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ORIGINAL ARTICLE

Comparison of Rapid Centrifugation Technique with Conventional Centrifugation for Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT) Testing

Ekta Chandler[1] · Naveen Kakkar[1] · Rupinder Kaur[1]

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Abstract Prothrombin Time (PT) and activated partial thromboplastin time (APTT) are frequently performed coagulation tests in patients with coagulation disorders especially in critical care areas and in monitoring patients on anticoagulation therapy. In coagulation testing, sample processing especially centrifugation is one of the most critical steps that affect turnaround time (TAT). This study was carried out over a period of 1 year. Three hundred paired samples from patients sent for PT and APTT estimation were included. One sample was centrifuged in a regular bench top centrifuge at 1500*g* for 20 min. The other sample was divided into two polypropylene aliquots and centrifuged in a microcentrifuge at 13000*g* for 3 min. The plasma obtained from both methods was tested for PT and APTT using the automated method on STA Compact coagulometer (Stago) using commercial thromboplastin STA^{R}-Neoplastine^{R} C1 Plus and phospholipid (cephalin), STA^{R}-C K PREST^{R} 5 respectively. Data were analyzed using descriptive statistics, Student *t* test, correlation coefficient and Bland–Altman plots. Mean PT, INR and APTT for both centrifugation methods was comparable with no statistically significant difference ($p > 0.05$). PT, INR and APTT also showed good correlation ($r > 0.98$) when compared between the two methods of centrifugation. Bland–Altman comparison between rapid and conventional methods of centrifugation for PT, INR and APTT also showed acceptable agreement. Rapid centrifugation technique for routine coagulation testing can be used safely with a significant reduction in the TAT. This can benefit patients in critical care settings and those on outpatient oral anticoagulant therapy.

Keywords Centrifugation · Conventional · Rapid · PT · APTT · TAT

Introduction

Prothrombin Time (PT) and activated partial thromboplastin time (APTT) are frequently performed tests for screening coagulation disorders and monitoring patients on anticoagulation therapy [1]. In routine hematology practice, the turn around time (TAT) for the complete blood count has reduced considerably with use of automated hematology analyzers. However, in coagulation testing, sample processing and preparation is one of the most critical steps that affect turnaround time [2].

Centrifugation of the blood sample for 15–30 min is the major rate limiting pre-analytical step in sample processing which increases the TAT of routine coagulation testing [2, 3]. This at times leads to delay in treatment of patients with bleeding disorders especially in critical care settings like operation theatres, emergency rooms or trauma units. Clinical decision making can be faster if the TAT for coagulation results in these settings can be reduced [4–6].

Studies done to reduce the TAT for coagulation testing have used centrifugation with higher centrifugal force and less spin time ranging from 1 to 10 min. The results have been shown to be comparable with routine centrifugation methods with a 50–85% reduction in spin-time [7–9]. This has enormous benefits in management of patients with acute bleeding in emergency settings and also reduces TAT significantly to benefit outpatients and inpatients in whom

✉ Naveen Kakkar
kakkar.naveen@gmail.com

[1] Department of Pathology, Christian Medical College and Hospital, Brown Road, Ludhiana, Punjab 141008, India

coagulation testing is ordered as faster clinical decisions can be made.

Materials and Methods

This prospective study was carried out in the hematology section of a tertiary care hospital over a period of 1 year (2014–2015). Three hundred paired samples from outpatients and inpatients sent for PT and APTT estimation were included. Samples which were overfilled, underfilled or lipemic were excluded. Demographic information and clinical diagnosis of the patient and indication for PT/APTT estimation was noted.

Sample Size

Sample size was calculated using the estimated difference from previous studies with power of 80 and a 95% confidence interval. A sample size of 130 was obtained. However, for the purpose of the study 300 paired samples were studied.

Procedure

For PT/APTT testing, paired blood samples were drawn with 3.2% sodium citrate anticoagulant and processed within 1 h. Tube 1 was centrifuged in a regular bench top centrifuge at 1500*g* (2500 rpm) for 20 min. The blood sample from tube 2 was divided into two polypropylene aliquots and centrifuged in the microtube slots of the fixed angle microcentrifuge, MK5 (Hawksley, England) at 13000*g* (with 11,800 rpm) for 3 min. No brake was used. The supernatant plasma thus obtained by both methods of centrifugation was tested for PT and APTT using the automated method on STA Compact coagulometer (Stago). For PT estimation, a rabbit brain derived commercial thromboplastin STA^{R}-Neoplastine^{R} C1 Plus with an International Sensitivity Index (ISI) of 1.25 was used. APTT was done using commercial rabbit brain derived phospholipid (cephalin), STA^{R}-C K PREST^{R} 5. Commercial Calcium chloride (0.025 M) (STAGO) was used for APTT testing.

Quality Control

Daily controls were run for PT and APTT using commercial plasma (STA- stago). To confirm the adequacy of platelet poor plasma (PPP), platelet count was done on the plasma obtained from 34 random samples out of the 300 samples included (once every week), after rapid centrifugation. Residual platelet results were assessed in accordance with the CLSI recommendations [3] and were less than 10,000/μL in the samples tested. Platelet count was done on BC 3000 plus (Mindray), 3-part automated hematology analyzer. Routine internal quality control for the automated analyser (including for platelet count) was done daily using trilevel (biorad) controls.

Data Analysis

PT, INR and APTT were compared between automated results obtained by conventional centrifugation and rapid centrifugation. Comparison was also done between INR values that were subtherapeutic (< 2.0), within therapeutic range (between 2.0 and 3.0) and those above therapeutic range (INR > 3.0). Data were analyzed using descriptive statistics, Student *t* test, correlation coefficient (r) and Bland–Altman plots for method comparison.

Ethical Approval

The study was approved by the institutional research and ethics committee. Informed consent was taken from all subjects.

Results and Analysis

Three hundred patients studied included children and adults with an age range of 4–87 years with mean age of 48.6 ± 17.6 years. The male to female ratio was 1.4:1. The various indications for coagulation testing in the study are shown in Table 1.

Of the 300 patients, 48 (16%) patients were on anticoagulant therapy. Of these, 39 (81.2%) were on Acitrom (acenocoumarol) and 9 (18.8%) were on heparin. The mean values for PT, INR and APTT for both conventional and rapid centrifugation were comparable with no statistically significant difference ($p > 0.05$) (Table 2). Good correlation was seen for PT (r = 0.993), INR (r = 0.993) and APTT (r = 0.985) when compared between the two methods of centrifugation (Fig. 1). Bland–Altman comparison between rapid and conventional methods of centrifugation for PT, INR and APTT also showed acceptable agreement (Fig. 2).

Of the 300 patients, 252 had INR values (< 2.0), 30 had INR between 2.0 and 3.0 and 18 patients had INR beyond therapeutic range (> 3.0). The INR difference seen between the two techniques of centrifugation for these subgroups was not statistically significant (Table 3, Fig. 3).

Only four patients showed an INR difference between subgroups when compared between rapid and conventional centrifugation techniques. In three patients, the conventional method showed marginally higher values compared to conventional centrifugation (3.0 vs. 2.95, 3.02 vs. 1.95

Table 1 Shows various indications for coagulation testing in the study (n = 300)

Indication	Number	Percentage
Cardiovascular and thromboembolic disorders	69	23
Hepatobiliary and gastrointestinal disorders	67	22.4
Renal and genitourinary disorders	54	18
Hematological diseases	31	10.3
Malignancies	25	8.3
Infective conditions	22	7.3
Cerebrovascular disorders	19	6.3
Respiratory disorders	8	2.7
Miscellaneous disorders	5	1.7

Table 2 Shows the comparison of Prothrombin Time (PT), international normalized ratio (INR) and activated partial thromboplastin time (APTT) between the conventional and rapid centrifugation groups

Parameter	Conventional centrifugation			Rapid centrifugation			*p* value
	Range	95% CI	Mean ± SD	Range	95% CI	Mean ± SD	
PT (s)	10.6–82.1	17.4–19.4	18.4 ± 8.6	10.9–81.4	17.5–19.4	18.4 ± 8.4	0.9999
INR	0.7–10.7	1.40–1.62	1.5 ± 1.1	0.7–10.6	1.38–1.62	1.5 ± 1.1	0.9999
APTT (s)	20.6–129.3	33.2–35.9	34.6 ± 11.8	20.3–125.2	33.4–36.0	34.7 ± 11.7	0.9170

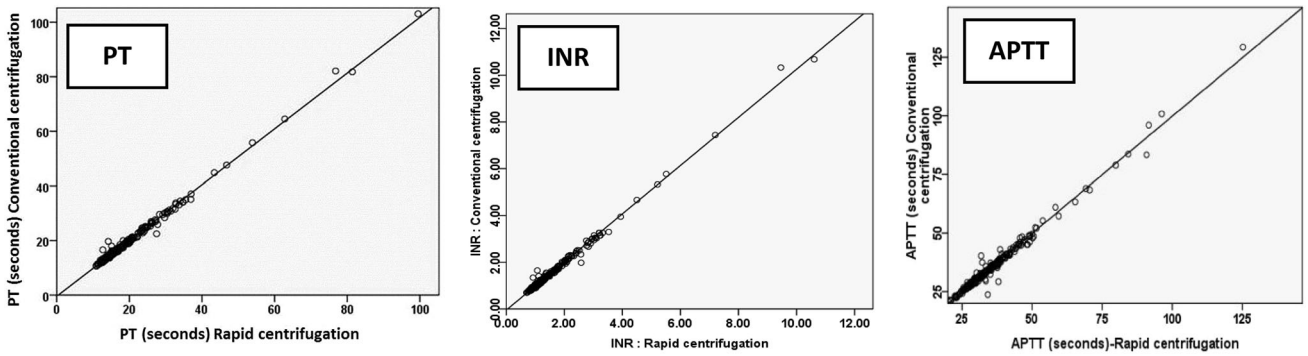

Fig. 1 Shows good correlation for PT (r = 0.993), INR (r = 0.993) and APTT (r = 0.985) values when compared between rapid and conventional methods of centrifugation

and 3.01 vs. 2.97). In one patient, the INR was marginally higher by rapid method when compared to the conventional method (3.02 vs. 2.95).

In all other 296 patients, the INR did not show a change across sub-therapeutic, therapeutic range or above therapeutic range that could potentially affect management decisions. None of the samples in the study showed hemolysis after centrifugation.

Discussion

The present study has shown that acceptable results can be obtained for PT/INR and APTT by rapid centrifugation of samples for 3 min, which are comparable to those obtained by conventional centrifugation for 20 min. The mean clotting times for PT and APTT were comparable ($p > 0.05$) between the rapid centrifugation and the conventional centrifugation groups. INR value was also comparable. Strong correlation was seen for PT ($r^2 = 0.993$), INR ($r^2 = 0.993$) and APTT ($r^2 = 0.985$) when compared between conventional and rapid centrifugation methods. Bland–Altman method showed acceptable bias and 95% limit of agreement for PT, INR and APTT values. No statistically or clinically significant difference between PT and APTT values was seen for both types of centrifugation.

A study from New York in 146 patients for routine coagulation testing compared rapid sample centrifugation for 2 min at 11200*g* with that for 10 min at 1150*g*. Similar to our study, good correlation was seen for PT (r = 0.992) and APTT (r = 0.971) with both centrifugation speeds [7].

Another study on 90 paired coagulation samples compared PT and APTT values by centrifugation of samples at 1400*g* for 20 min and at 11000*g* for 2 min. Mean PT and APTT values were comparable between the two groups.

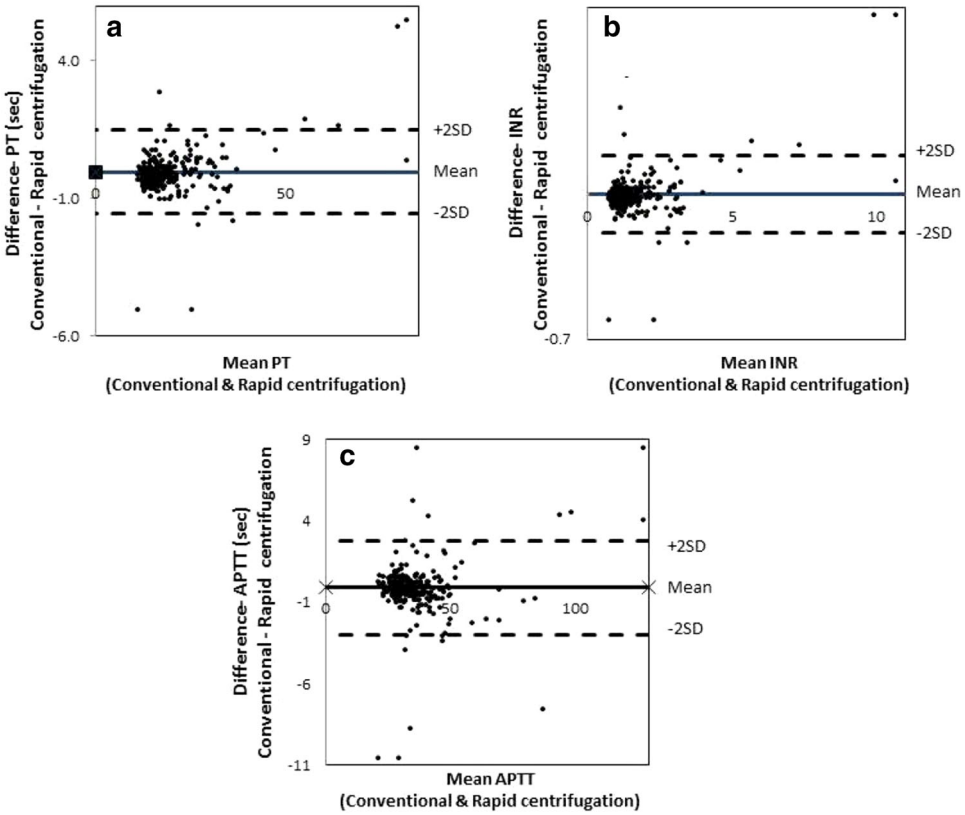

Fig. 2 Bland Altman plots showing acceptable agreement for PT (**a**), INR (**b**) and APTT (**c**) values when compared between rapid and conventional methods of centrifugation

Table 3 Shows the comparison of international normalized ratio (INR) for patients with INR < 2.0, between 2.0 and 3.0 and those with INR > 3.0 between the conventional and rapid centrifugation groups

INR groups	Number of patients	Conventional centrifugation Mean ± SD	Rapid centrifugation Mean ± SD	*p* value
<2.0	252	1.17 ± 0.29	1.18 ± 0.29	0.6989
2.0–3.0	30	2.39 ± 0.31	2.38 ± 0.33	0.9041
>3.0	18	4.60 ± 2.47	4.52 ± 2.31	0.9206

Similar to our study, this study reported good correlation for PT (r = 0.99) and APTT (r = 0.97) between the two centrifugation methods [10].

Nelson et al. [11] used routine centrifugation at 1800*g* for 15 min and rapid centrifugation at 11000*g* for 2 min for comparing PT and APTT. They also reported good correlation for PT (r = 0.99) and APTT (r = 0.96) [11].

A Taiwan based study compared PT values of 10 volunteers (healthy subjects) by centrifugation of samples at 7000*g* for 1 min and with centrifugation at 1500*g* for 15 min. There was no statistically significant difference between the two methods for PT ($p = 0.192$) and APTT ($p = 0.918$).

Both PT and APTT also showed good correlation (r = 0.996 r = 0.984) respectively. INR values also showed good correlation ($p = 0.224$, r = 0.996) [9]. Another study on 152 PT and 146 APTT samples also good correlation for PT (r = 0.992) and APTT (r = 0.971) with both conventional and rapid centrifugation [12]. Sultan et al. while comparing conventional and rapid techniques reported a mean of 1.01 ± 0.05 and 1.01 ± 0.05 respectively for INR similar to the present study. The authors found no statistical difference between INR ($p > 0.05$) using conventional and rapid centrifugation. However, unlike the present study, this study involved only healthy subjects [13].

Other studies have also reported good correlation for coagulation testing between conventional and rapid centrifugation [8, 14–16].

In our study, only patients showed an INR difference between subgroups when compared between rapid and

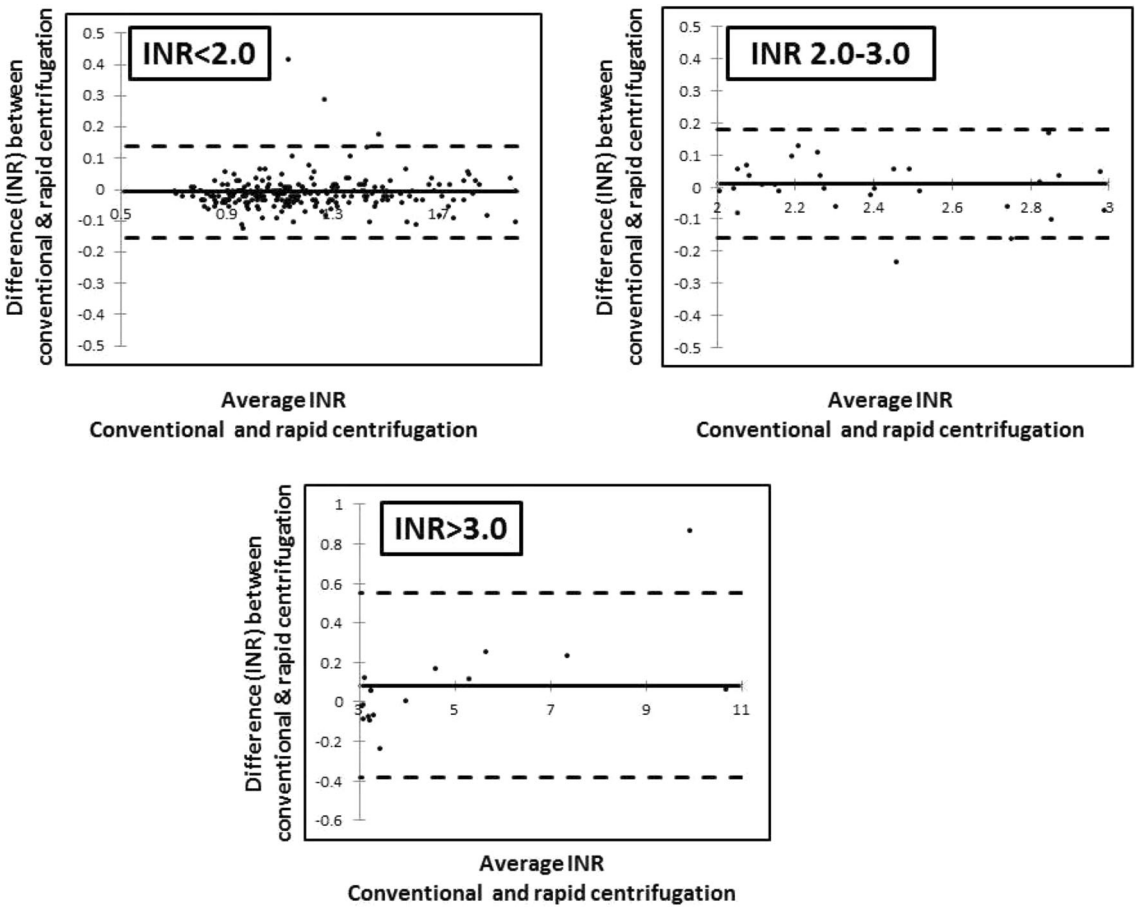

Fig. 3 Bland Altman plots showing acceptable agreement for INR values for different subgroups when compared between rapid and conventional methods of centrifugation

conventional centrifugation techniques. However this was marginal and would not potentially affect dosage decisions in patients on anticoagulant therapy.

Our study did not show any significant hemolysis due to rapid centrifugation. No brake was used on the centrifuge which could have been the reason for smooth rapid centrifugation. Also the time for which the samples were centrifuged was very short (3 min).

Hemolysis of blood samples can adversely affect sample quality for coagulation testing with interference in automated determination especially in optical coagulometers [17, 18]. The problems anticipated with high centrifugation speed of blood samples for coagulation tests include risk of hemolysis, loss of activity of coagulation factors due to generation of heat and introduction of preanalytic errors by dividing samples in aliquots. Also, laboratories would need to invest in more expensive high speed centrifuges. A study on the effect of hemolysis on PT and APTT showed the results are not significantly affected and heat generation does not seem to affect the factor activity adversely as the time for spin is very short [19].

The findings of the present study have the potential to significantly reduce the TAT for routine PT/APTT testing by 17 min, an 85% reduction in the existing centrifugation time of 20 min. In coagulation testing which has a TAT of 45–60 min, this is a significant reduction and can positively impact management decisions in patients especially those in critical care settings like trauma units and operation theatres. This will also reduce the waiting time of outpatients on oral anticoagulant monitoring. Although this study was done in patients requiring routine PT and APTT testing, the results may have implications for reducing TAT even for specialized coagulation testing like fibrinogen levels and factor assays for which more studies need to be done.

Conclusion

The present study showed comparable results between conventional and rapid centrifugation techniques for PT and APTT estimation with a significant reduction in the TAT. This can benefit patients in critical care settings and those on outpatient oral anticoagulant therapy.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no competing interests.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No patient/subject identifying information has been disclosed in the manuscript. No patient/subject intervention was done and the subjects were not exposed to any risks during the study.

Informed Consent Informed consent was taken from the patients included in the study.

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