366-0.774) between FVIIIc in citrate and FVIIIag in EDTA. The lines do not pass through the origin because clotting activity was proportionately higher than antigen concentration.

Discussion

The anticoagulant action of citrate solutions was discovered more than a century ago and the anticlotting effects of EDTA in 1944. Citrate forms un-ionized calcium salts; adding calcium reverses the anticoagulation. On the other hand, EDTA irreversibly chelates calcium and permanently prevents progression of the coagulation cascade. Therefore, citrate has been the anticoagulant of choice for bioassay of coagulation factors. However, citrate may fail to prevent activation of coagulation if technical difficulties permit thrombin generation. For example, a difficult venipuncture may cause hemolysis and expose blood to tissue fluids, delays in transferring the blood from the collection device to the citrate tube may allow time for thrombin formation, and improper or inadequate mixing of the sample with the citrate may permit activation of coagulation by calcium ions. Under these circumstances, citrate plasma is unsuitable for measurement of clotting factor concentrations. We performed this study to answer the question of whether immunoassay of clotting factors performed using EDTA-anticoagulated plasma could substitute for assays using citrate-anticoagulated plasma.

The mean values for FVIIag in citrate and EDTA were identical and for FVIIIag differed by only 0.04 IU/mL. Scatter plots for the 2 factors in citrate and EDTA showed correlation coefficients of 0.89 and 0.93, respectively, indicating that there is no loss of antigen when EDTA is used as the anticoagulant. This is in marked contrast with the major
loss of FVIII clotting activity that is observed with EDTA-anticoagulated plasma. Many years ago, EDTA was shown to inactivate FVIII, probably by disrupting calcium ion–dependent noncovalent interactions among the FVIII domains. This is not surprising because calcium ions are integral to the structural integrity of the FVIII molecule. Recent studies have shown that 2 calcium ions are located within the molecule.

One is in the A1 domain, tightly coordinated by a single glutamate and 3 aspartate residues. It may function to maintain a stable metal ion–dependent linkage of the heavy chain (the A1, A2, and B domains) and the light chain (the A3, C1, and C2 domains). The second is in the A2 domain coordinated by 2 aspartate residues; it functions to weakly associate the A1 domain with the A2 domain through electrostatic interactions.

EDTA likely disrupts these critical interactions and accounts for the loss of procoagulant activity.

The mean values for FVIIc and FVIIag were close to 1 IU/mL, and the correlation coefficient between the 2 was 0.81. This result likely reflects the different standards used for each method. Kleinveld et al suggest that the differences among the various assays are probably due to intercompany variations in standardization rather than to differences attributable to the anticoagulant. On the other hand, FVIIIc in citrate plasma was higher than FVIIIag measured in citrate or EDTA plasma, and the correlation coefficient was only 0.61 between the clotting assay and the antigen assay. FVIII levels more than 1.0 U/dL in the FVIII clotting assay were found to correlate imperfectly with levels measured by a chromogenic substrate assay for FVIII. Although it is possible that some activation of FVIII occurs during sample collection, storage, or assay, a more likely explanation is that the higher values again reflect a problem with assay standardization.

In this study, we examined the feasibility of assaying FVII, FVIII, and vWF in EDTA-anticoagulated blood. It has previously been shown that fibrinogen, assayed in EDTA-anticoagulated plasma, provides results comparable to clotting assays using citrate-anticoagulated plasma. Using clot-rate assays, the investigators reported a correlation coefficient of 0.972.

A limitation of our study is that we measured hemostatic factors in healthy volunteers, and the results may not be generalizable to patients with high or low clotting factor levels. However, as shown in Table 1, the range of values in these volunteers was quite broad and included values more than 1.50, which implies that the assays would be suitable for epidemiologic studies of thrombotic risk factors.

Measurements of FVII, FVIII, and vWF antigens in EDTA-anticoagulated plasma from healthy volunteers gave values comparable to similar measurements in citrate-anticoagulated plasma. Although antigen assays using EDTA are not recommended for patients who have coagulopathies, they may be suitable for population-based studies, especially when there is concern about the quality of citrate-anticoagulated samples.

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References