Effect on Routine and Special Coagulation Testing Values of Citrate Anticoagulant Adjustment in Patients With High Hematocrit Values

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Abstract

Recommendations to adjust citrate concentration for blood coagulation specimens with high hematocrit values are based on indirect experimental studies and not direct studies of patient samples with high hematocrit values. We compared the effect of adjusted and non–adjusted citrate concentrations on coagulation test results in samples from 28 patients with high hematocrit values (55%-72% [0.55-0.72]). Prothrombin time (PT) and activated partial thromboplastin time (aPTT) results from nonadjusted and adjusted samples were statistically different and exponentially increased with increasing hematocrit values. Results for fibrinogen, factor VIII, and protein C activity were statistically different and increased linearly with increasing hematocrit values; however, the difference was not as clinically significant. The protein C antigen value increased with increasing hematocrit values but was not significant. The effects on PT and aPTT are due to a dilutional effect of plasma and an interference effect of the higher final citrate concentration on the clotting test result. For patients with high hematocrit values, citrate concentrations must be adjusted for accurate results.

Many preanalytic variables affect the results of routine coagulation assays. To improve laboratory testing, it is critical to identify and understand the underlying mechanisms these variables impart and realize their potential impact on results. In addition, advances in laboratory instrumentation have improved the reproducibility and sensitivity of the analytic phase, creating greater dependence on specimen integrity. Activated partial thromboplastin time (aPTT) and prothrombin time (PT) determinations are among the most frequently ordered tests in the clinical laboratory with the greatest potential for preanalytic variability and error. These assays are used in the evaluation of a wide variety of clinical conditions and also form the basis of many special coagulation tests.

Since the introduction of these assays, efforts have been made to standardize their testing protocols, thus providing more accurate clinical information.1-3 Many standards set by the Clinical and Laboratory Standards Institute (CLSI; formerly the National Committee for Clinical Laboratory Standards) for testing in the coagulation laboratory have been developed in an effort to improve reproducibility, precision, and accuracy.3,4 Despite documentation, there is still a lack of standardization among clinical laboratories regarding preanalytic variables. Some of the clinical laboratory procedures currently in practice and/or used by regulatory agencies are founded on “tradition,” whereas others are based on data published using outdated methods.3,4 As a result, a number of inconsistencies and possible nebulous standards or regulations can arise.

One such variable is the effect of elevated hematocrit values on coagulation testing values. Based on antiquated studies and indirect experimental studies, recommendations have been made to adjust the citrate anticoagulant used for blood
specimens with elevated hematocrit values.\textsuperscript{1,3,5,7} In blood samples with an elevated hematocrit value, the citrate concentration is increased in the plasma in the collection tube and present in great excess after binding the free-ionized calcium of blood.\textsuperscript{5,6} When the plasma then is added to the clotting test reagents, the residual excess citrate binds a significant amount of calcium that is added to the clotting test reaction.\textsuperscript{5,6} This causes an artifactual increase in clotting time.\textsuperscript{5} In indirect studies, high hematocrit values increase the citrate concentration to a level that will influence clotting results.\textsuperscript{5} These early studies demonstrated that the optimum calcium concentration in the coagulation cascade is narrow and the citrate concentration in the plasma will have a significant effect on the clotting time.\textsuperscript{5,6,8} Studies were performed on samples with “normal” hematocrit values with increasing citrate concentrations to simulate high hematocrit values and demonstrated the necessity to adjust the citrate concentration.\textsuperscript{6,7} No direct studies on actual samples from patients with high hematocrit values, however, have been reported to validate the need for citrate adjustment in this setting.

Two laboratory standards organization currently recommend citrate adjustments for high hematocrit values. However, Midyett\textsuperscript{9} questioned whether adjustment of citrate in the collection tube is necessary based on a lack of direct data. We studied the need to adjust citrate concentrations for high hematocrit values and evaluated the current guidelines.\textsuperscript{3,5,7}

\section*{Materials and Methods}

Paired citrate-adjusted and non–citrate-adjusted blood specimens were obtained from 28 people with high hematocrit values (>55% [>0.55]) during a 2-year period at the Denver Veterans Affairs Medical Center, Denver, CO. The Human Subject Guidelines for Research of the University of Colorado Health Sciences Center were followed. The causes for the elevated hematocrit values were as follows: polycythemia vera, 23 cases; dehydration, 4 cases; and overtransfusion, 1 case. The hematocrit values ranged from 55% to 72% (0.55-0.72).

Hematocrit values were determined on a Coulter STK-S (Beckman-Coulter, Brea, CA) using a standard instrument protocol. The adjusted and nonadjusted samples were obtained in random order when obtained together from each patient by standard venipuncture. Samples in one standard, 5-mL, 3.2% sodium citrate tube (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ) with 0.5 mL of trisodium citrate (final citrate/blood ratio, 1:9) and one 5-mL, 3.2% sodium citrate tube (Becton Dickinson Vacutainer Systems) with citrate adjustment based on the hematocrit value (per CLSI guidelines\textsuperscript{3}) were obtained at the same time or within 15 minutes (after the hematocrit value was determined).

For adjusted citrate tubes, a sterile tuberculin syringe was inserted through the stopper and the appropriate volume of anticoagulant removed. The vacuum on the adjusted tube was retained and a normal fill volume obtained. Both samples were centrifuged immediately (2,500g for 15 minutes), and plasma was removed from the cell mass and maintained at room temperature (20°-22°C) for determination of PT, aPTT, and fibrinogen values. Aliquots were frozen at −80°C for determination of factor VIII and protein C activity and antigen values. For 5 patients who were receiving heparin, the adjusted and nonadjusted samples were obtained at the same time in random order.

The PT and aPTT values were ascertained using the coagulation reagents Recombiplastin and Sythasil, respectively (Instrumentation Laboratories, Lexington, MA), and testing was completed on an MLA model 1400 (Instrumentation Laboratories).\textsuperscript{2,7} The aPTT and PT assays for the adjusted and nonadjusted samples were performed in duplicate and the results averaged. Fibrinogen\textsuperscript{10} (by the von Clauss clotting method\textsuperscript{11}), factor VIII activity (standard clinical method using factor VIII–deficient plasma),\textsuperscript{12} protein C activity according to the manufacturer’s instructions (Protein C Clotting Activity kit, Dade Behring, Deerfield, IL),\textsuperscript{13,14} and protein C antigen value (by enzyme-linked immunosorbent assay as previously described) also were determined on both samples. For all assays, the adjusted and nonadjusted samples were analyzed together in random order.

Each patient sample pair was evaluated by determining the change in the coagulation value between the citrate-adjusted sample (considered clinically correct) and the non–citrate-adjusted sample, and the percentage change was calculated for each sample pair using the following equation:

\[
\% \text{ Change} = (\text{PT}_{\text{adjusted}} - \text{PT}_{\text{nonadjusted}})/\text{PT}_{\text{adjusted}} \times 100\%
\]

A clinically relevant difference was defined as a change between the citrate-adjusted coagulation test value of greater than 10%.\textsuperscript{2} Statistical significance was determined using the Wilcoxon matched-pairs signed-rank test (GraphPad InStat computer program, Graph Pad Software, San Diego, CA).

\section*{Results}

Paired citrate-adjusted and non–citrate-adjusted blood specimens were compared for 28 cases. The specimens obtained for the aPTT assay ranged in the citrate-adjusted samples from 36 seconds (within the normal range) to 97 seconds. The average difference for the aPTT between the citrate-adjusted and non–citrate-adjusted samples was a 19.29% increase in the nonadjusted sample and ranged from 4.2% to 46.7% \textsuperscript{1}Table II. The PT in the citrate-adjusted samples ranged from 10.2 seconds (within the normal reference range) to 52.5 seconds. The average difference between the
citrate-adjusted and non–citrate-adjusted samples was 25.26% with a range of 5.1% to 52.2% (Table 1). The percentage differences for the aPTT and PT exponentially increased with hematocrit values [Figure 1] and [Figure 2].

In the citrate-adjusted samples, fibrinogen levels ranged from 74 to 407 mg/dL (0.74-4.07 g/L) and for factor VIII, from 38% to 256% (0.38-2.56) [Figure 3]. In the citrate-adjusted samples for protein C activity and antigen, levels ranged from 12% to 125% and from 26% to 135%, respectively (Figure 3). Fibrinogen, factor VIII, and both protein C assays had a much narrower difference in the range between the citrate-adjusted and non–citrate-adjusted samples than for aPTT and PT (Table 1). These values increased linearly as the hematocrit increased.

The differences in values of citrate-adjusted and non–citrate-adjusted samples for the global coagulation tests (PT and aPTT) were statistically significant (P < .0001) [Table 2], and the majority of samples (21/28 for aPTT and 24/28 for PT) were clinically different (>10% difference) (Table 2). Differences in fibrinogen, factor VIII activity, and protein C activity values also were statistically significant (P < .0001), but fewer results were clinically significant (>10% difference). The protein C antigen values were not statistically different, and only 5 of 28 had greater than a 10% difference.

**Discussion**

In the present study, the aPTT and PT results for 28 samples that were citrate-adjusted for high hematocrit (>55% [>0.55]) and samples not citrate-adjusted were statistically and clinically significantly different. For PT and aPTT, the differences between citrate-adjusted and non–citrate-adjusted samples exponentially increased as the hematocrit value

| Table 1 | Overall Characteristics of the Differences Between Citrate-Adjusted and Non–Citrate-Adjusted Collection Tubes for High Hematocrit Values |
| --- | --- | --- |
| Percentage Difference | Average | SD | Range |
| Activated partial thromboplastin time | 19.29 | ± 12.21 | 4.2 to 46.7 |
| Prothrombin time | 25.26 | ± 13.89 | 5.1 to 52.2 |
| Fibrinogen | 10.43 | ± 3.26 | 6.8 to 18.9 |
| Factor VIII | 8.37 | ± 4.06 | 0.8 to 14.8 |
| Protein C activity | 9.90 | ± 4.82 | 0 to 20.0 |
| Protein C antigen | 2.67 | ± 4.47 | 0 to 14.6 |

[Figure 1] Percentage difference for activated partial thromboplastin time values between citrate-adjusted and non–citrate-adjusted samples in patients with elevated hematocrit values. The percentage difference is the change between the citrate-adjusted and the non–citrate-adjusted samples. See the “Materials and Methods” section for details.

[Figure 2] Percentage difference for prothrombin time values between citrate-adjusted and non–citrate-adjusted samples in patients with elevated hematocrit values. The percentage difference is the change between the citrate-adjusted and the non–citrate-adjusted samples. See the “Materials and Methods” section for details.
increased from 55% to 72% (0.55-0.72; Figures 1 and 2). The exponential increase in the aPTT and PT assay results shows the effect is due to an increase in citrate concentration (a dilutional effect with increasing hematocrit) and increasing levels of citrate in the blood sample interfering with the coagulation reactions. However, the change in the values for fibrinogen, factor VIII, and protein C activity and antigen changed in a linear manner, suggesting the cause is primarily dilution from the citrate rather than citrate interference with the assay. Because patient plasma is diluted before performance of the assays for fibrinogen, factor VIII, and protein C activity, the citrate interference effect is muted. The dilutional effect and the assay interference effect prolonged the clotting times more in the higher hematocrit samples.5

Although there were statistical differences between the citrate-adjusted and non–citrate-adjusted samples for fibrinogen, factor VIII, and protein C activity, there was only a moderate difference clinically with increasing hematocrit values. The protein C antigen value determined by immunoassay was not statistically or clinically different. Because the difference with increasing hematocrit values was small for the specific factors, it is much less of a problem clinically.

Table 2
Summary of Statistical and “Clinical” Differences Between Citrate-Adjusted and Non–Citrate-Adjusted Collection Tubes for High Hematocrit Values in 28 Cases

<table>
<thead>
<tr>
<th></th>
<th>P</th>
<th>&gt;10% Difference†</th>
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<tbody>
<tr>
<td>Activated partial thromboplastin time</td>
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<td>21</td>
</tr>
<tr>
<td>Prothrombin time</td>
<td>&lt;.0001</td>
<td>24</td>
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<tr>
<td>Fibrinogen</td>
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<td>Factor VIII</td>
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<td>.0802</td>
<td>5</td>
</tr>
</tbody>
</table>

* Using the Wilcoxon matched-pairs signed-rank test.
† Data are given as the number of cases.

Figure 3 Percentage difference for fibrinogen (A), factor VIII (B), and protein C activity (C) and antigen (D) values between citrate-adjusted and non–citrate-adjusted samples in patients with elevated hematocrit values. The percentage difference is the change between the citrate-adjusted and the non–citrate-adjusted samples. See the “Materials and Methods” section for details.
There are numerous articles in the clinical laboratory literature (non–research-based) recommending that for patients with high hematocrit values (>55% [>0.55]), the citrate anticoagulant concentration be adjusted (decreased) in the blood collection tube to compensate for the decreased amount of plasma present in the blood sample.\textsuperscript{5,16} However, in these articles there is not direct evidence that demonstrates the necessity for citrate concentration adjustment in blood samples with high hematocrit values.

One of the first and most referenced studies involved an indirect evaluation of the effect of hematocrit in which the authors used apheresis plasma and isolated RBCs to create various hematocrit values.\textsuperscript{5} These authors found that in this artificial system, the PT and aPTT were increased exponentially with increasing hematocrit values. The clotting times elevated more with increasing hematocrit values than found in the present study. Also, Koepke et al\textsuperscript{5} used 3.8% citrate in these studies, which was the common practice at that time, whereas the present study used 3.2% citrate.

A number of studies have been published using “short draws” in which the tube was underfilled to simulate the increased citrate concentrations found in samples with high hematocrit values.\textsuperscript{1,5-7} These studies investigated 3.8% citrate and 3.2% citrate. These studies showed the same significant effect of increased clotting times for PT and aPTT except the effect was much greater in the 3.8% citrate tubes.\textsuperscript{1,5-7} Our study provides direct evidence, confirming the results of previous indirect studies, that coagulation clotting times increase with increasing sample hematocrit values unless the citrate anticoagulant concentration is adjusted.

Although the recommendations for adjusting the citrate concentration in blood coagulation tubes for patients with high hematocrit values have been expounded in the clinical laboratory literature,\textsuperscript{15,16} reviews of preanalytic variables,\textsuperscript{17} and guidelines of clinical standards organizations and regulatory agencies,\textsuperscript{3,4} these recommendations were based on indirect studies of underfilled tubes or artificially constructed hematocrit values.\textsuperscript{1,5-7} Because direct research was not available for establishing this standard, the best indirect evidence was used, which has been the basis for this practice for many years.

The present direct comparison demonstrates that adjusting the citrate concentration and volume for a high hematocrit value is valid and must be performed. Had adjustments not been made, several of the patients might have had incorrect diagnosis or treatment! For example, a patient receiving heparin (case 26) with a hematocrit of 66% (0.66) had aPTT values of 106 seconds in the nonadjusted sample and 74 seconds in the adjusted sample. The therapeutic range for heparin is 58 to 80 seconds. If the value for the unadjusted tube had been accepted as correct, the patient would have been considered overtanticoagulated and treated accordingly.

The CLSI recommended that the final citrate concentration be adjusted for patients with hematocrit values of more than 55% (>0.55).\textsuperscript{3,4} This recommendation is correct, and the adjustment method described in the appendix of the CLSI document must be followed using a nomogram or a mathematical formula.\textsuperscript{3} Both methods provide the appropriate citrate concentration for elevated hematocrit values.\textsuperscript{3} Based on the work of Siegel et al,\textsuperscript{17} low hematocrit values do not affect PT and aPTT test results. One should note that the experiments were performed in 3.8% citrate, the common anticoagulant tube used at that time.\textsuperscript{17} Although 3.2% citrate probably will not change the conclusion, studies using 3.2% citrate will have to be performed to confirm those observations. The College of American Pathologists accredits the majority of laboratories in the United States and has requirements for adjusting the citrate concentration in elevated hematocrit values (HEM.22830). The issue is a strong recommendation (phase I) but not a requirement. It probably should be made a requirement (phase II) because it will provide better patient care.

**Recommendations**

For patients with a hematocrit value of more than 55% (>0.55), the citrate concentration must be adjusted (usually by removing volume) to an appropriate level to maintain an optimal citrate concentration in the resulting plasma sample. Failure to adjust the citrate concentration can lead to erroneous clotting results. The following is the recommended procedure:

1. For patients with a hematocrit greater than 55% (>0.55), the evacuated tube or syringe should have the appropriate volume of 3.2% citrate removed.

2. To calculate the amount of citrate to be present in the blood drawing tube or syringe, use the following formula\textsuperscript{3,15,16}:

   \[ C = (1.85 \times 10^{-3})(100 - \text{Hct})(V_{\text{Blood}}) \]

   where \( C \) is the volume of citrate remaining in the tube, \( \text{Hct} \) is the hematocrit of the patient, and \( V \) is the volume of blood to be added. (If a 5-mL tube is used, the volume is 4.5 mL.)

3. A nomogram can be used (see appendix of CLSI document H21-A4 or the most current version\textsuperscript{3}).

4. Add the correct amount of blood to the tube containing the adjusted citrate concentration.

5. Mix and process the sample in the same manner as the other coagulation samples.

6. A note should be added to the laboratory record and patient record stating that the hematocrit value was elevated and the citrate concentration adjusted.

7. For hematocrit values of less than 25% (<0.25), the citrate concentration does not need to be adjusted.\textsuperscript{17}
We believe that laboratory personnel need to challenge practices that rely on tradition, ie, practices without good solid evidence (direct or good indirect evidence) to back up the laboratory practice. If there is not good evidence, it is the responsibility of the clinical laboratory community to make sure that evidence is generated (whether direct evidence or good indirect studies) and the results made available to all laboratories.

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References