

Does Platelet Contamination Really Affect the Coagulation Tests? A Prospective Study

Rajalakshmi Rajashekar, Vani Krishnamurthy, Srinivasa Murthy Doreswamy

Rajalakshmi Rajashekar, Vani Krishnamurthy, Srinivasa Murthy Doreswamy, JSS Medical College, JSS University, Mysuru, Karnataka, India

Correspondence to: Vani Krishnamurthy, 70, BEML 2nd Stage, Rajarajeshwari nagara, Mysuru, Karnataka, India.

Email: vanidsri@gmail.com

Telephone: +91-9980941161

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ABSTRACT

AIM: Coagulation tests are often requested as urgent tests by the intensive care clinicians. This is one of the many tests which is grossly influenced by pre analytical errors. Platelet contains phospholipids and pro coagulants which can interfere with the coagulation results. Preparation of platelet poor plasma by centrifuging at 1500 g for 15 mins is the standard recommended procedure. In order to decrease the turnaround time, several authors have centrifuged the sample at high speeds to obtain platelet poor plasma and reported unaffected coagulation test results. However, the effect of platelet contamination on the results of the coagulation tests was less well studied.

METHODS: We prospectively analysed the coagulation test results after centrifuging at 1000 g (Standard spin) and 500 g (Intervention spin) respectively. A total of 31 samples were studied out of which, 15 had normal coagulation and 16 had abnormal coagulation results. Agreement of values between the two preparations were determined with Bland Altman analysis.

RESULTS: Median platelet count in standard spin group was 4000/cumm and with intervention spin was 25000/cumm. Estimated mean difference (95% CI) between intervention and standard spin for prothrombin time was -0.59 secs (-0.96 to -0.03), for INR was -0.059 (-0.11 to -0.001) and for activated partial

thromboplastin time was -0.59 secs (-1.5 to +0.3).

CONCLUSIONS: Platelet contamination of plasma insignificantly shorten the coagulation test results and remains clinically useful.

Key words: Activated partial thromboplastin time; Coagulation tests; Platelet contamination; Prothrombin time

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INTRODUCTION

Laboratory investigations are an integral part of patient management in modern medicine. These investigations are expected to provide quick and precise results in order to aid proper diagnosis, management and prognostication. Coagulation tests are one of the many investigations which is requested to be done on urgent basis by the intensive care clinicians and also grossly influenced by pre analytical errors. On top of this, imprecise results lead to erroneous management. Sample collection and transport are the commonest areas of pre analytical error before the sample reaches the laboratory^[1]. In the laboratory, preparing platelet poor plasma is an important step to prevent pre analytical error. Platelets can provide the phospholipid surface for activating clotting factors and hence interfere with the laboratory diagnosis^[2]. CLSI has recommended to keep the platelet count of the prepared plasma below 10000/cumm by centrifuging the sample at 1500 g for 15 minutes^[3].

In the busy laboratories multiple samples are centrifuged at higher speeds in batches so as to reduce the turnaround time. Following the CLSI protocol results in need for separate setup and system of performing coagulation tests. This has both time and resource

implications.

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^[4-6]. Consequent to this, shorter centrifuge time is successfully used to fulfill the clinical need of shorter turnaround time^[6-9]. Only few studies have assessed the platelet count in the plasma prepared from rapid centrifugation for a shorter duration. They have produced contradictory results with respect to platelet contamination of plasma^[4,10]. In this light, studies addressing the issue of possible platelet contamination during short centrifuge time and its effect on coagulation test results are needed. We undertook a detailed analysis of agreement of values between platelet poor plasma and platelet contaminated plasma in contrast to other small number of studies which have compared the summarized values.

Objectives

Primary objective: To evaluate the effect of platelet contamination on the coagulation screening tests (Prothrombin time and activated partial thromboplastin time).

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

MATERIALS AND METHODS

This is a prospective analytical study conducted in the month of January 2016 at JSS Hospital, Mysuru, Karnataka, India which is a tertiary care center.

Sample size

We could not find any similar study determining the agreement between the two values on literature search. Hence, we chose a convenient sample size of thirty for this study. We recruited a total of 31 samples over a period of one month.

Sample selection

We recruited only the 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.

Sample processing

The samples were obtained from either surgical or medical intensive care units. The blood was collected in the commercially available citrated vacutainer (BD vacutainer, 0.109 M/3.2% Citrate). After the initial receiving formalities and thorough mixing, the sample was divided into two equal parts. Thus separated portions were labeled as "S" for standard spin and "I" for Intervention spin.

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.

Statistical analysis

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 analysed 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 priori cut-off of less than 10% difference between the two techniques was considered clinically acceptable.

RESULTS

A total of 31 samples were analysed. 15 of them had coagulation parameters in the normal range and rest 16 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 13.7 & 97 sec and with standard spin was 14.3 & 98.5 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 -0.96 to -0.03. 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.

Table 1 Baseline characteristics.

Total	31
Age in years -Median (IQR)	42.5 (28 - 52.75)
Male-n(%) / Females n (%)	18(58%) / 13(42%)
Normal / Abnormal coagulation n (%)	15 (48.3%) / 16 (51.7%)
IQR - inter quartile range	

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 21.6 seconds to 180 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 -7.1 to -3.9 seconds. The 95% upper LoA was 4.38 seconds with a 95% CI of 2.7 to 5.9 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.

DISCUSSION

Our study has shown that the certain amount of platelet contamination does not significantly alter the coagulation tests. Platelet contamination was achieved by altering the centrifugation technique. Reducing the speed and time of centrifugation does result in visibly

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

PT* (in seconds)	Median (IQR)^	Mean difference
Standard spin	18.4 (15.6 - 27.3)	-0.5
Interventional spin	18.3 (15.5 - 27.7)	
PT (Normal)		0.1
Standard spin	15.5 (15 - 17.1)	
Interventional spin	15.25 (15 - 16.8)	
PT (Prolonged)		-0.58
Standard spin	25.8 (23.3 - 33.9)	
Interventional spin	26.9 (22.8 - 33.8)	
INR\$		-0.06
Standard spin	1.34 (1.1 - 2.2)	
Interventional spin	1.33 (0.96 - 2.3)	
INR (Normal)		0.01
Standard spin	1.2 (1.0 - 1.3)	
Interventional spin	1.1 (1.1 - 1.3)	
INR (increased)		0.1
Standard spin	2.7 (2.1 - 4.1)	
Interventional spin	2.7 (1.9 - 4.1)	
APTT#(in seconds)		-0.59
Standard spin	36.7 (29.1 - 45.7)	
Interventional spin	35.3 (30.2 - 44)	
APTT (Normal)		-0.1
Standard spin	28.2 (25 - 30.6)	
Interventional spin	29 (25.1 - 31.3)	
APTT (Prolonged)		1.1
Standard spin	40.9 (37.9 - 48.3)	
Interventional spin	42 (39 - 47.5)	

*PT = Prothrombin time, \$ INR = Internationalized normal ratio, # APTT = Activated partial thromboplastin time, ^ Interquartile range.

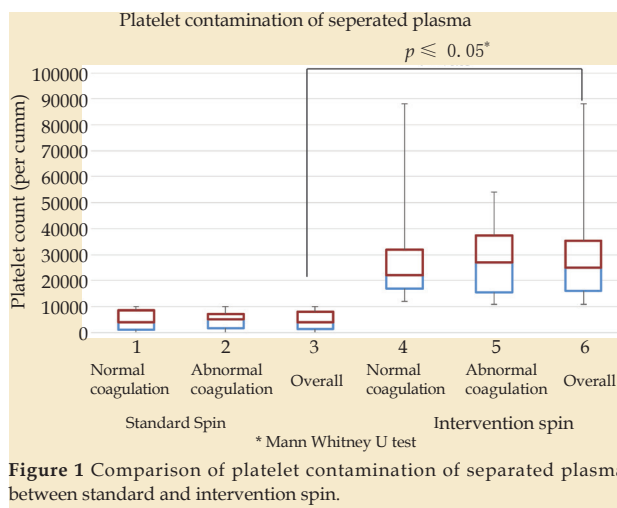


Figure 1 Comparison of platelet contamination of seperated plasma between standard and intervention spin.

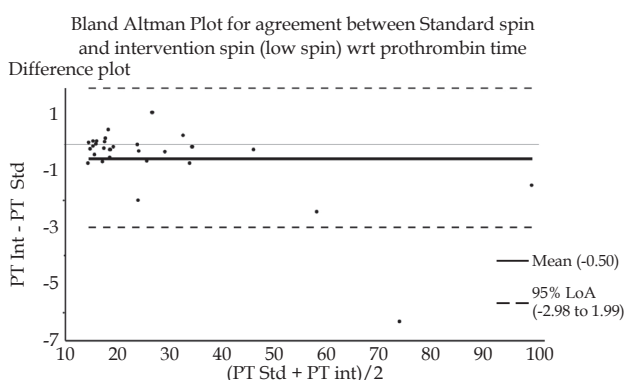


Figure 2 Bland Altman plot showing agreement between standard and intervention spin with respect to prothrombin time.

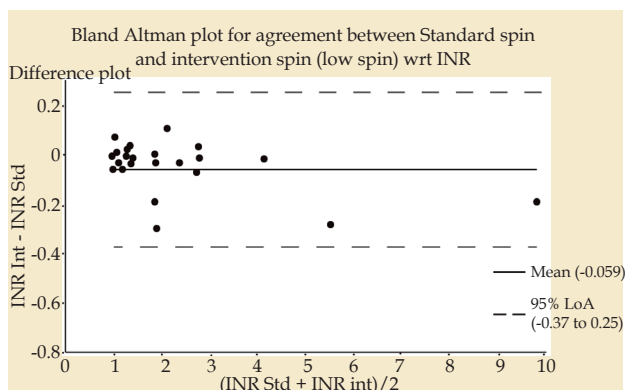


Figure 3 Bland Altman plot showing agreement between standard and intervention spin with respect to International normalized ratio.

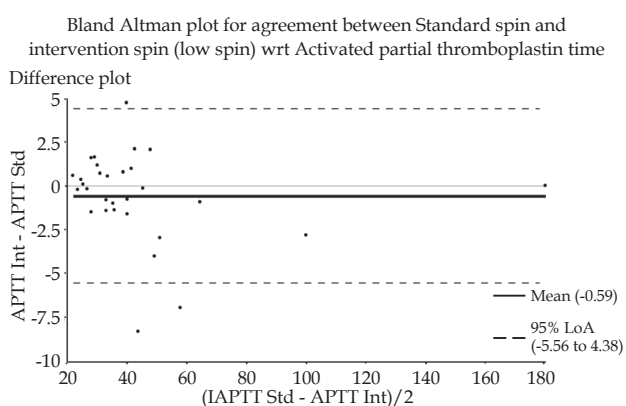


Figure 4 Bland Altman plot showing agreement between standard and intervention spin with respect to activated partial thromboplastin time.

clear plasma but not platelet poor plasma^[11]. Our intervention spin resulted in nearly 6-fold increase in platelet count in the supernatant plasma which was statistically significant. Standard spin technique always produced plasma with platelet count consistently less than 10000/cumm whereas intervention spin always produced plasma with platelet count of greater than 10000/cumm. The effect of such contamination interfering with coagulation tests is poorly understood.

The standard recommendation for pre-analytical processing is to obtain as low platelet count 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 tests^[2]. 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^[12]. Azlin et al have studied the effect of platelet contamination and found there was high correlation between the coagulation tests conducted with platelet poor plasma and platelet contaminated plasma^[10]. We believe, agreement between the two values is more important than just correlation. We have attempted to find out the agreement between two values obtained from platelet poor and platelet contaminated plasma in order to make the results clinically more useful.

Our study has shown the mean difference between the two plasma preparations with respect to prothrombin time was only 0.5 seconds and the 95% CI was below one second suggesting that platelet contamination up to a maximum of 35000/cumm will not alter the coagulation tests significantly from the clinical perspective. The mean differences between standard spin group and intervention spin group for INR was minus 0.06 and APTT was minus 0.59. Table 2 depicts the mean difference for the subgroups as well. All these values are insignificant fraction of the median values of the respective parameters. Only notable difference is the values for abnormal APTT. The median value minus the lower limit of agreement of – 5 seconds would bring down the values close to normal range. Larger sample size in this group can be more informative. Our study hence suggests minimal insignificant effect of platelet contamination on routine coagulation tests. However, there still remains some ambiguity if the APTT values are marginally prolonged but are in the high normal range. It has to be noted that our intervention group had platelet count of 35000/cumm. Our results may not be applicable to samples with higher contamination with platelets.

Providing timely results to the clinicians is one of the quality attribute of laboratory services^[13]. In order to achieve quick and quality service, we need to have optimal protocols for preparing good quality plasma^[14,15]. Our study show, centrifuging at a slower rate for shorter duration does not alter the coagulation test results significantly and hence can be considered for revising the plasma preparation protocol.

Our results are based on small sample size which needs to be validated with a bigger sample. However, once validated, this result suggested that coagulation test values can be used even if pre-analytical processing is suboptimal leading to platelet contamination. This could save sizable number of reject samples and re tests in busy hospitals. These results may also help the experts to revise the standard recommendation for pre-processing of samples obtained for coagulation tests.

CONCLUSIONS

Platelet contamination of the plasma up to the tune of 35000/cumm will not significantly alter the coagulation tests. 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 values.

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REFERENCES

- 1 Van Geest-Daalderop JH, Mulder AB, Boonman-de Winter LJ, Hoekstra MM, van den Besselaar AM. Preanalytical variables and off-site blood collection: influences on the results of prothrombin time/ international normalized ratio test and implications for monitoring of oral anticoagulant therapy. *Clin Chem* 2005; **51**: 561-68.
- 2 Mendelsohn EE, Solum NO, Brosstad F. Effects of platelets and platelet-derived material on the activated partial thromboplastin time (Cephotest) coagulation test. *Scand J Clin Lab Invest* 2005; **65(4)**: 321-32.
- 3 Clinical and Laboratory Standards Institute: Collection, transport, and processing of blood specimens for testing plasma-Based coagulation assays and molecular hemostasis Assays; approved guideline H21–A5, 5th ed. Wayne, PA. *CLSI*, 2009: 5-15.
- 4 A A Pappas, S K Palmer, D Meece, L M Fink. Rapid preparation of plasma for coagulation testing. *Arch Pathol Lab Med* 1991; **115(8)**: 816-17.
- 5 Sultan A. Five-minute preparation of platelet-poor plasma for routine coagulation testing. *East Mediterr Health J* 2010; **16(2)**: 233-6.
- 6 Giuseppe L, Gian LS, Martina M, Guidi GC. Preparation of a Quality Sample: Effect of Centrifugation Time on Stat Clinical Chemistry Testing. *Laboratory Medicine* 2007; **38**: 172-76.
- 7 Valenstein P. Turnaround time. Can we satisfy clinicians' demands for faster a service? Should we try? *Am J Clin Pathol* 1989; **92**: 705-6.
- 8 Nelson S, Pritt A, Marlar RA. Rapid preparation of plasma for 'Stat' coagulation testing. *Arch Pathol Lab. Med* 1994; **18**: 175–76.
- 9 Solano C, Saal RJ. Statspin – an effective urgent coagulation service enhancement. *Aust J Med Sci* 2003; **24**: 1.
- 10 Azlin L, Azma RZ, Aidifitri RK, Hamidah NH. Effect of higher centrifugation speed and shortened centrifugation time on prothrombin and activated thromboplastin time. *Med & Health* 2011; **6(1)**: 68 – 72.
- 11 Giuseppe Lippi, Gian Luca Salvagno, Martina Montagnana, Franco Manzato, Gian Cesare Guidi Influence of the centrifuge time of primary plasma tubes on routine coagulation testing. *Blood Coagul Fibrinolysis* 2007; **18(5)**: 525-28.
- 12 Chih-Hsiung Kao, Ling-Chen Shu, Wen-Hsin Yen. Evaluation of a High-speed Centrifuge with Rapid Preparation of Plasma for Coagulation Testing to Improve Turnaround Time. *J Biomed Lab Sci* 2010; **22(1)**: 23-27.
- 13 Joan HH, Peter JH. Laboratory Results- Timeliness as a Quality Attribute and Strategy. *Am J Clin Pathol* 2001; **116**: 311-5.
- 14 Suchsland J, Friedrich N, Grotevendt A, Kallner A, Lüdemann J, Nauck M, Petersmann A. Optimizing centrifugation of coagulation samples in laboratory automation. *Clinical Chemistry and Laboratory Medicine* 2014; **52(8)**: 1187– 91.
- 15 Dimeski G, Solano C, Petroff MK, Hynd M. Centrifugation

protocols: tests to determine optimal lithium heparin and citrate plasma sample quality. *Ann Clin Biochem* 2011; **48**: 218-22.

Peer reviewers: Yeling Lu, Department of Laboratory Medicine, Ruijin Hospital affiliated to Medical College of Shanghai JiaoTong University, No.197 Ruijin Er Road,

Shanghai, 200025, P. R. China; Elena Masselli, MD, Hematology and BMT Unit, Parma University Hospital, Via Gramsci n. 14, 43126 Parma, Italy; Istanbul Kanuni Sultan Süleyman Education and Research Hospital, Pediatric Hematology/Oncology Clinic, 34303 Kucukcekmece/İstanbul, Turkey.