Do Elevated Hematocrits Prolong the PT/aPTT?

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ABSTRACT

The Clinical and Laboratory Standards Institute guidelines require special processing of whole blood specimens with hematocrits greater than 55% due to the possibility of spurious prolongation of routine coagulation studies (PT, aPTT). As samples with hematocrits above 60% are rare at our institution, our study seeks to determine the effect of relative citrate excess on routine coagulation studies in samples with hematocrits of 60% to determine whether special processing is necessary. A calculated volume of 3.2% citrate was added to 1 mL aliquots of 40 whole blood samples in citrated tubes from adult patients to simulate a hematocrit of 60%. A dilutional control was created by adding an equivalent volume of saline to a separate 1 mL aliquot. Routine coagulation studies (PT, aPTT) were run on both samples on the STA Compact Analyzer in accordance with manufacturer instructions. While a paired Student's *t*-test demonstrated a clinically significant change in both PT and aPTT with the addition of citrate ($p = 0.0002$ for PT and $p = 0.0234$ for aPTT), clinical management would not have been altered by any observed change. More interestingly, we observed a shortening of 27/40 PTs and 23/40 aPTTs rather than the expected prolongation. Based on our data, no adjustment of citrate volume appears to be necessary in samples with hematocrits less than or equal to 60%.

ABBREVIATIONS: CLSI - Clinical and Laboratory Standards Institute, PT - prothrombin time, aPTT - activated partial thromboplastin time , CBC - Complete Blood Count

INDEX TERMS: Prothrombin Time, Partial Thromboplastin Time, Hematocrit, Blood Coagulation Tests, Citrate

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INTRODUCTION

The Clinical and Laboratory Standards Institute (CLSI) guidelines require special processing of whole blood specimens with hematocrits greater than 55% due to the possibility of spurious prolongation of routine coagulation studies.[1] As sodium citrate only equilibrates within the plasma component, samples with an elevated hematocrit will have a relative excess of citrate anticoagulant in relation to plasma volume.[2,3,4] Excess citrate will in turn bind calcium added to initiate the clotting process and slow its initiation, leading to prolonged prothombin times (PT) and activated partial thromboplastin times (aPTT).[4,5]

In cases where polycythemia is chronic and stable, these patients may be pre-identified by nursing and phlebotomy staff and appropriate modification of the citrate volume in the empty blood tube can be accomplished prior to phlebotomy. However, there are many instances (e.g. markedly dehydrated patients) where the polycythemia is acute and transient and therefore impossible to identify prior to performance of a complete blood count (CBC). Repeat phlebotomy may be difficult or impossible, particularly in the outpatient or Emergency Department setting, with potential downstream effects including cancellation of procedures or surgery due to the inability to interpret results with confidence.

Previous studies have demonstrated that relative citrate

excess as a result of either polycythemia or short draws results in spuriously prolonged PT and aPTT results;[2-8] however, it is important to note that some of the original studies were performed with 3.8% citrate[2,5,6] rather than the 3.2% citrate[2,3,6,7] that is currently the standard while other studies evaluated samples with hematocrits up to 72%. As hematocrits greater than 60% are rare at our institution in the absence of known, chronic polycythemia, we sought to determine the effect of relative citrate excess on routine coagulation studies (PT and aPTT) in samples with hematocrits of 60% and to determine whether clinical management would be altered by any observed change.

MATERIALS AND METHODS

Materials

Forty whole blood samples in standard 3-mL plastic tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ) containing 0.3 mL of 3.2% buffered sodium citrate (final citrate/whole blood ratio of 1:9) with hematocrits ranging from 21-46% were selected at random from existing specimens submitted for routine coagulation studies. Two 1-mL aliquots were removed from each tube and transferred to empty 12x75 mm polypropylene tubes. A sufficient volume of 3.2% buffered sodium citrate was added to one of each pair of 1-mL aliquots to simulate a hematocrit of 60% (final citrate/plasma ratio of 1:3.6). An equivalent volume of 0.9% sodium chloride was added to the second 1-mL aliquot as a dilutional control. Both samples were centrifuged at 3,600g for 2 minutes, and the plasma was removed and maintained at room temperature prior to determination of the PT and aPTT.

Methods

Hematocrit values were determined on concurrently drawn EDTA samples using a Sysmex XE 2100 (Sysmex America, Inc, Mundelein, IL) according to the manufacturer's instructions. The PT and aPTT values (see Table 1 for reference ranges and assay variance) were determined on the STA Compact Analyzer (Diagnostica Stago, Asnieres, France) using the Neoplastin CI$^+$ and PTT Automate reagents, respectively (Diagnostica Stago, Asnieres, France); all assays were performed according to the manufacturer's package inserts. The unadjusted, citrate-adjusted and saline-diluted aliquots for each sample were analyzed simultaneously. The change in PT and aPTT between the citrate-adjusted and unadjusted samples was calculated in seconds.

Studies on human subjects were carried out according to the principles of the Declaration of Helsinki. All samples were anonymized. The study was classified as a Nonhuman Subjects Protocol under the category of Use of Non-Identifiable Biological Specimens by the University of Washington (Seattle) Human Subjects Review Committee.

Data Analysis

Statistical significance was determined using a two-tailed paired Student's *t*-test with $p<0.05$ chosen as the cutoff for statistical significance, and linear regression was used to evaluate the correlation between citrate-adjusted and citrate-unadjusted PT and aPTT values (Microsoft Excel, Redmond, WA). Change in PT and aPTT was further evaluated from the perspective of the reference range and institutional clinical guidelines for PT and aPTT to determine whether clinical workup or management would be altered by any observed change.

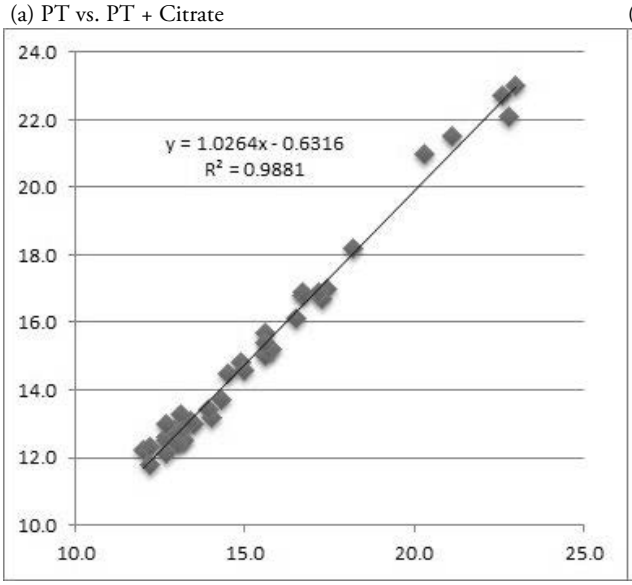
RESULTS

The PT and aPTT were compared for the citrate-adjusted and unadjusted samples, and overall results are presented in Table 2. The change in PT with the addition of citrate ranged from a shortening of 0.8 seconds to a prolongation of 0.7 seconds. The change in aPTT with the addition of citrate ranged from a shortening of 10 seconds to a prolongation of 8.5 seconds. On average, the PT was shortened by 1.6% and the aPTT was shortened by 2.7% with the addition of citrate. The PT and aPTT of the citrate-adjusted and unadjusted samples correlated well as shown in Figures 1 and 2 with PT values correlating better than aPTT. Both the change in PT and aPTT were statistically significant with p values and average changes in PT and aPTT shown in Table 1. Three of forty samples demonstrated a change that crossed the upper limit of the reference range for PT and four of forty samples demonstrated a similar change in aPTT and required further evaluation for any clinical significance.

DISCUSSION

While a statistically significant change in both PT and aPTT was observed with the addition of sufficient citrate to simulate a hematocrit of 60%, the actual average change was a shortening in both PT and aPTT, and the majority of samples demonstrated a shortening

(a) PT vs. PT + Citrate



(b) Change in PT with Addition of Citrate

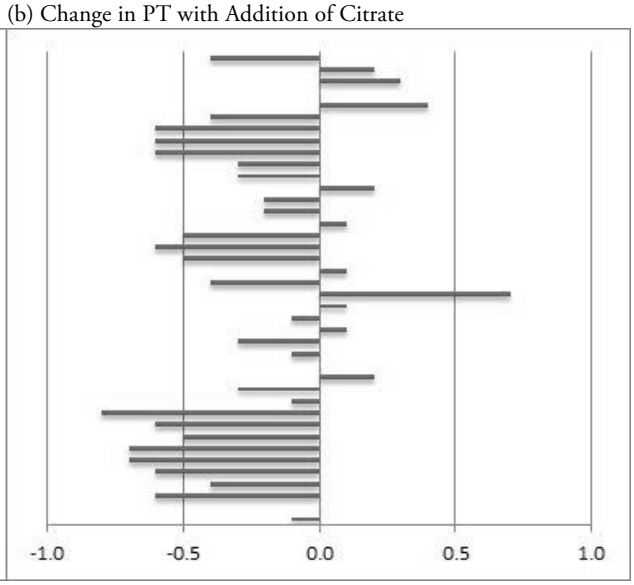
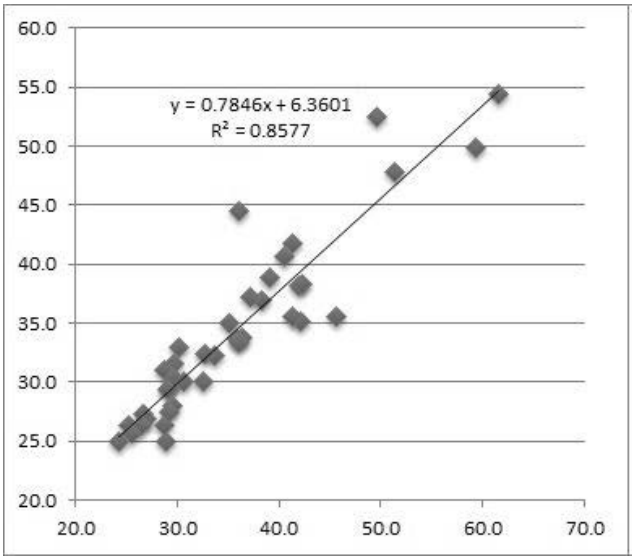


Figure 1. There was excellent correlation between PT and PT + Citrate (a), and the majority of PTs were shortened with addition of citrate (b).

(a) PTT vs. PTT + Citrate



(b) Change in PTT with Addition of Citrate

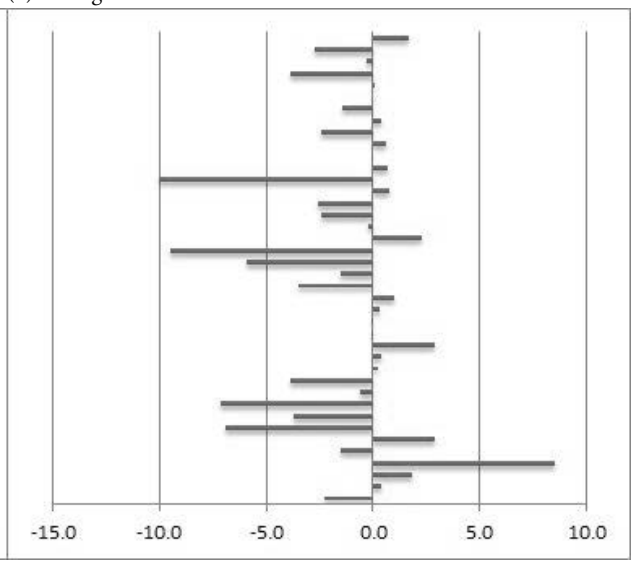


Figure 2. The correlation coefficient between aPTT and aPTT + Citrate is 0.8577 (a), and the majority of aPTTs were shortened with addition of citrate (b).

in PT and aPTT rather than the prolongation expected based on results of previous studies.[2-7] Given the small variance of both assays, it is not surprising that a minor change was deemed statistically significant. Many of the observed changes were within the accepted inter-assay variance for both PT and aPTT at our institution, so this is one potential explanation for the observed change. As demonstrated in Table 1 and Figure 3, the PT and aPTT demonstrated an average prolongation with addition of saline, effectively ruling out a dilutional effect as the cause of the changes observed with addition of citrate.

The most recent publication on the effects of elevated hematocrit on coagulation studies set clinical significance/difference as a 10% change in the PT or aPTT, and the majority of samples they evaluated had hematocrits >60%.[3] We chose to evaluate the clinical

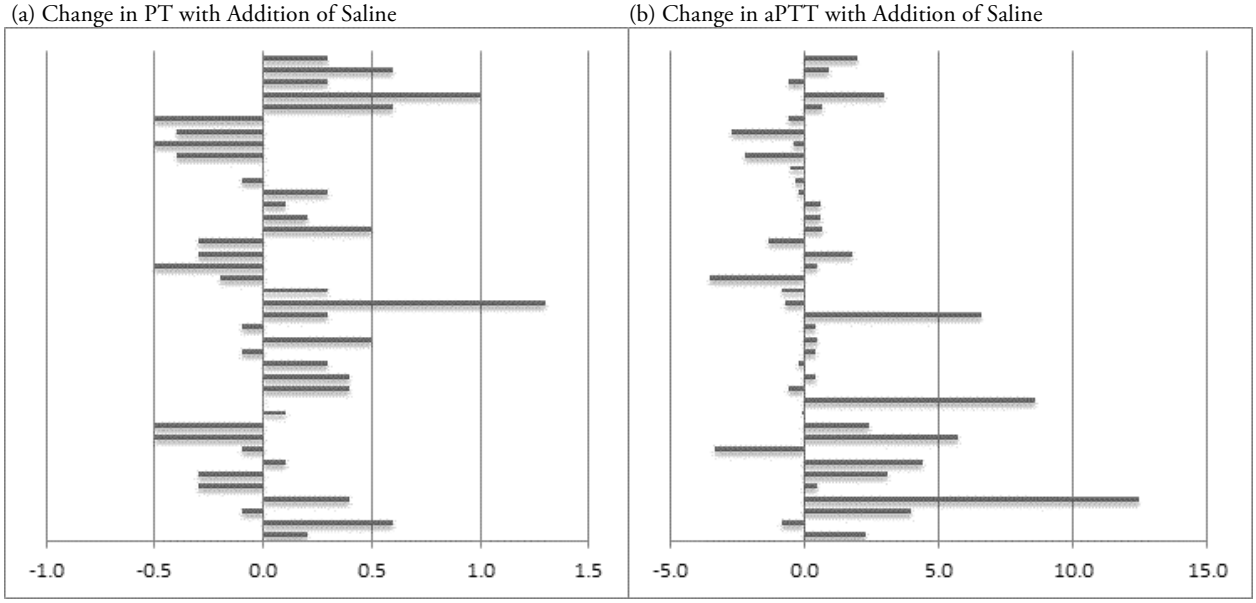


Figure 3. In contrast to the effect seen with addition of citrate, the majority of samples demonstrated a prolongation in PT (a) and aPTT (b) with addition of saline.

Table 1. PT and aPTT Summary Data

	PT	PTT
Average Unadjusted (secs)	15.3	35.4
Average with Citrate Excess (secs)	15.1	34.2
Average Change with Citrate Excess (secs)	-0.2	-1.3
Paired T-test (two tailed)	p = 0.0002	p = 0.0234
Average with Saline (secs)	15.4	36.5
Average Change with Saline (secs)	+0.1	+1.1
Paired T-test (two tailed)	p = 0.1898	p = <0.0001
Reference Range (secs)	10.7-15.6	22-35
Accepted Inter-Assay Variance (secs)	0.4	1.1

implications of any observed change in PT or aPTT based on current guidelines for the stratification and evaluation of patients based on routine coagulation studies in use at our institution. Three of the 40 samples had a change in PT with addition of citrate sufficient to potentially change the clinical interpretation (gray highlighted in Table 2). In each case, the PT of the unadjusted sample was within 0.2 seconds of the upper limit of the reference range. In two samples, the PT shortened with the addition of citrate (15.8 to 15.2 and 15.7 to 15.2 seconds respectively); in the third case, the PT was prolonged to just above the upper limit of the reference range (15.6 to 15.7 seconds). However, since the INR is the primary means by which patients are stratified at our institution, the clinical interpretation would not have been changed because the INR of both the unadjusted and citrate-adjusted samples was normal.

Four different samples also had a change in aPTT with addition of citrate sufficient to cross the upper limit of the reference range; in each case, a shortening of the aPTT was observed (36 to 34 seconds, 42 to 35 seconds, 36 to 34 seconds, and 36 to 33 seconds) (gray highlighted in Table 2). As the clinical standard for evaluation of a marginally elevated aPTT is to repeat the assay, and each of these patients subsequently had a normal aPTT study drawn within close proximity to this sample, the elevated unadjusted aPTTs were values that would have been unlikely to result in a substantial clinical workup on their own. The normal aPTT values seen in their citrate-adjusted counterparts would therefore have been unlikely to change ultimate clinical management had they been viewed in isolation.

CONCLUSIONS

While it is clear from previous data[2-5] that adjustment of citrate concentration in samples with markedly elevated hematocrits is necessary, our data suggest that routine coagulation studies from samples with hematocrits up to 60% may be safely performed and interpreted with confidence. It is important to note, however, that due to variance in phlebotomy technique, handling, processing, and coagulation instrumentation and reagents, each institution should independently validate our findings within their patient population.

Table 2. PT and aPTT Results

Original Hct (%)	PT (secs)	PT with Citrate Excess (secs)	Change (secs)	Clinically Significant	PTT (secs)	PTT with Citrate Excess (secs)	Change (secs)	Clinically Significant
37	14.9	14.8	-0.1	No	35.9	33.6	-2.3	No
38	23.0	23.0	0.0	No	41.3	41.7	0.4	No
25	15.8	15.2	-0.6	No	29.7	31.5	1.8	No
29	17.4	17.0	-0.4	No	36.0	44.5	8.5	No
43	13.1	12.5	-0.6	No	29.5	28.0	-1.5	No
34	13.2	12.5	-0.7	No	30.1	33.0	2.9	No
25	22.8	22.1	-0.7	No	42.1	35.2	-6.9	No
46	13.5	13.0	-0.5	No	41.9	38.2	-3.7	No
34	17.3	16.7	-0.6	No	61.5	54.4	-7.1	No
27	14.0	13.2	-0.8	No	30.7	30.1	-0.6	No
21	13.1	13.0	-0.1	No	28.9	25.0	-3.9	No
22	17.2	16.9	-0.3	No	40.5	40.7	0.2	No
37	12.0	12.2	0.2	No	29.6	30.0	0.4	No
24	14.5	14.5	0.0	No	49.6	52.5	2.9	No
44	12.7	12.5	-0.2	No	35.1	35.0	-0.1	No
39	13.4	13.1	-0.3	No	27.0	26.9	-0.1	No
34	12.2	12.3	0.1	No	29.2	29.5	0.3	No
44	13.0	12.9	-0.1	No	25.3	26.3	1.0	No
36	15.6	15.7	0.1	No	51.3	47.8	-3.5	No
35	20.3	21.0	0.7	No	33.7	32.2	-1.5	No
24	16.5	16.1	-0.4	No	41.4	35.5	-5.9	No
37	16.7	16.8	0.1	No	59.3	49.8	-9.5	No
41	13.9	13.4	-0.5	No	28.7	31.0	2.3	No
29	15.6	15.0	-0.6	No	39.1	38.9	-0.2	No
25	15.7	15.2	-0.5	No	32.5	30.1	-2.4	No
42	22.6	22.7	0.1	No	36.4	33.8	-2.6	No
21	12.7	12.5	-0.2	No	24.2	25.0	0.8	No
30	15.6	15.4	-0.2	No	45.6	35.6	-10.0	No
45	13.1	13.3	0.2	No	26.6	27.3	0.7	No
36	13.1	12.8	-0.3	No	26.5	26.4	-0.1	No
41	13.0	12.7	-0.3	No	29.7	30.3	0.6	No
43	12.7	12.1	-0.6	No	28.8	26.4	-2.4	No
36	14.3	13.7	-0.6	No	29.0	29.4	0.4	No
41	13.0	12.4	-0.6	No	38.3	36.9	-1.4	No
46	12.2	11.8	-0.4	No	25.6	25.6	0.0	No
37	21.1	21.5	0.4	No	37.1	37.2	0.1	No
25	18.2	18.2	0.0	No	42.2	38.3	-3.9	No
45	12.7	13.0	0.3	No	32.7	32.4	-0.3	No
28	16.7	16.9	0.2	No	36.0	33.3	-2.7	No
27	15.0	14.6	-0.4	No	29.2	27.5	-1.7	No

PT: Prothrombin Time, aPTT: activated Partial Thromboplastin Time, Hct: Hematocrit, nl: normal, INR: International Normalized Ratio

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