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Original Research Article

A Prospective Comparative Assessment of Effect of Platelet Contamination on the Coagulation Screening Tests

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Abstract

Objectives: To evaluate the effect of platelet contamination on the coagulation screening tests (Prothrombin time and activated partial thromboplastin time) and to evaluate and compare effect of platelet contamination on the normal and abnormal coagulation screening tests (Prothrombin time and activated partial thromboplastin time).

Material & Methods: This is a prospective analytical study conducted over a period of six months at the Department of Pathology, Darbhanga Medical College, Laheriasarai, Darbhanga, Bihar, India.

Results: A total of 40 samples for this study. A total of 40 samples were analysed. 19 of them had coagulation parameters in the normal range and rest 21 had abnormal coagulation studies. The mean difference in APTT values between intervention and standard spin technique was 0.1 second and 95% LoA was -1.5 to +1.2 in samples with normal APTT values. Analysis of the samples with prolonged APTT values yielded a mean difference of 1.1 seconds with 95% LoA of -4.1 to +6.3.

Conclusions: Platelet contamination of plasma insignificantly shortens the coagulation test results and remains clinically useful.

Keywords: Activated partial thromboplastin time; Coagulation tests; Platelet contamination; Prothrombin time

Introduction

Prothrombin time (PT) and activated partial thromboplastin time (APTT) are the 2 most commonly requested routine coagulation tests for the screening of acquired and inherited coagulation disorders and the monitoring of anticoagulation therapy [1]. Quality care requires reliable test results and prompt turnaround times. Reliable results can be achieved by carefully controlling preanalytical variables, which are responsible for 64% of all errors in testing [2, 3]. PT and APTT tests represent about 90% of coagulation tests ordered, and it takes 40–180 minutes or more for

reporting results with standard laboratory methods [4, 5].

Several studies have been undertaken to address this issue by centrifuging the sample at a higher speed for a shorter duration so as to achieve platelet poor plasma. These studies have demonstrated non-significant effect on routine coagulation tests [6-8]. Consequent to this, shorter centrifuge time is successfully used to fulfill the clinical need of shorter turnaround time [8-11].

Hence the present study was conducted to evaluate and compare effect of platelet contamination on the normal and abnormal coagulation screening tests (Prothrombin time and activated partial thromboplastin time).

Material & Methods:

This is a prospective analytical study conducted over a period of six months at the Department of Pathology, Darbhanga Medical College, Laheriasarai, Darbhanga, Bihar, India. We recruited a total of 40 samples for this study.

Samples obtained during working hours in our lab. 15 consecutive samples which yielded normal coagulation results and 16 consecutive samples which yielded abnormal coagulation results were recruited. Sample tubes which failed to maintain proper anticoagulant blood ratio were rejected.

Centrifugation: The samples were centrifuged at 3500 rpm (1000 g) for 15 mins. This is considered as 'standard spin' for our study.

Prior to this study, we conducted an experiment to obtain platelet contaminated plasma. We centrifuged the sample at various speed for variable time. After trial and error, we noticed that centrifugation at a speed of 2500 rpm (500 g) for 5 min yielded clear supernatant in the citrated samples with platelet count of the separated plasma exceeding ten thousand. We considered this centrifugation speed and time for our 'intervention spin' in this study.

One of the authors did the initial mixing, dividing and labeling the samples received. Two technicians who were in charge of conducting the coagulation studies were trained to do intervention spin along with standard spin. They did the centrifugation and further procedures of the coagulation tests. Supernatant plasma of both the standard spin and intervention spin were subjected to platelet count by another author. Platelet count was done on automated cell counter - Sysmex 1000 XN. Coagulation tests were done within 10 min on fully automated coagulometer (Destiny plus, Trinity Biotech, Texas, US).Coagulation parameters studied were Prothrombin time (PT), international normalized ratio (INR) and activated partial thromboplastin time (APTT).

Demographic details of the patient were collected from the laboratory information system.

All the results were entered in Microsoft Excel.The numerical data was summarized as either mean & standard deviation or median and interquartile range depending upon the distribution. The comparison of the median was done using Mann-Whitney U test. The results of coagulation screening tests obtained with two different techniques were analyzed for the agreement using Bland Altman analysis. All the statistical tests and construction of Bland - Altman plot was done in Microsoft Excel. Both the whisker box plots and Bland-Altman plots were recreated in Adobe illustrator (Adobe creative suit,) for the sake of picture clarity. A priory cut-off of less than 10% difference between the two techniques was considered clinically acceptable

Results:

A total of 40 samples were analyzed. 19 of them had coagulation parameters in the normal range and rest 21 had abnormal coagulation studies. Baseline

characteristics of the samples recruited are depicted in table 1.

The median platelet count in the standard spin group was 4000 with and interquartile range of 1500 to 8000. In contrast, the median platelet count in intervention spin group was 25000/cumm with an interquartile range of 16000 to 35500/cumm. Comparing the difference between two groups with Mann Whitney U test yielded a Z score of -6.75 and U value of zero. The p value was 0.00 suggesting the difference was significant. Figure 1 illustrates the median, range and inter quartile range as whisker box plot for both normal and abnormal coagulation group with standard and intervention spin.

Minimum and maximum prothrombin time recorded with intervention spin was 12.9 & 89 sec and with standard spin was 13.5& 97.0 sec respectively. Figure 2 illustrates the Bland Altman plot for the mean difference and 95% limits of agreement. Estimated mean difference between the intervention and standard spin was minus 0.59 seconds with a 95% Confidence interval (CI) of -0.95 to -0.05. Sub group analysis of samples with normal Prothrombin time showed a mean difference of 0.1 sec with 95% limits of agreement (LoA) between – 0.5 to +0.70. Analysis of the samples with prolonged prothrombin time showed a mean difference of – 0.58 with 95% LoA between – 1.2 to +2.4.

The range of INR was 0.96 to 9.78 for intervention spin and 1.00 to 9.97 for standard spin. Figure 3 illustrates the Bland Altman plot for mean difference and 95% limits of agreement for INR. The mean difference in INR between intervention spin and Standard spin was -0.059 with 95% confidence interval of -0.11 to -0.001. The mean and 95% CI for lower LoA was -0.37 and -0.47 to -0.26. Mean and 95% CI values for upper LoA was 0.25 and 0.15 to 0.35. samples with normal INR yielded a mean difference between intervention and standard spin techniques of 0.01 and lower and upper LoA of -0.03 &+0.04. Similar values for abnormal INR were a mean difference of 0.1 with 95% LoA of -0.15 to +0.35 for INR.

Figure 4 depicts the Bland Altman plot for mean difference and 95% LoA between Intervention spin and Standard spin techniques for activated partial thromboplastin time (APTT). APTT values ranged from 22.2 seconds to 180 seconds in intervention spin group and 20.3 seconds to 178 seconds in standard spin group. The mean difference was -0.59 seconds with 95% CI of -1.5 to 0.3 seconds. 95% lower LoA was -5.56 seconds with 95% CI of -7.6to -3.6 seconds. The 95% upper LoA was 4.40 seconds with a 95% CI of 2.5 to 5.7 seconds. The mean difference in APTT values between intervention and standard spin technique was 0.1 second and 95% LoA was -1.5 to +1.2 in samples with normal APTT values. Analysis of the samples with prolonged APTT values yielded a mean difference of 1.1 seconds with 95% LoA of -4.1 to +6.3.

The summarized value and the corresponding mean difference of coagulation tests with the subgroups of normal and abnormal values are depicted in table 2.

Table 1: Baseline characteristics:

Total	40
Age In years – Median (IQR)	41.4 (29.3 – 56.72)
Gender	
Males	22 (55%)
Females	18 (45%)
Coagulation	

Normal	19 (47.5)
Abnormal	21 (52.5)

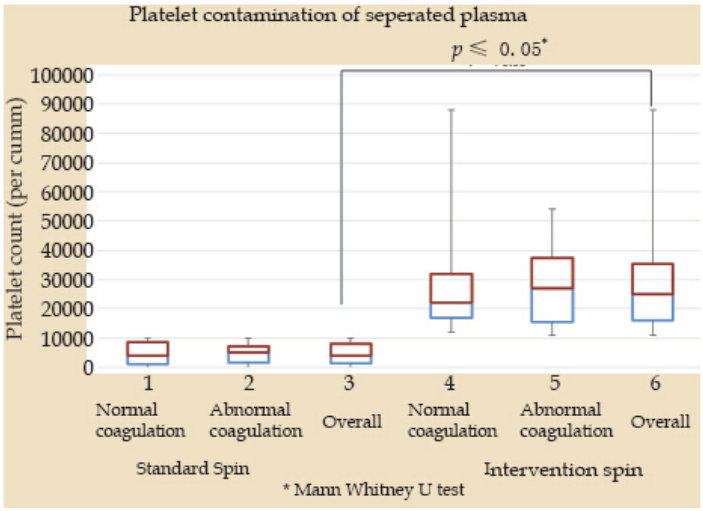

Figure 1: comparison of platelet contamination of separated plasma between standard and intervention spin.

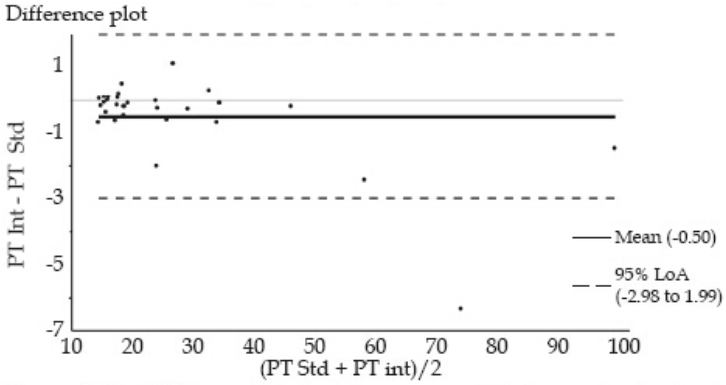

Figure 2: Bland Altman Plot showing agreement between standard and intervention spin with respect to prothrombin time

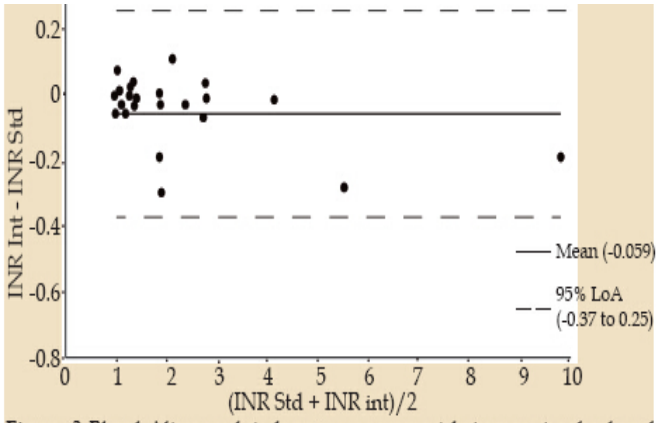

Figure 3: Bland Altman Plot showing agreement between standard and intervention spin with respect toInternational Normalized ratio

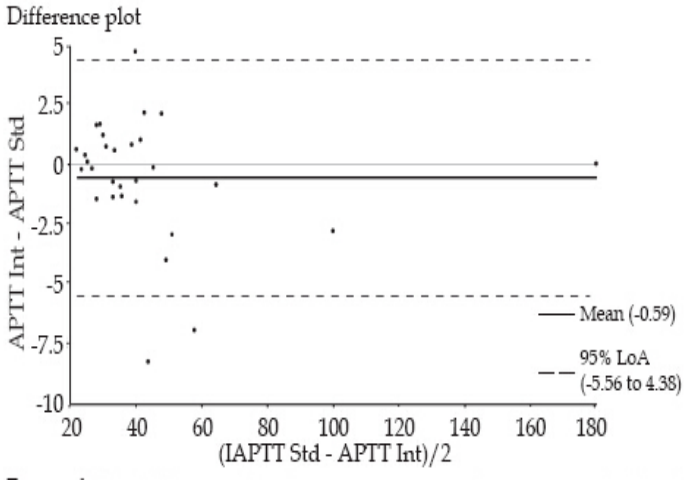

Figure 4: Bland Altman Plot showing agreement between standard and intervention spin with respect to Activated partial thromboplastin time

Table 2: Comparison of summarized values and mean difference of coagulation tests between standard and intervention spin

PT (in seconds)	Median (IQR)	Mean difference
Standard spin	19.5 (14.2 – 28.6)	- 0.5
Intervention spin	19.9 (14.6 – 28.9)	
PT (Normal)		
Standard spin	16.4 (14.2 – 18.5)	0.10
Intervention spin	16.7 (14.3 – 17.3)	
PT (Prolonged)		
Standard spin	25.4 (22.4 – 31.5)	-.0.60
Intervention spin	26.7 (22.7 – 31.9)	
INR		
Standard spin	1.36 (1.03 – 1.10)	- 0.05
Intervention spin	1.39(1.05 – 0.96)	
INR (Normal)		
Standard spin	1.4 (1.1 – 1.5)	0.01
Intervention spin	1.5 (1.1 – 1.4)	
INR (Increased)		
Standard spin	2.9 (2.2 – 4.5)	- 0.21
Intervention spin	2.9 (2.21– 4.7)	
APTT		
Standard spin	35.4 (28.0 – 43.8)	- 0.73
Intervention spin	34.7 (28.3 – 43.6)	
APTT(Normal)		
Standard spin	27.4 (26.7 – 31.2)	- 0.25
Intervention spin	26.3 (25.1 – 30.3)	
APTT (Prolonged)		
Standard spin	39.0 (38.3 – 45.7)	2.47
Intervention spin	41.3 (39.1 – 44.8)	

Discussion:

Platelet contamination was achieved by altering the centrifugation technique. Reducing the speed and time of centrifugation does result in visibly clear plasma but not platelet poor plasma [12].

The standard recommendation for pre-analytical processing is to obtain as low platelet counts as possible due to its possible interference with the coagulation tests. Platelets are believed to provide phospholipid surface for the coagulation factors to get activated and spuriously shorten the coagulation time during laboratory tests. It is also known to contain procoagulant substances which may interfere with the coagulation test [13]. However, the optimal quantity of platelets to interfere in coagulation tests is so far unknown. Earlier studies have adopted higher centrifugation speed to achieve platelet poor plasma and have compared the mean values of coagulation tests. Though there was no significant statistical difference between the two groups, in this scenario, summarized values are not suitable for adopting the results [14].

Whenever a delay in transport is expected, it might be advisable to perform local centrifugation and separation.Plasma is generally prepared by centrifugation of a whole blood sample. A temperature-controlled centrifuge is required for processing routine coagulation assays. Centrifugation should take place at room temperature (15–25 °C) [15]. The effect of centrifugation temperature on MP determination is still unknown [16].

The preparation of platelet-rich plasma (PRP) for platelet function analysis requires that centrifugation is performed at 200–250 g for 10 min without application of a rotor brake [17]. These centrifugal forces appear to be the best condition for preparing PRP for light transmission aggregometry (LTA) studies, both in terms of the degree of contamination of PRP by other blood cells and of platelet reactivity [17].

Age, gender, ethnicity, and blood group might influence reference values for certain parameters of laboratory hemostasis, and/or generate variable test results for some tests. [18]For example, FVIII and VWF and platelet function tests are generally influenced by such factors.

Alternatively, anticoagulant therapy will affect the detected levels of the natural anticoagulants, as mentioned previously (viz, heparin therapy may influence antithrombin detection, warfarin therapy may influence protein C and protein S levels, and heparin and warfarin therapy may influence APCR testing). Heparin and warfarin therapy may also influence the appropriate identification of LA. Recent audits of clinical practice indicate that up to 1/3 of samples destined for thrombophilia investigations are from patients on warfarin and/or heparin therapy, or the sample is otherwise heparin contaminated, and thus representing high potential for diagnostic error. Considered another way, upwards of 80% of abnormal thrombophilia test results may be a reflection of inappropriate testing while on anticoagulant therapy. [19]

Providing timely results to the clinicians is one of the quality attribute of laboratory services [20]. In order to achieve quick and quality service, we need to have optimal protocols for preparing good quality plasma [21-23].

Conclusion:

Platelet contamination of plasma insignificantly shortens the coagulation test results and remains clinically useful. A marginal shortening of the test values still remains clinically useful. This holds good for both patients who have normal and abnormal coagulation. Caution should be exercised in interpreting marginally prolonged APTT value.

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