Effect of Higher Centrifugation Speed and Shortened Centrifugation Time on Prothrombin and Activated Thromboplastin Time

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ABSTRAK

Pengemparan spesimen darah untuk menghasilkan plasma kurang platelet merupakan aspek penting bagi ujian koagulasi. Pengurangan masa yang diambil untuk memproses spesimen tanpa menjejaskan kualiti keputusan ujian yang dihasilkan adalah ideal bagi ujian rutin segera. Pengemparan untuk mendapatkan plasma kurang platelet selama 15 minit pada 3580 rpm secara rutin bagi ujian koagulasi akan menambahkan tempoh masa pengujian tersebut dan tempoh masa pengujian bagi ujian segera. Kajian ini dijalankan untuk mengetahui kesan pengurangan masa pengemparan ujian koagulasi bagi memenuhi tuntutan masa ujian segera (30 minit). Ujian *prothrombin time* (PT), *activated partial thromboplastin time* (APTT), fibrinogen dan platelet dijalankan ke atas 79 sampel darah yang diterima untuk ujian koagulasi rutin, dengan menggunakan dua masa pengemparan berbeza untuk setiap sampel iaitu 15 minit pada 3580 rpm dan lima minit pada 4000 rpm. Ujian *paired sample t-test* menunjukkan terdapat perbezaan yang signifikan bagi pengiraan platelet di antara dua kumpulan tersebut (p=0.001). Walaubagaimanapun, tidak terdapat perbezaan signifikan bagi ujian APTT normal (p=0.16), APTT tidak normal (p=0.80), PT tidak normal (p=0.43) dan fibrinogen (p=0.36) bagi kedua-dua kumpulan tersebut. Kesimpulannya, pengemparan yang dijalankan dalam masa yang singkat iaitu lima minit pada 4000 rpm tidak menjejaskan keputusan ujian koagulasi rutin (PT, APTT dan fibrinogen). Justeru, keputusan kajian ini akan memberi manfaat dalam mengeluarkan keputusan segera, untuk ujian koagulasi khususnya.

Kata kunci: ujian koagulasi, pengemparan singkat, variasi pra-analisis, *prothrombin time*, *activated partial thromboplastin time*

ABSTRACT

Centrifugation of blood samples to produce platelet-poor plasma is one of the important steps for coagulation testing. Reduction of the time required for specimen processing without affecting quality of results should be ideal for tests which require immediate results. Centrifugation of platelet-poor plasma (3580 rpm) for 15 minutes performed for routine coagulation tests would prolong the turn-around time for an

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urgent test (30 minutes). This study was done to determine the effect of reducing centrifugation time for routine coagulation tests in order to meet the turn-around time (TAT) for urgent tests. Seventy-nine blood samples sent for routine coagulation tests, were assayed for prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen level and platelet counts, using two different centrifugation speed for plasma preparation: centrifugation at 3580 rpm for 15 minutes and rapid centrifugation at 4000 rpm for five minutes. Paired sample t-test showed that there was a significant difference in the platelet count between the two groups (p=0.001). However, there was no significant difference in the normal APTT (p=0.16), abnormal APTT (p=0.80), abnormal PT (p=0.43) and the results of fibrinogen levels (p=0.36). In conclusion, rapid centrifugation at 4000 rpm for five minutes does not modify results of routine coagulation tests (PT, APTT and fibrinogen). It would be beneficial in providing rapid results for urgent coagulation tests.

Key words: coagulation test, rapid centrifugation, pre-analytical variable, prothrombin time, activated partial thromboplastin time

INTRODUCTION

One of the important objectives of quality assurance for a laboratory is to deliver accurate, timely, and clinically relevant diagnostic reports to the customers i.e. the clinicians and patients. The quality of this service is thus related, among other factors, to the accurateness and timeliness of the reports. For a pathology report to be beneficial to the clinicians, results should be available on time especially for urgent requests from critical units.

The turn-around time (TAT) can be defined as the time taken from when the specimen arrives at the laboratory to the time results are released. It is one of the indicators of quality and efficacy, principally in laboratories performing urgent analyses (Hilborne et al. 1989). This is of importance especially when processing samples for coagulation tests where standard processing methods may not be fast enough for urgent requests.

A survey in the United States showed that the prothrombin time (PT) and activated partial thromboplastin time (APTT) tests represent more than 90% of coagulation tests ordered (Shahangian et al. 2005) which mirrors the requested tests in our laboratory. In coagulation testing, platelet poor plasma (PPP) is required to prevent interference by the phospholipid surface on the platelets. With standard laboratory methods (Adcock et al. 2008), the duration for reporting coagulation tests is about 40–180 minutes. A long TAT may cause adversity in medical situations associated with critical, surgical and emergency cases, and good medical care could be denied (Steindel & Howanitz 2001).

Strategies to reduce the time essential for specimen processing without affecting quality should be recognized, especially for laboratories performing urgent analyses. In our laboratory, urgent cases are mainly requested by the Emergency Department. The standard methods for processing coagulation tests do not meet the current TAT for urgent requests (30 minutes) in our centre. This study was performed to determine whether a higher centrifugation speed and a shorter centrifugation time would affect coagulation results as compared to centrifugation using the standard time

(10-15 minutes) for PPP preparation (Adcock et al. 2008).

MATERIALS AND METHODS

Blood samples for routine coagulation test were randomly selected from patients from November through December 2010. The age range for the study population was from 28 to 83-years-old.

Platelet-poor plasma (PPP) was prepared at room temperature within one hour of sample collection using two table-top centrifuge machines (Rotina 38, Hettich Zentrifugen, Germany). Each sample was thoroughly mixed and separated into equal volumes in two separate plastic vacuum tubes (with 3.2% sodium citrate). The first tube was centrifuged at 4000 rpm for five minutes (protocol A), while the second tube was centrifuged at 3580 rpm for 15 minutes (protocol B) simultaneously. A plastic pipette was used to carefully transfer approximately 1ml of supernatant plasma from the middle of the sample away from the buffy coat to avoid contamination with excess platelets.

All paired samples were prepared and tested for PT, APTT and fibrinogen at room temperature immediately after centrifugation using a coagulation analyzer with the required controls and reagents (STA-Compact, All-Eights, France). The PPP samples were tested for platelet count within 30 minutes of collection using a haematology analyser (LH 750, Beckmann Coulter, USA).

Data were entered using Microsoft Excel and descriptive statistical analyses (mean, range, standard deviation (SD) and paired Student t-test) were computed using SPSS, version 14.

RESULTS

A total of 79 samples were randomly selected for this study. Of these 79 samples, 49 samples showed abnormal PT, 30 samples showed normal PT, 34 samples showed abnormal APTT and 44 samples showed normal APTT readings. One APTT reading (21.5s) was much lower than the normal reference range and was thus excluded from the data analysis. The normal reference range for PT and APTT are 11.4-14.2 seconds and 31.3-46.1 seconds respectively.

Platelet counts less than 10×10^9/L were produced from all 79 PPP samples centrifuged for 15 minutes at 3580 rpm except for two samples (2.5%) which showed a platelet count of more than 10×10^9/L (12×10^9/L and 14×10^9/L). However, when samples were centrifuged for five minutes at 4000 rpm, only 15 samples (19%) showed platelet counts less than 10×10^9/L. The difference

Table 1: Comparison of platelets, PT, APTT and fibrinogen levels using 5 minutes and 15 minutes centrifugation.

Test	5 minutes at 4000 rpm Protocol A			15 minutes at 3580 rpm Protocol B		p-value
	(n)	Mean(SD)	Range	Mean (SD)	Range	
Platelet (x 10^9/L)	79	19.9 (10.03)	5.0 - 43.0	4.7 (2.14)	1.0 – 14.0	<0.001
Fibrinogen(g/L)	79	3.3 (0.84)	1.9 – 7.5	3.3 (0.79)	1.6 – 7.6	0.36
Normal PT (s)	30	13.3 (0.57)	12.2 – 14.5	13.1 (0.58)	12.2 – 14.4	0.01
Abnormal PT(s)	49	25.6 (11.76)	13.5 – 79.8	25.7 (11.76)	14.3 – 81.2	0.43
Normal APTT(s)	44	39.0 (4.21)	29.5 – 45.3	39.3 (4.38)	28.6 – 47.3	0.16
Abnormal APTT (s)	34	60.2 (13.40)	44 – 109.9	60.1 (13.04)	48.4 – 111.4	0.80

between these two groups were statistically significant (p<0.001).

Descriptive statistics for platelet count, PT, APTT and fibrinogen levels prepared for five minutes at 4000 rpm (protocol A) and 15 minutes at 3850 rpm (protocol B) are shown in Table 1. We found a significant difference (p=0.01) between the normal PT levels when comparing centrifugation between 15 minutes (standard time) and five minutes. However, there was no significant difference in the results of normal APTT, abnormal PT and APTT between the two protocols. There was also good correlation between the results of PT, APTT and fibrinogen levels of protocol A and B respectively (Figure 1, 2 and 3).

DISCUSSION

Preparation of blood specimens can be a major time limiting factor in the laboratory throughput. In order to obtain a faster TAT, while maintaining sample quality and accuracy of results, the time consumed on coagulation sample collection, handling, processing and testing should be looked into (Lawrence 2003). This is especially so in critical areas where results are needed urgently.

In our laboratory, it takes about 60 minutes to release routine coagulation results to the wards or clinics. The time may be further prolonged if repeat testing is necessary. This will not fulfill the TAT requirement for urgent tests for critical units such as the Emergency Department and Intensive Care Unit. The shortest recommended centrifugation time to produce PPP with platelet counts less than 10×10^9/L is 10-15 minutes at 3850 rpm using regular centrifuges (Adcock et al. 2008). In this study, we looked at whether centrifugation at five minutes would affect coagulation results (PT/APTT), as compared to centrifugation at 15 minutes recommended time.

We found that 15-minutes centrifugation, achieved a better PPP than a five minutes centrifugation. A shorter centrifugation time (five minutes) causes an increase in platelet count in the sample tubes in 81% of cases. Our results differed from a previous report by Sultan (2010) on 46 healthy volunteers which showed nearly all samples centrifuged at five minutes had platelet count less than 10×10^9/L. Barnes and colleagues (2002) showed that 10 minutes was the minimum centrifugation time required to consistently meet the recommended standards.

However, although platelet counts were significantly different in both protocols, the coagulation results between 15 minutes recommended centrifugation time and five minutes centrifugation time did not differ significantly for abnormal PT, nor-
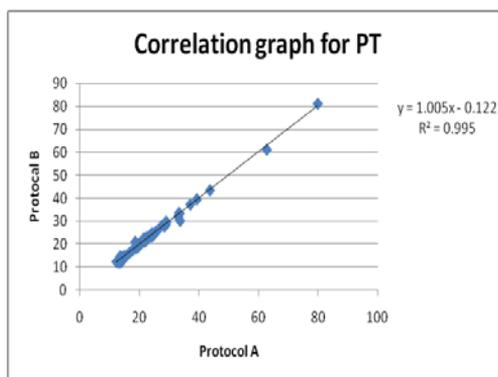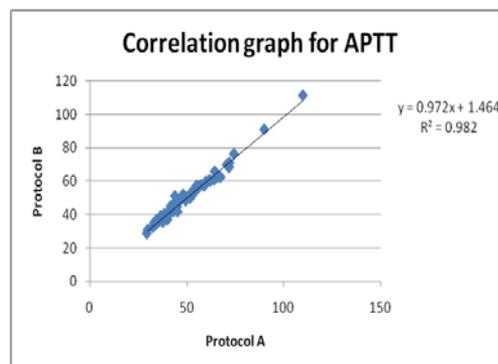

Figure 1: Correlation graph for PT

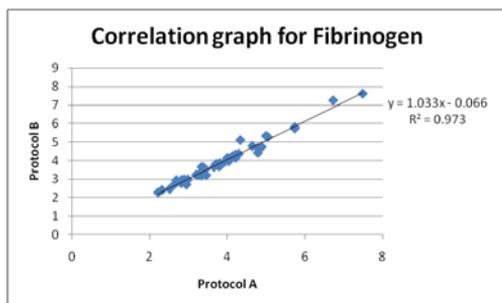Figure 2: Correlation graph for APTT

Figure 3: Correlation graph for Fibrinogen

mal APTT, abnormal APTT and fibrino-gen results (Table 1). This is in agree-ment with a previous study (Barnes et al. 2002). For normal PT results, the differ-ence between 15 minutes centrifugation and five minutes centrifugation had a p value of 0.01. Nevertheless, the mean (13.1s vs 13.3s) and SD (0.58 vs 0.57) did not differ much. In our opinion, this unexpected result may be due to statis-tical reason as a result of a small number of samples for this subgroup, rather than the platelet content in the PPP itself. APTT is more sensitive to platelet con-tamination than PT. Therefore platelet contamination will affect the APTT result more than the PT result, a finding of which was not evident in this study.

When we combined both normal and abnormal PT and APTT results, we ob-serve a good correlation between the two different protocols used in this study (Figure 1 and 2). This is also true for fi-brinogen levels (Figure 3). In view of this, we feel that although the platelet count can be high when a sample is centrifuged for five minutes, it does not affect coa-gulation results in general.

CONCLUSION

This study showed that, although there was presence of residual platelet counts in PPP prepared with five minutes cen-trifugation at 4000 rpm, no significant difference was found for the results of PT, APTT and fibrinogen values. We conclude that this centrifugation time can be used for urgent test requests without compromising the quality and reliability of the results. This contributes to reducing the overall turnaround time for coagula-tion STAT results and thus provides bet-ter medical care for patients with coagu-lation test requests.

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