Comparison of Plasma With Whole Blood Prothrombin Time and Fibrinogen on the Same Instrument

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Key Words: Whole blood; Coagulation; Fibrinogen; Prothrombin time; Point of care; Hematocrit

Abstract

We compared plasma with whole blood (WB) international normalized ratio (INR) and fibrinogen using the same instrument and reagents. WBINRs were 50% higher than plasma INRs. After increasing the WB sample volume 40% and adjusting the International Sensitivity Index, WBINRs were similar to plasma INRs [adjusted WBINR = 0.99(plasma INR) – 0.02; \( r^2 = 0.98; n = 155 \)], but the average difference in WB vs plasma INR was 4-fold higher than duplicate plasma INRs. Variation in hematocrit was a major determinant of the accuracy of the WBINR, with increased error at high INRs. The WB fibrinogen assay was highly dependent on the sample hematocrit (\( r^2 = 0.83 \)), even after the sample volume was adjusted. Accurate WB fibrinogen measurements required a mathematical hematocrit correction. We conclude that WBINR and fibrinogen assays can be performed on point-of-care or automated analyzers, but sample volume must be adjusted to account for hematocrit. Accuracy is limited by variations in hematocrit with worsening accuracy for samples with high INRs or low fibrinogen levels.

Whole blood (WB) prothrombin time (PT) and international normalized ratio (INR) testing is used for outpatient monitoring of warfarin therapy and to reduce turnaround time. WB testing typically uses finger-stick or citrated WB specimens, whereas plasma INR, the reference method, uses citrated plasma specimens. Studies comparing WBINR with plasma INR generally show correlations of \( r^2 = 0.70 \) to \( r^2 = 0.96 \), but WBINR tends to be biased high, with the bias becoming worse as the INR rises.1,6-12

In contrast with WBINR testing, direct WB testing for individual coagulation factors is not widely used or widely available. Indirect WB fibrinogen viscoelastic assays have been devised based on the fact that the maximal amplitude is primarily a function of the platelet count and fibrinogen concentration in these assays.13,14 The maximum amplitude is most closely related to the fibrinogen concentration in the presence of specific fibrinogen activators (such as reptilase) or glycoprotein IIb/IIIa receptor antagonists that block platelet binding to fibrin.15-17 However, correlation between viscoelastic fibrinogen and plasma fibrinogen measurements is relatively weak (\( r^2 < 0.61 \)) with limited positive predictive value for low fibrinogen levels (<55%).15,17

Potential sources of inaccuracy between plasma and WB methods include thromboplastin sensitivity,12,18 differences in clot detection mechanisms,19 citrate,20 coagulation factor levels,9 technical expertise,21,22 intrinsic imprecision of WB methods, and hematocrit values.23 When point-of-care instruments are compared with automated analyzers, it is difficult to determine which source of inaccuracy between WB and plasma assays is most important because multiple differences are present in the same comparison. The purpose of our study was to evaluate the effect of hematocrit on WB coagulation testing, while eliminating other sources of variation. Hematocrit error...
applies to all WB assays and is, therefore, an important variable to understand. Our study compares WB and plasma PT and fibrinogen measurements using the same instruments, reagents, and technical expertise. This design allowed us to control for many preanalytic and analytic variables and determine the inherent performance limitations of WBINR and WB fibrinogen measurements. It also allowed us to assess the feasibility of performing WB coagulation testing on an automated coagulation analyzer.

Materials and Methods

Subjects and Samples

The study was approved by the University of Washington Human Subjects Review Committee (Seattle). Venous blood samples were anticoagulated by adding 2.7 mL of blood to 0.3 mL of 0.109 mol/L citrate. All samples were gently mixed manually and then split. The aliquot for plasma studies was centrifuged at 3,600g for 2 minutes at room temperature and analyzed.

PT and INR Measurements

All PT and INR measurements were made on Diagnostica Stago analyzers (STA-R Evolution and STA-Compact, Diagnostica Stago, Parsippany, NJ) using Neoplastine CI Plus reagents (Diagnostica Stago). The International Sensitivity Index (ISI) and geometric mean normal for the PT reagent reagents (Diagnostica Stago, Parsippany, NJ) using Neoplastine CI Plus were 1.25 and 13.1 seconds, respectively. For the plasma PT and INR Measurements Index (ISI) and geometric mean normal for the PT reagent was 0.30; average for patients having stat coagulation testing ordered). In the AWBPT assay, well-mixed WB samples were loaded singly instead of in batches, and a 70-μL sample of citrated WB was incubated for 1 minute, followed by addition of 100 μL of prothrombin reagent.

Effect of Hematocrit Value on PT

We evaluated the effect of hematocrit on the PT in samples at a range of INRs by spiking split plasma samples with increasing amounts of WB cellular components from the same subject.

Plasma Fibrinogen Measurements

All measurements were made on Diagnostica Stago analyzers (STA-R Evolution and STA-Compact) using STA Fibrinogen reagent (Diagnostica Stago) and Owren Koller diluent (Diagnostica Stago). To measure plasma fibrinogen levels, 10 μL of citrated plasma was added to 90 μL of diluent followed by incubation at 37°C for 4 minutes. After incubation, 50 μL of STA fibrinogen reagent was added. For the adjusted WB fibrinogen (AWBFIB) assay, well-mixed samples of citrated WB were loaded singly instead of in batches, 14 μL of WB was added to 86 μL of diluent, and the diluted sample was incubated at 37°C for 1 minute, followed by addition of STA fibrinogen reagent.

For hematocrit-corrected WB fibrinogen (CWBFIB) measurements, we applied an additional arithmetic correction using the hematocrit value of each WB sample. The corrective factor we used was derived from the relationship between the plasma and WB fibrinogen and hematocrit values:

\[
\text{CWBFIB} = \frac{\text{WBFIB}}{1.4294 - (0.0137 \times \text{Hematocrit})}
\]

Statistical Analysis

Deming regression was used to compare WB with plasma results for PT, INR, and fibrinogen levels. Linear regression was used to compare adjusted WBINR (AWBINR) and AWBFIB with hematocrit values. A 2-sided unpaired t test was used to compare plasma INR with the average AWBINR at different hematocrit values in individual patients. To determine the effect of WB tests on clinical classification of patients, we stratified patient groups according to their INRs—INR less than 2, INR 2 to 4, INR greater than 4—and compared agreement of the plasma and WB-based cohorts (concordance). Analyse-it statistics add-in software (Analyse-it, Leeds, England) for Excel (Microsoft, Redmond, WA) was used to do the analysis.

Results

WB vs Plasma PT and INR

With the same 50-μL sample volume for plasma and WBPT assays, WBPT values were on average about 40% longer than plasma PT measurements. After
Comparison of whole blood (WB) vs plasma prothrombin time (PT)/international normalized ratio (INR) testing (n = 154). **Figure 1** A and B, WBPT/INR vs plasma PT/INR using the same 50-μL sample volume for both WB and plasma assays. Deming regression analysis equation for the line WBPT = 1.43x – 5.39; \( r^2 = 0.966 \) (A); and for the line WBINR = 1.55x – 0.60; \( r^2 = 0.960 \) (B). C, Adjusted WBPT (AWBPT) using a 40% larger, 70-μL WB sample volume. AWBPT = 0.92x + 0.97; \( r^2 = 0.978 \). D, Adjusted WBINR (AWBINR) used a 40% larger, 70-μL WB sample volume and an adjusted International Sensitivity Index of 1.30. AWBINR = 0.99x – 0.02; \( r^2 = 0.980 \). E and F, Difference plots for AWBPT vs plasma PT (E) and AWBINR vs plasma INR (F). The dashed lines delineate the 95% limits of agreement.
adjusting the WBPT sample volume to 70 μL to deliver approximately the same plasma volume used in the plasma assay, the AWBPT produced less bias between WB and plasma assays but still showed substantial variability. Duplicate plasma PTs showed an average ± SD difference of 0.2 ± 0.5 second, whereas duplicate AWBPTs showed an average ± SD difference of 0.1 ± 0.8 second. For plasma PT vs AWBPT, the average ± SD difference was –0.8 ± 1.8 seconds, nearly 4-fold more than the difference for duplicate plasma PTs.

With the same 50-μL sample volume for plasma and WBINR assays and the original plasma ISI of 1.25, WBINR values were on average more than 50% higher than the plasma INR (Figure 1). The AWBINR had 2 modifications. First, the AWBPT method used the increased 70-μL WB sample volume, and second, the ISI was adjusted to 1.30 to provide the best fit between WB and plasma INR values. On average, there was little bias between plasma INR and AWBINR results but, again, significant variability. Duplicate plasma INRs showed an average ± SD difference of 0.02 ± 0.05. Duplicate AWBINRs showed an average ± SD difference of 0.01 ± 0.1. For plasma INR vs AWBINR, the average ± SD difference was overall –0.04 ± 0.2, which increased to –0.11 ± 0.4 for INRs greater than 2.

**Figure 2** shows a comparison of plasma INR vs AWBINR at different hematocrit values in the same sample. For samples with plasma INRs of 1.24 and 2.17, the mean AWBINR was not significantly different from the plasma INR, and there was no correlation between AWBINR and hematocrit values. For plasma INRs of 3.04 and 4.69, the mean AWBINR was significantly less than the plasma INR, but there was no correlation between AWBINR and hematocrit values. For a plasma INR of 5.7, the AWBINR was significantly correlated with hematocrit ($r^2 = 0.98; P = .001$).

We studied the concordance of the AWBINR vs plasma INR using INR-defined cohorts **Table 1**. The groups were INR less than 2, 2 to 4, and more than 4. The overall concordance was 97% between the 2 assays for the 154 samples studied, but it worsened with increased INR. At INRs less than 2, the concordance was 98%, and at INRs more than 4, the concordance was 88%.

**WB Fibrinogen**

The AWBFIB level was on average only 2% to 4% lower than the plasma fibrinogen level but showed substantial variation **Figure 3**. For plasma fibrinogen levels less than or equal to 150 mg/dL (1.5 g/L), the average ± SD difference between plasma and AWBFIB was 6 ± 20 mg/dL (0.06-0.2 g/L). For plasma fibrinogen levels greater than 150 mg/dL (1.5 g/L), the average ± SD difference between plasma and AWBFIB was –23 ± 56 mg/dL (–0.23 ± 0.56 g/L). Even though the AWBFIB sample volume was adjusted to increase the sample plasma volume to approximately the same level as the plasma assay, hematocrit still had a major effect on the fibrinogen level measured in WB **Figure 4** ($r^2 = 0.80$). If the hematocrit value was known, a CWBFIB level could be derived that substantially reduced the variation between WB and plasma fibrinogen measurements (Figure 3). The average ± SD difference between CWBFIB and plasma fibrinogen levels was 0.5 ± 21 mg/dL (0.005-0.21 g/L).

**Discussion**

**WB Results Are Affected by Hematocrit Values and Sample INRs**

Hematocrit was an important determinant of the accuracy of WBINR and fibrinogen measurements. Point-of-care and viscoelastic coagulation assays are designed to

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**Table 1**

<table>
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<th>Plasma INR</th>
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<th>&gt;4</th>
<th>Concordance (%)</th>
<th>No. of Samples</th>
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<td>&lt;2</td>
<td>110</td>
<td>0</td>
<td>0</td>
<td>98</td>
</tr>
<tr>
<td>2-4</td>
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<td>28</td>
<td>0</td>
<td>93</td>
</tr>
<tr>
<td>&gt;4</td>
<td>0</td>
<td>14</td>
<td>14</td>
<td>88</td>
</tr>
<tr>
<td>Total</td>
<td>112</td>
<td>28</td>
<td>14</td>
<td>88</td>
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INR, international normalized ratio.
* Overall concordance, 97%.
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correct for an average hematocrit value in patient samples to address this issue. However, our data demonstrate that a single assumed sample hematocrit value or a single arithmetic correction is not sufficient. The impact of hematocrit values remains significant, especially in samples with hematocrit values more than 10% from the corrected-for hematocrit value and for samples with high INRs. Hence, the sickest patients with high or low hematocrit values (eg, patients with anemia, substantial blood loss, polycythemia, or dehydration) are most at risk for a clinically significant error, particularly if they have INRs more than 3.0. A majority of studies comparing WB with plasma INR testing demonstrated biases that were more pronounced at higher INRs, with the magnitude and direction of discrepancies and biases varying significantly even between sites in a single study. Our results suggest that an important determinant of the magnitude and direction of the bias is how different the hematocrit value of a given sample is from the corrected-for hematocrit value of the WB testing platform in question.

Figure 3A and B, Deming regression analysis and difference plots for the adjusted whole blood fibrinogen (AWBFIB) vs plasma fibrinogen (FIB) (n = 158) equation for the line AWBFIB = 0.98x – 11.83; \( r^2 = 0.929 \). C and D, Deming regression analysis and difference plots for the corrected whole blood fibrinogen (CWBFIB) vs plasma FIB (n = 126) equation for the line CWBFIB = 1.01x – 4.76; \( r^2 = 0.984 \). CWBFIB values were adjusted mathematically for differences in hematocrit values.
van den Besselaar et al\textsuperscript{23} investigated the impact of hematocrit values on differences between WB and plasma INRs and concluded that the hematocrit value had a statistically but not clinically significant effect on the INR in the hematocrit range from 37\% to 51\% (0.37-0.51). However, their data showed that samples with hematocrit values less than 35\% (0.35) would result in WB vs plasma INR differences more than 0.65 INR based on hematocrit values alone and that the discrepancy due to hematocrit increases as samples get further away from their corrected-for hematocrit value of 40\% (0.40).

Our data, in addition to showing similar trends, demonstrate that the effect of hematocrit values on INR measurements is amplified at higher INRs. The relationship of INR to plasma factor level is exponential.\textsuperscript{25} Hence, low INRs are relatively insensitive to factor level changes and, thus, plasma sample volume differences. The INR becomes increasingly sensitive to factor level and, thus, sample plasma volume differences as factor levels decrease. This exponential relationship between the PT/INR and factor levels precludes the use of a simple hematocrit-based arithmetic correction to improve WB coagulation testing accuracy and implies an analytic limitation for WBINR testing on current platforms. An 18-month–28-coagulation testing accuracy and implies an analytic limitation sensitive to factor level and, thus, sample plasma volume differences. The INR becomes increasingly sensitive to factor level and, thus, sample plasma volume differences as factor levels decrease. This exponential relationship between the PT/INR and factor levels precludes the use of a simple hematocrit-based arithmetic correction to improve WB coagulation testing accuracy and implies an analytic limitation for WBINR testing on current platforms.

First, because the coagulation analyzer was not designed to mix samples as hematology analyzers are, only a few WB samples could be analyzed at a time before cell settling occurred. Second, if WB testing is contemplated, adjustment of the sample volume is needed to account for the average hematocrit value of patient samples that will be tested. This may vary depending on whether the population is an outpatient population with a near normal hematocrit value or a postoperative or trauma population that may have a lower hematocrit value. Third, as described, differences in hematocrit values produced variations in the measured PT/INR and fibrinogen values between WB and plasma results. This

**WB Fibrinogen Testing**

Like INR, the hematocrit value is an important determinant of the accuracy of WB fibrinogen assays. Our study shows that although the accuracy and usefulness of WB fibrinogen assays is improved by accounting for the impact of hematocrit values, a single arithmetic correction based on a population hematocrit value is not sufficient. Even after applying the population-hematocrit–based correction, we still had differences of up to 200 mg/dL (2.0 g/L) between plasma and WB fibrinogen levels for a single specimen with a study design that used identical instruments and reagents. Samples from patients with values 10\% or more from the corrected-for hematocrit value would require an individual correction to have clinically reliable accuracy, which may limit the turnaround time benefit of WB viscoelastic fibrinogen testing.

**WB Testing in the Clinical Laboratory**

With regard to WB testing on a standard coagulation analyzer, we found that several modifications were necessary. First, because the coagulation analyzer was not designed to mix samples as hematology analyzers are, only a few WB samples could be analyzed at a time before cell settling occurred. Second, if WB testing is contemplated, adjustment of the sample volume is needed to account for the average hematocrit value of patient samples that will be tested. This may vary depending on whether the population is an outpatient population with a near normal hematocrit value or a postoperative or trauma population that may have a lower hematocrit value. Third, as described, differences in hematocrit values produced variations in the measured PT/INR and fibrinogen values between WB and plasma results.
variation was not so severe as to preclude use of WBPT/INR, particularly for INRs near or below the therapeutic range, but it may be unacceptable for WB fibrinogen measurement unless a hematocrit correction is used. This requires that a hematocrit value be obtained with each WB fibrinogen value, which may be acceptable for certain stat panels, but may limit use of WB fibrinogen testing when the hematocrit value is not available. If these modifications are incorporated, WB testing is sufficiently reliable for clinical testing.

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References