Effect of 3.2% vs 3.8% Sodium Citrate Concentration on Routine Coagulation Testing

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The effects of 3.2% and 3.8% sodium citrate concentration on the results of routine coagulation assays (prothrombin time [PT] and activated partial thromboplastin time [aPTT]) were evaluated by means of two sets of reagents, one responsive and the other nonresponsive. Five groups were entered in the study: healthy volunteers; outpatients receiving stable oral anticoagulant therapy; and hospitalized patients receiving intravenous (IV) heparin therapy, both IV heparin and oral anticoagulant therapy, or no anticoagulant therapy. With use of nonresponsive PT and aPTT reagents, varying the citrate concentration has little clinical significance except in patients receiving IV heparin therapy. In contrast, when responsive PT and aPTT reagents are used, the concentration of sodium citrate anticoagulant has a significant effect on assay results. Eighteen percent of samples from patients receiving stable oral anticoagulant therapy demonstrated a change of less than 0.7 INR (International Normalized Ratio) units between citrate concentrations. Nineteen percent of patients receiving IV heparin therapy had a greater than 7-second difference when aPTT results were compared. These data demonstrate that citrate concentration affects the results of coagulation tests. On the basis of these data, it is recommended that 3.2% citrate be used for all coagulation tests. (Key words: Activated partial thromboplastin time; Anticoagulant; Coagulation testing; Preanalytic variables; Prothrombin time) Am J Clin Pathol 1997;107:105-110.

Many preanalytic variables may affect the results of routine coagulation assays. To improve the precision and accuracy of laboratory testing, it is important to identify these variables and realize their potential effects on testing. Preanalytic variables pertinent to routine coagulation testing can be classified into three major categories: specimen collection, specimen transportation and storage, and specimen processing. Variables associated with specimen collection alone are numerous. Some have little to no effect; others significantly affect assay results. Few studies document the influence of preanalytic variables. Such studies are difficult to reference; many have been published in nonpeer-reviewed publications such as manufacturer’s technical bulletins, “throw away journals,” or as Letters to the Editor.1-3 Many of these studies were performed 20 to 30 years ago. Since then, a number of changes have been made to reagents, instruments, and evacuated tube systems.

In the United States, the majority of laboratories use an evacuated tube collection system rather than a syringe for routine blood collection.4 A variety of vacuum tubes are marketed for routine coagulation testing. Vacuum tubes may differ in citrate concentration (3.2% or 3.8%), type of stopper (standard rubber stopper, foil stopper, or plastic-capped stopper), tube composition (plastic or glass), and lubrication and silicongization. Vacuum tubes may also vary based on proprietary differences among manufacturers. The effects of these differences in evacuated tube systems on routine coagulation testing is unappreciated and largely unknown in most clinical laboratories. In our laboratories, we are in the process of systematically studying a number of these variables related specifically to evacuated tube systems to determine their clinical importance on routine coagulation testing.

We evaluated the effect of the two commonly available sodium citrate concentrations on routine coagulation assays (ie, prothrombin time [PT] and activated partial thromboplastin time [aPTT]). Both 3.2% (109 mmol/L) and 3.8% (129 mmol/L) citrate
concentrations are acceptable for routine coagulation testing, according to guidelines established by the National Committee for Clinical Laboratory Standards (NCCLS).\(^5\) The NCCLS does not state whether either citrate concentration is preferred. We studied the variability and interchangeability of 3.2% and 3.8% citrate tubes on five populations: healthy volunteers; hospitalized patients not receiving anticoagulant therapy, receiving intravenous (IV) heparin therapy, or receiving both IV heparin and oral anticoagulant therapy; and outpatients receiving oral anticoagulant therapy. To determine whether reagent sensitivity is a significant factor, two PT and aPTT reagents were used; one set was responsive, and the other was relatively nonresponsive.

**MATERIALS AND METHODS**

After informed consent was obtained, blood was collected by means of standard venipuncture. Three milliliters to 10 mL of blood was drawn into a standard red-stoppered tube. Then two Vacutainer glass tubes (Becton Dickinson, San Jose, Calif) of blood were drawn, one with 3.2% citrate and the other with 3.8% citrate. The order in which the two citrate tubes were filled was randomized. Plasma was centrifuged at 2,500g for 15 minutes at room temperature (platelet count <10,000/L). Samples were maintained at room temperature and were evaluated within 2 hours of venipuncture. Samples were tested against two reagent sets: (1) PT: Innovin (International Sensitivity Index [ISI] = 1.0) and Thromboplastin C+ (ISI = 1.94); and (2) aPTT: Actin FS (Factor Sensitive) and Actin. (All reagents were obtained from Dade, Miami) All assays were performed with a model 850, 900, or 1000 instrument (Medical Laboratory Automation, Pleasantville, NY). Each reagent was used according to the manufacturer’s instructions. Both citrate samples from each patient were evaluated with the same instrument in random order. Both aPTT and PT assays were performed in duplicate, and the results were averaged.

Five populations were included in the study. Blood from 20 healthy volunteers, 13 randomly chosen hospitalized patients not receiving anticoagulant therapy, 24 patients receiving IV heparin therapy, and 10 patients receiving both IV heparin and oral anticoagulant therapy was tested with all four reagents and both citrate concentrations. In addition, blood from 33 outpatients receiving stable oral anticoagulant therapy was tested with Innovin and Actin FS, and blood from 78 patients was tested with Thromboplastin C+.

Results were evaluated with the paired t-test (Sigma Stat; Jandel Scientific, San Raphael, Calif). If normalcy failed, the Wilcoxon signed rank test or Mann-Whitney rank sum test was used. Significance was reached at \(P<0.05\). Presence of outlying values was evaluated during plotting of values. Normal ranges were determined in the usual manner by establishing the mean ± 2 SD. Delta values for INR, PT, and aPTT were derived by determining the arithmetic difference between results either in seconds or International Normalized Ratio (INR) units.

**RESULTS**

The normal ranges for both PT and aPTT are shown in Table 1 for the four reagent sets and the two citrate tubes. A statistical difference is noted between the responsive reagents (Innovin and Actin FS) and the two citrate concentrations, whereas no difference is found with the nonresponsive reagents (Thromboplastin C+ and Actin). The normal ranges shift higher when samples are drawn into 3.8% citrate compared with 3.2% citrate with Actin FS and Innovin. It is surprising that the nonresponsive reagents demonstrated slightly higher mean normal values for PT and aPTT testing when drawn into 3.2% citrate compared with 3.8% citrate. Less variation in the normal ranges is noted between the citrate concentrations with the nonresponsive reagents.

The PT results for the four treatment groups are shown in Table 2. Samples drawn into 3.8% citrate displayed a consistently higher mean PT value with Innovin. In contrast, Thromboplastin C+ produced less variation in results between the citrate concentrations, and the higher citrate concentration did not yield consistently higher PT values. Samples assayed with Innovin demonstrated a statistical difference between citrate concentrations. However, this is not a consistent finding with the nonresponsive PT reagent.

**TABLE 1. NORMAL RANGE FOR EACH CITRATE CONCENTRATION**

<table>
<thead>
<tr>
<th>Citrate</th>
<th>Actin FS aPTT (s)</th>
<th>Innovin PT (s)</th>
<th>Actin aPTT (s)</th>
<th>Thromboplastin C+ PT (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.2%</td>
<td>21.6-31.4</td>
<td>8.6-10.7</td>
<td>23.0-33.0</td>
<td>11.8-14.5</td>
</tr>
<tr>
<td>3.8%</td>
<td>23.8-33.0</td>
<td>9.2-11.4</td>
<td>21.8-31.4</td>
<td>11.5-14.3</td>
</tr>
<tr>
<td>(P)</td>
<td>&lt;.001*</td>
<td>&lt;.001*</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Statistically significant.
NS = not significant.
TABLE 2. COMPARISON OF PROTHROMBIN TIME BETWEEN CITRATE CONCENTRATIONS

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Innovin</th>
<th>Thromboplastin C+</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.2%</td>
<td>3.8%</td>
</tr>
<tr>
<td>No anticoagulant</td>
<td>11.2 ± 2.5</td>
<td>11.8 ± 2.3</td>
</tr>
<tr>
<td>IV heparin</td>
<td>16.4 ± 8.3</td>
<td>17.8 ± 9.9</td>
</tr>
<tr>
<td>IV heparin + PO anticoagulant</td>
<td>25.1 ± 28.0</td>
<td>26.3 ± 28.0</td>
</tr>
<tr>
<td>PO anticoagulant</td>
<td>27.6 ± 13.0</td>
<td>34.3 ± 17.0</td>
</tr>
</tbody>
</table>

All values represent mean ± SD.
*Statistically significant.

TABLE 3. COMPARISON OF ACTIVATED PARTIAL THROMBOPLASTIN TIME BETWEEN CITRATE CONCENTRATIONS

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Actin FS</th>
<th>Actin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.2%</td>
<td>3.8%</td>
</tr>
<tr>
<td>No anticoagulant</td>
<td>28.2 ± 4.0</td>
<td>30.3 ± 3.5</td>
</tr>
<tr>
<td>IV heparin</td>
<td>44.0 ± 11.0</td>
<td>48.6 ± 14.0</td>
</tr>
<tr>
<td>IV heparin + PO anticoagulant</td>
<td>65.5 ± 16.0</td>
<td>69.0 ± 14.0</td>
</tr>
<tr>
<td>PO anticoagulant</td>
<td>40.2 ± 9.7</td>
<td>44.1 ± 13.0</td>
</tr>
</tbody>
</table>

All values represent mean ± SD.
*Statistically significant.

The 3.8% concentration produced consistently longer aPTT values with Actin FS. The 3.8% concentration did not give consistently longer values with Actin reagent. Statistical testing revealed a significant difference between samples drawn into 3.2% citrate compared with 3.8% citrate when samples from the four treatment groups were assayed with the more responsive reagent.

For aPTT results, the greatest change was observed in patients receiving IV heparin therapy. aPTT values varied significantly with both aPTT reagents in this treatment group (Fig 1). Five of 22 heparinized samples had a greater than 7-second difference between the two citrate concentrations with Actin FS. In contrast, 14 of 22 samples had a less than 2-second change with Actin (see Fig 1).

In patients receiving stable oral anticoagulant therapy, a significant change in INR values between citrate concentrations was noted with Innovin (Fig 2). Six of 33 samples demonstrated a change of more than 0.7 INR units. However, less variation in INR values was found with Thromboplastin C+ (Fig 3). Ninety-five percent of these samples demonstrated a change of less than 0.5 INR units.

DISCUSSION

Sodium citrate is the anticoagulant of choice for coagulation testing and is used by nearly all laboratories in the United States. According to the NCCLS
guidelines, either 3.2% (109 mmol/L) or 3.8% (129 mmol/L) citrate is acceptable for coagulation testing. Laboratories enrolled in the College of American Pathologists Comprehensive Coagulation Proficiency Testing Survey were recently polled, and of the 942 respondents, approximately 21% of laboratories use 3.2% sodium citrate and 79% use 3.8% sodium citrate. In contrast, the majority of European laboratories use 3.2% citrate.

The concentration of citrate used as an anticoagulant can vary clotting time because the amount of citrate present directly affects the calcium concentration. Either 3.2% or 3.8% citrate binds all of the patient calcium present in the test tube. However, the different citrate concentrations alter the amount of added calcium available for clotting in the assay mixture. A higher citrate concentration binds more assay-added calcium, making less calcium available to promote clot formation. Therefore, PT and aPTT typically are longer in 3.8% citrate tubes than in 3.2% tubes. This was demonstrated consistently in our study when responsive reagents were used. With a nonresponsive reagent, 3.8% citrate did not yield consistently longer results, which emphasizes that the interplay of different variables may create unexpected outcomes.

Citrate concentration may also alter hematocrit, because citrate concentrations above 3.0% are hyperosmolar with blood. Ingram and Hills reported that as citrate concentration rises, a fairly uniform decrease in hematocrit (0.2% per 0.1 mol/L increase in anticoagulant) occurs. Therefore a higher citrate concentration would have a greater effect on clotting times with use of an under-filled blue-stoppered tube (<90% filled) or a sample with a significantly abnormal (reduced or elevated) hematocrit.

Our data demonstrate that results of routine coagulation tests depend on the concentration of citrate used as an anticoagulant. The most consistent and greatest effect is noted in patients receiving anticoagulation therapy who are tested with responsive reagents. In patients receiving stable oral anticoagulant therapy, PT and INR results differ significantly when a responsive reagent is used (see Table 1). In general, use of a responsive PT reagent yields higher INR values when samples are drawn into 3.8% citrate. Twenty-seven percent of samples varied from 0.7 to 2.7 INR units between the two citrate concentrations. Only 15% of samples showed no change in INR, whereas 58% of samples varied from 0.1 to 0.6 INR units (see Fig 2).

The effect of citrate concentration on INR values is evident also when samples are drawn into one citrate concentration and the INR is calculated using a mean based on the second citrate concentration. This can occur, for example, when a laboratory that routinely uses 3.2% citrate receives a sample for monitoring oral anticoagulant therapy that was drawn into 3.8% citrate, or vice versa. With a responsive reagent, 48% of samples deviate by more than 0.5 INR units when drawn into 3.8% citrate and the INR is calculated from a 3.2% PT mean. In comparison, when blood is drawn into 3.2% citrate and INR is calculated from the 3.8% mean, 45% of samples vary by more than 0.5
INR units (see Fig 2). This difference is not so striking when a less responsive PT reagent is used. In 50% of patients receiving stable oral anticoagulant therapy, INR values did not show any difference when samples were drawn into different concentrations of citrate. Of the samples that did show change, only 5% differed by more than 0.5 INR units (see Fig 2).

In hospitalized patients not receiving anticoagulation therapy, little clinically significant variation in PT results was noted with the two citrate concentrations (see Table 2). This suggests that the effect of citrate concentration on PT results may not be important when factor levels are in the normal range.

Effects of sodium citrate concentration on aPTT were statistically significant in healthy volunteers and in the four patient treatment groups when a responsive reagent was used (see Table 3). A nonresponsive reagent yielded statistical significance between citrate concentrations only in patients receiving IV heparin therapy.

Many recent publications have reported variations in INR values between laboratories.10-12 The various reasons for differences in INR values cited include biologic variation, ISI determination and standardization, instrument variation, and proper use of the equation to determine INR. Only one other study, by Duncan et al,13 examined the effect of citrate concentration on INR. Their study showed that citrate concentration is an important variable in the calculation of INR. First, patients receiving oral anticoagulant therapy had significantly higher INR values in samples drawn into a 3.8% citrate tube compared with a 3.2% citrate tube. Second, the effect of citrate concentration was reagent dependent, and less responsive reagents had less effect on INR. Third, ISI values were determined using different citrate concentrations and demonstrated a 10% difference between samples. We also demonstrated significantly higher INR values with 3.8% citrate compared with 3.2% citrate, which is reagent dependent. Although we did not study the effect of citrate concentration on ISI determination, we concur with Duncan and coworkers that laboratories should collect blood with the same citrate concentration used to calibrate the reagent ISI used in their laboratory.

Many laboratory staff may not be aware that two citrate concentrations are routinely available in standard blood collection tubes and that citrate concentration can alter PT and aPTT results. Part of the confusion is related to the fact that manufacturers do not record citrate concentration on the collection tubes. Some laboratories may unknowingly stock 5.0 mL vacuum tubes with 3.8% citrate concentration and 3.0 mL pediatric tubes with 3.2% citrate concentration. A similar situation can occur in laboratories that test samples drawn outside their facility. The normal and therapeutic ranges are established with one citrate concentration, but samples from other facilities may have been drawn into a different citrate concentration. Inasmuch as two citrate concentrations are currently offered for coagulation testing, it is important that manufacturers distinguish collection tubes as containing 3.2% or 3.8% citrate. This can be accomplished by using different colored stoppers, marking the glass tubes, or indicating the citrate concentration on the label.

The effect of citrate concentration needs to be considered in reference laboratories that perform tests for a variety of hospitals, because many special coagulation assays are based on clotting assays. The aPTT is the basis for most factor assays, most plasma mixing studies, protein C and protein S activity assays, and a variety of lupus anticoagulant assays (eg, platelet neutralization, hexagonal phospholipid assay). Until a uniform citrate concentration is used, special coagulation laboratories should state which citrate concentration their reference ranges are based on and ask that all plasma samples submitted use the same citrate concentration. If this is not feasible, normal ranges for both citrate concentrations should be determined for each assay. This could create major logistic and expense problems for these laboratories. In addition, variation in citrate concentration may cause inconsistencies in proficiency testing and interlaboratory comparison studies.

A single concentration of sodium citrate should be used within a laboratory, and ideally, nationally. Adoption of a uniform citrate concentration will become increasingly important as laboratories use more responsive PT reagents with ISI closer to 1.0. We recommend that the NCCLS advocate only one anticoagulant and one concentration, specifically, 3.2% (109 mmol/L) sodium citrate. This concentration is consistent with the recommendation of the International Society for Thrombosis and Hemostasis Expert Panel and is the current standard in Europe. Using an anticoagulant with an osmolarity closer to plasma, such as 3.2% citrate, potentially could decrease the variability in clotting times related to variances in hematocrit and filling volume.

Until a standard citrate concentration is adopted, laboratory staff should become more aware of this difference and (1) make certain that all blue-stoppered tubes used in their institutions are of the same citrate concentration, whether samples are drawn within one facility or sent from another site; (2) make certain that the normal range is established using the same citrate concentration as the standard collection tubes; (3) use the same
citrate concentration that the reagent ISI was determined from; and (4) use the same citrate concentrate as the special coagulation reference laboratory does.

In conclusion, the concentration of citrate used as an anticoagulant for routine coagulation testing can significantly affect clinical results of PT and aPTT testing, especially if responsive reagents are used. In an effort to standardize testing and provide more precise, accurate clinical information, the sodium citrate concentration used should be consistent within a laboratory. A concentration of 3.2% citrate is the recommended anticoagulant for coagulation testing and should be adopted worldwide.

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REFERENCES