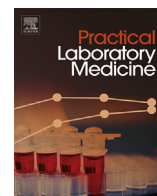




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Effect of anticoagulant adjustment on prothrombin time test using two different PT reagents in patients with elevated hematocrit

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ABSTRACT

The recommendations for adjustment of citrate volume in sample tubes with high hematocrit (Ht) are based on indirect studies of underfilled tubes or artificially constructed Ht values. The aim of this study was to evaluate the effect of citrate volume adjustment in sample tubes from patients with hematocrit >55% using two different prothrombin time (PT) tests.

Methods: Paired citrate-adjusted and unadjusted blood specimens were obtained from 181 patients from the pulmonary hypertension ambulatory with high Ht values and on warfarin therapy. The samples were tested using recombinant human tissue factor (RTF) and reagents extracted from rabbit brain (HS Plus). The results are expressed as the international normalized ratio (INR). The correlation and percent change (% change) between sample pairs were calculated.

Results: INR-RTF results from adjusted and unadjusted citrate blood specimens showed a strong correlation ($R^2 = 0.8226$, $p < 0.0001$). The INR median was 2.25 (95% CI 2.10 to 2.41) for citrate-adjusted samples and was 2.22 (95% CI 2.06 to 2.38) for citrate-unadjusted samples. For samples with Ht >62%, the % change between sample pairs was >10%. Results using HS Plus showed a moderate correlation between citrate-adjusted and unadjusted samples ($R^2 = 0.4267$, $p < 0.0001$). The INR median was 2.51 (95% CI 2.35 to 2.68) for citrate-adjusted samples and 3.45 (95% CI 3.11 to 3.80) for citrate-unadjusted samples. For samples with Ht >55%, the % change between sample pairs was higher than 10%.

Conclusion: Our data demonstrate that in patients with polycythemia on warfarin therapy, INR-RTF does not require anticoagulant adjustment for assessment of samples with Ht <62%.

1. Introduction

Preanalytical variables may affect the results of routine coagulation assays [1]. One important variable is hematocrit (Ht), which affects coagulation testing results. A sample with high Ht has lower plasma quantity, resulting in a dilution effect on the sample by an excess of the anticoagulant sodium citrate [2]. This excess of citrate binds a significant amount of the calcium added to the test and increases the clotting time, as evidenced by the prothrombin time (PT)/international normalized ratio (INR) tests whose results are altered [3].

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The PT/INR test is the assay used to monitor dose-adjustments of vitamin K antagonist (VKA) treatment [4]. Thus, for patients on monitored oral anticoagulant therapy who present an elevated Ht, adjustment of sodium citrate volume is essential [5]. Otherwise, these patients would have an incorrect diagnosis or treatment.

The Clinical and Laboratory Standards Institute (CLSI) [6] guidelines and reviews [1,7,8] of preanalytical variables recommend adjustments in the ratio of anticoagulant solution/blood volume when Ht values are above 55%. Nevertheless, these recommendations are based on indirect studies of under filled tubes or artificially constructed Ht values [2,9–11].

Furthermore, none of these studies have investigated different PT tests with different thromboplastin sensitivity, which is equally as important as elevated Ht for the PT/INR test [5]. Thromboplastin variability explains the differences in results obtained for plasma from the same patient when tested at different laboratories [12]. Therefore, it is important to validate the need for citrate adjustment in this setting.

The aim of this study was to evaluate the effect of citrate volume adjustment in patients with hematocrit >55% using two different PT tests.

2. Materials and Methods

The Ethics Committee of the Heart Institute of the University of São Paulo approved the study, and written informed consent was obtained from all participants.

In this study, 181 patients from our pulmonary hypertension ambulatory with elevated Ht (>55%) and on warfarin therapy were enrolled. All patients were diagnosed with polycythemia due to pulmonary hypertension and received anticoagulation treatment at the anticoagulation ambulatory center.

For each patient, one EDTA tube (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ) of blood was collected for Ht value determination using a Sysmex XE 2100 (Sysmex America, Inc, Mundelein, IL) according to the manufacturer's instructions.

For patients with Ht >55%, two citrate tube (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ) blood samples were collected (1 adjusted and 1 unadjusted volume of 3.2% sodium citrate) by standard venipuncture. Platelet-poor plasma was obtained by centrifugation performed at room temperature at $1500 \times g$ for 15 min.

The PT test was performed using two different thromboplastin reagents that are routinely used in our laboratory: the first was the HemosIL RecombiPlasTin 2G, with a thromboplastin (ISI 1.00) of lyophilized recombinant human tissue factor (RTF), and the second reagent was HemosIL PT-Fibrinogen HS Plus with a thromboplastin (ISI 1.24) lyophilized from rabbit brain extract (HS Plus). Both reagents were produced by Instrumentation Laboratories (Lexington, MA, USA) and kindly provided by Werfen Medical (Barcelona, Spain). The ISI was calibrated for each thromboplastin against reference plasma that had been primarily calibrated against the international reference standard, in accordance with guidelines [13]. The adjusted and unadjusted samples were analyzed together in random order in the same laboratory using an automated coagulometer ACL TOP 500 from the same manufacturer.

2.1. Statistical analyses

The Kolmogorov-Smirnov normality test was used to analyze data normality. Data were expressed as the median and 95% confidence interval (95% CI). Comparison between groups was performed using the Wilcoxon test for nonparametric variables. The significance level adopted for the statistical tests was 5% ($p < 0.05$). Linear regression analyses were performed to determine the straight line that best describes the relationship between the adjusted plasma and the unadjusted plasma. Statistical analyses were performed using MedCalc Statistical Software version 14.12.0 (MedCalc Software bvba, Ostend, Belgium; 2014).

Each patient sample pair was evaluated by determining the percent change (% change) between the adjusted citrate sample (considered clinically correct) [3] and the unadjusted citrate sample. The % change in each sample pair was evaluated considering the allowable total error (TE) of 10% as defined for the PT test (Clinical Laboratory Improvement Amendments/CLIA) [14].

The % change was calculated for each sample pair using the following equation: $\% \text{ change} = (\text{INR}_{\text{adjusted}} - \text{INR}_{\text{unadjusted}}) / \text{INR}_{\text{adjusted}} \times 100\%$.

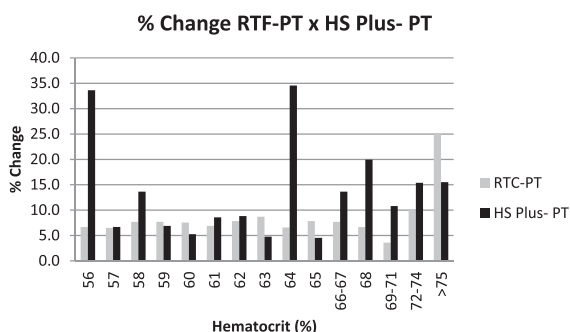


Fig. 1. Percentage difference between RTF-PT and HS Plus PT. The percentage difference is the change between the citrate-adjusted and unadjusted samples. See the “Materials and Methods” section for details.

Sample pair results were clinically accepted with a difference of less than 0.5 units of INR, a difference accepted according to CLSI-H57A [15].

3. Results

For the PT/INR test performed with the RTF reagent, the results from adjusted and unadjusted citrate did not differ significantly, with a median INR of 2.25 (95% CI: 2.10 to 2.41) for adjusted citrate and 2.22 (95% CI: 2.06 to 2.38) for unadjusted citrate, $p = 0.771$. The INR-RTF results from adjusted and unadjusted citrate blood specimens showed a strong correlation ($R^2 = 0.8226$, $p < 0.0001$).

Of the 181 patients, 158 (87%) presented sample pairs with a % change below the allowable TE (<10%). All the remaining patients (24) had a % change above 10% and Ht values above 62% (Fig. 1).

The INR of 7.7% of the 181 samples had a difference >0.5 INR units in comparing unadjusted and adjusted citrate in PT tests performed with RTF reagent. This difference of >0.5 INR units was observed only for Ht values above 62%.

For PT tests performed with HS Plus reagent, the median citrate-adjusted INR was 2.51 (95% CI: 2.35 to 2.68), and the median citrate-unadjusted INR was 3.45 (95% CI: 3.11 to 3.80), $p < 0.0001$. The results using HS Plus showed a moderate correlation between citrate-adjusted and unadjusted samples ($R^2 = 0.4267$, $p < 0.0001$).

In contrast to the RTF reagent, the HS Plus reagent identified 90 (49%) sample pairs whose % change was above the allowable TE, and these results were independent of Ht values. Considering the clinical significance of these differences, the HS Plus-thromboplastin presented discrepancies between sample pair results for Ht >55% (Fig. 1).

For fifty-five samples (30.4%), the INR presented a difference above 0.5 units for results from unadjusted and adjusted citrate in tests performed with HS Plus reagent, independent of Ht values.

4. Discussion

In the present study, all 181 patients on VKA anticoagulation therapy presented elevated Ht (>55%). For this group of patients, citrate correction is extremely important due to the narrow therapeutic index of warfarin and the high risk of bleeding or clotting if the dose is incorrect [5].

There are several articles in the literature with this recommendation, but most of them are reviews [1,6–9], with few research-based articles [3,16]. There is a specific recommendation for using citrate-adjusted tubes from CLSI that can be found in NCCLS document H21-A5 [6]. Additionally, it must be emphasized that since 2002, the College of American Pathologists Hematology checklist, HEM.22, 830, contains the following question: “Are there documented guidelines for detection and special handling of specimens with elevated hematocrit?” [17].

One of the first studies to address this issue was from Koepke et al. [10] in 1975. This study involved plasma at various concentrations to achieve different simulated hematocrit levels for testing. The use of citrate adjustment for specimens with elevated hematocrit was recommended to correct for false prolongation results.

In 1990, a study by Pai HS et al. [2] used samples that mimicked polycythemic patients, using insufficiently filled citrate tubes, to compare the PT test results in samples from both normal patients and those under warfarin treatment. According to the authors, the samples with altered hematocrit do not need citrate adjustment for the PT test. However, these results were obtained from a small number of samples, and there is no information on the PT reagent used.

In 2013, Austin M et al. [16] used thromboplastin extracted from rabbit brain to perform PT tests on samples with artificially high Ht, in which a sufficient volume of 3.2% buffered sodium citrate was added to aliquots to simulate a hematocrit of 60% (final citrate/plasma ratio of 1:3.6). The results of this analysis suggested that the PT test from samples with Ht up to 60% may be performed and interpreted with confidence. This result is in agreement with the RTF reagent data reported in our study, even with a different thromboplastin.

However, a study of the effects of elevated hematocrit on coagulation tests by Marlar et al. [3] concluded that the citrate concentration must be adjusted once Ht levels are >55%.

This study analyzed samples from 28 patients with high Ht values (heterogeneous group without warfarin therapy mention), using Recombiplastin PT reagent, the same thromboplastin source used in our study. Although this thromboplastin was extracted from recombinant tissue factor, the reagent constitution may be different.

Differences in PT results related to thromboplastin reagent sensitivity have been well recognized [5], and it is a misconception to assume that for an individual patient's plasma, the INR interassay results will always be identical [18]. Recombinant thromboplastins are manufactured using recombinant human tissue factor produced by *Escherichia coli*. These thromboplastins are highly sensitive to factor deficiencies and oral anticoagulant-treated patient plasma samples (with ISI close to 1.0) [19]. PT HS PLUS is a thromboplastin prepared from lyophilized rabbit brain extract with a higher ISI (1.24) and is less sensitive for anticoagulant-treated patient samples.

Furthermore, the PT test contains synthetic phospholipids, with different percentages of phosphatidylserine (PS), phosphatidylethanolamine (PE), and phosphatidylcholine (PC). These differences in the phospholipid percentage and other components could vary among the reagents and have not been characterized in detail by the manufacturer.

We did not investigate the reagent components of the kits that could be underlying the difference presented in the current study. Additionally, as we analyzed only two different PT reagents, our results are specific to them and to the instrument system. It will be important that each laboratory validate their PT reagents to establish the need for sodium citrate volume adjustment for the different percentages of hematocrit.

One limitation of our study was that we did not study other coagulation tests in these samples.

In conclusion, the adjustment of citrate concentration in samples with markedly elevated hematocrit depends on the PT reagent used

in the routine tests, and each laboratory must perform a validation assay. Our data suggest that with RecombiPlasTin 2G reagent, samples of patients with polycythemia on warfarin therapy with Ht up to 62% could be obtained in an unadjusted citrate concentration tube. Using this PT reagent, we could reduce the number of collection tubes and length of patient stay and avoid recall of patients, meaning lower treatment costs and faster patient care.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.plabm.2020.e00177>.

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