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EVALUATION OF aPTT AND PT RESULTS: A COMPARISON BETWEEN POINT-OF-CARE TESTING AND OPTICAL FULLY AUTOMATIC METHODS.

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ABSTRACT

A coagulometer is a tool used to check coagulation parameters, such as prothrombin time (PT) and activated partial thromboplastin time (aPTT), for which there are currently various detection methods. Examining PT and aPTT coagulation function requires a fast turnaround time and immediate results because the stability of coagulation factors is less than four hours, so a fast and precise examination is needed. Point-of-care testing (POCT) is a good alternative. This study aimed to determine the difference in aPTT and PT values between POCT and optical fully automatic. This comparison will be useful when selecting coagulometer devices. Methods: This study used a cross-sectional study with a non-probabilistic sample of 100 citrate samples. Each sample was tested for aPTT and PT using POCT and optical fully automatic testing. The results of each examination were analyzed using the Mann–Whitney, Bland-Altman Plot, and correlation statistical tests. In this study, precision tests were also carried out on both methods. methods. There was a significant difference ($p < 0.001$) in the comparative test results for the aPTT and PT values obtained using the POCT method compared to the optical fully automatic method. The average difference between the two methods was 6.6 seconds for the aPTT parameter and 4.98 for the PT parameter. Fourteen samples were found to have different interpretation values for the aPTT parameter, and 28 samples for the PT parameter. There is a significant difference between the POCT method and the optical fully automatic method. The precision test on the POCT method produced good results.

Keywords: aPTT; optical fully automatic; point-of-care testing; PT

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INTRODUCTION

Coagulation tests are performed to identify blood clotting disorders. Prothrombin time (PT) and activated partial thromboplastin time (aPTT) are blood tests used to assess the blood's ability to clot. PT and aPTT are common hemostasis tests, especially before surgery. (Pagana KD et al., 2019) Activated Partial Thromboplastin Time (aPTT) is a screening test used to detect abnormalities in the intrinsic and shared pathway clotting factors, including F XII, F XI, F IX, F VIII, F X, F V, prothrombin, and fibrinogen. If the aPTT is prolonged, it indicates a deficiency in these pathway factors. Prothrombin time (PT) is a test used to evaluate hemostasis in the extrinsic and shared pathways, which involve fibrinogen, prothrombin, factor V, factor VII, and factor X, and to monitor patients on oral anticoagulants. (Bachler M et al., 2019) A coagulometer is a device used for PT and aPTT tests, and it employs various detection methods. These methods can be manual, semi-automatic, or fully automatic. The coagulometer's measurement principles include electromechanical and photo-optical techniques. The electromechanical method uses a rotating cuvette with a steel ball and measures the impedance of the rotating ball, while the photo-optical method detects light scattering during the coagulation test. (Hernaningsih Y & Butarbutar T., 2019) The coagulation function examination of PT and aPTT requires a fast turnaround time, immediate

results, and stability of coagulation factors within less than 4 hours, so it needs a quick and accurate test. Therefore, the availability of point-of-care testing (POCT) is a good alternative. Coagulation testing with POCT has been shown to be useful in speeding up emergency care. (Ebner M et al., 2017) The main advantage of the POCT device is the short duration of the test (25-45 minutes) compared to conventional laboratory tests for coagulation (40-60 minutes). (Mohammadi Aria M et al., 2019) In Kok et al's study, there was a lack of concordance between POCT aPTT results and those obtained from the coagulometer in the laboratory. Meanwhile, Fitch et al demonstrated a significant correlation of PT/INR and aPTT values between POCT and laboratory instruments. The discrepancies in results between these two studies may be due to differences in the principles used to detect clotting time, such as photoelectricity versus viscoelasticity. (Karigowda L et al., 2019) Based on previous studies, there are still differences in the accuracy of POCT compared to laboratory coagulometers, likely due to different research designs. Therefore, this researcher aims to determine the difference in aPTT and PT values between POCT and optical fully automatic method.

METHOD

This type of research is observational and analytical with a cross-sectional design, where data collection involves patient laboratory examination data to compare aPTT and PT values obtained through the POCT method with those from the optical fully automatic method. The study was conducted at Adam Malik Hospital, using samples that met both the inclusion and exclusion criteria. The inclusion criteria involved citrated venous whole blood samples for the POCT method and sufficient citrate sample volume (blood/anticoagulant ratio 9:1). Exclusion criteria included haemolysis, icteric, and lipemic (HIL) samples, as well as plasma citrate samples collected more than 4 hours before testing. For each sample, an aPTT and PT/INR test was performed using both POCT and optical fully automatic method. The researchers also did a within-run precision test using one normal sample that was repeated 21 times for aPTT and PT parameters across both methods. Data analysis involved statistical comparisons using the Mann-Whitney, Bland Altman and corelation to assess differences in aPTT and PT values between the POCT and fully automatic optical methods. All statistical tests with a p-value <0.05 was considered statistically significant.

The POCT method uses the Wondfo OCG-102 device with a 20 µL sample of citrated venous whole blood. The sample reacts with aPTT and PT reagents, initiating the coagulation system. An optical method detects the occurrence of clots, and the results are expressed in seconds for the aPTT value and the PT value (in International Normalized Ratio, INR). The POCT method uses disposable test strips containing dry reagents.

Optical Fully Automatic Method uses the Sysmex CN 3000 device with venous citrate plasma samples at a blood-to-anticoagulant ratio of 9:1. The aPTT reagent is Dade Actin FS (purified soy phosphatides and plasma activator), and the PT reagent is Dade Innovin (human recombinant thromboplastin and calcium). In the fully automatic optical method, the examination measures the change in plasma turbidity in response to plasma activation with the aid of light.

RESULT

aPTT and PT tests were conducted on 100 samples. A total of 100 participants were included in the study. The median age of the participants was 45 years (range: 5–74 years). Of these participants, 58 (58%) were male and 42 (42%) were female. As can be seen in Table 1, the median PT value was 15.45 seconds for the POCT method and 11.9 seconds for the optical fully automatic. Median for INR value was 1.37 for the POCT method and 1.09 for the optical fully automatic method. For the aPTT parameter, the median values were found to be 32.65 seconds and 26.4 seconds,

respectively. A significant difference was found in the results of the INR value comparison test between the two examination methods (p<0.001). Similarly, a significant difference in aPTT values was found between the two methods (p < 0.001).

Table 1.
Results of PT, INR and aPTT examinations using the POCT method with Optical Fully Automatic

Variable	POCT	Optical Fully Automatic	P Value*
	Median (Min-Max)	Median (Min-Max)	
PT	15.45 (11.5 – 90.0)	11.9 (9.6 – 80.0)	<0.001
INR	1.37 (1.00 – 9.01)	1.09 (0.86 – 6.01)	<0.001
aPTT	32.65 (25.90 – 55.70)	26.4 (19.90 – 99.60)	<0.001

**Mann-Whitney Test*.

The mean difference in APTT values between POCT and optical fully automatic measurements was 4.35 ± 7.66, with a limit of agreement of 2.827–5.868. The *one sample t-test* yielded a *p*-value of <0.001, indicating a statistically significant difference in the mean APTT values between the two methods. Therefore, it can be concluded that there is no agreement between POCT and optical fully automatic methods for APTT measurement. (Table 2)

Table 2.
Conformity test of APTT measurement based on POCT and Optical Fully Automatic.

Parameters	APTT
Range	-51,7 – 18,10
Mean ± SD	4,35 ± 7,66
95% CI	2,827 – 5,868
p value	<0,001

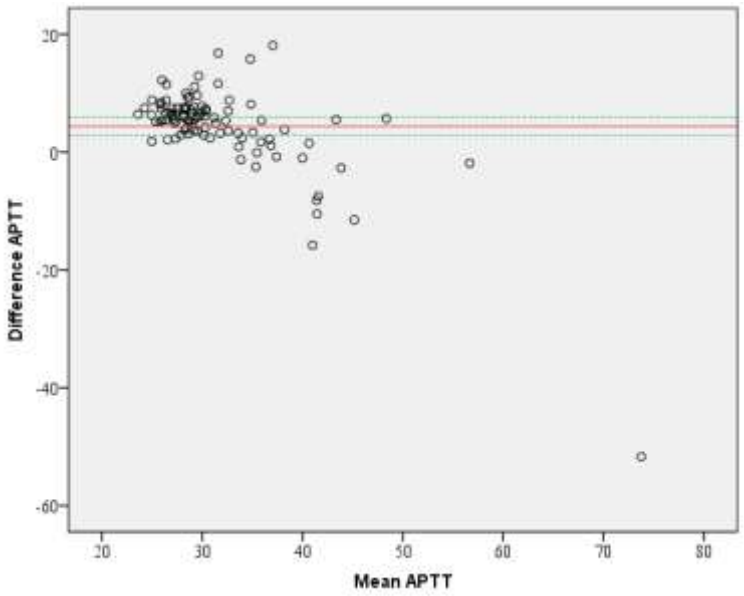

Figure 1. Bland-Altman plot graph of APTT test result accuracy based on measurements from POCT and optical fully automatic devices.

The Bland-Altman plot in Figure 1 demonstrates that several data points fall outside the limit of agreement (2.827–5.868), which are considered outliers. The presence of these outliers indicates a significant discrepancy between the two measurement methods, further supporting the finding that APTT results obtained from POCT and optical fully automatic methods are not in agreement. Table 3 presents the agreement analysis of PT values between POCT and optical fully automatic methods. The mean difference in PT values was 4,73 ± 6,47, with a limit of agreement of 3,445 – 6,013. The *one sample t-test* yielded a *p*-value of <0.001, indicating a statistically significant difference in the

mean PT values between the two measurement methods. Therefore, it can be concluded that there is no agreement between POCT and optical fully automatic methods for PT measurement.

Table 3.
Conformity test of PT measurement based on POCT and Optical Fully Automatic

Parameters	PT
Range	-12,6 – 46
Mean ± SD	4,73 ± 6,47
95% CI	3,445 – 6,013
p value	<0,001

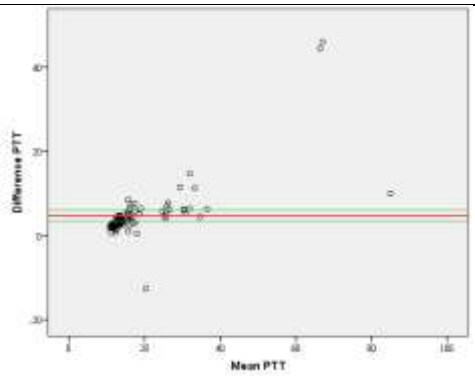

Figure 2. Bland-Altman plot graph of PT test result accuracy based on measurements from POCT and optical fully automatic devices.

Figure 2. shows that the Bland-Altman plot indicates many points outside the limit of agreement (3.445 - 6.013), which are therefore outliers. These outliers suggest a significant difference between the two measurement methods.

Table 4.
APTT, PT And INR Correlation Test Based on POCT and Using a Optical Fully Automatic

		Optical Fully Automatic	
		p*	r
APTT	POCT	<0,001	0,728
PT	POCT	<0,001	0,886
INR	POCT	<0,001	0,886

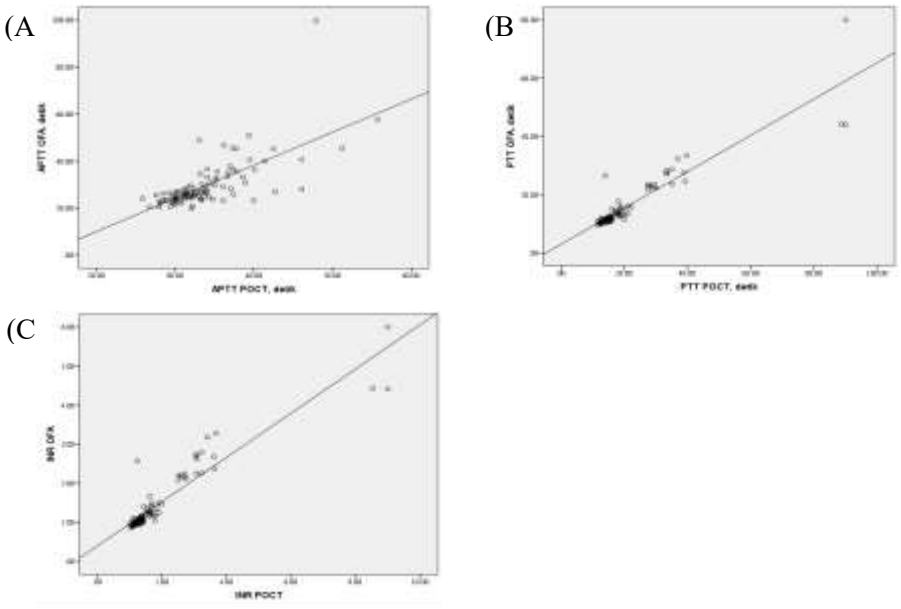

Figure 3. Scatterplot graph illustrates the correlation between APTT (A), PT (B), and INR (C) values obtained through POCT and optical fully automatic testing.

Spearman's correlation test demonstrated a significant correlation between APTT measurements obtained using POCT and optical fully automatic methods (p<0.001). The correlation coefficient was 0.728, indicating a strong positive correlation (r >0.6–0.8). For PT, the analysis also revealed a significant correlation between the POCT and optical fully automatic test results (p<0.001). The correlation value obtained was 0.886, indicating a positive correlation of very strong intensity (r >0.8-1) between the two tests. A significant correlation was also found between the POCT and optical fully automatic test results for the INR parameter (p<0.001).The correlation coefficient was 0.886, suggesting a very strong positive correlation (r >0.8–1).

Table 5.
Precision testing of within-run aPTT and PT in POCT and Optical Fully Automatic methods

Test	aPTT		PT	
	Optical Fully Automatic	POCT	Optical Fully Automatic	POCT
1	28,1	32,8	12,5	13,4
2	27,7	32,9	12,5	13,6
3	27,4	32,2	12,2	13,2
4	27,9	33,2	12,4	12,9
5	27,5	32,8	12,4	13,5
6	27,2	32,7	10,6	14,2
7	27,8	32,3	12,4	13,9
8	27,6	33,3	10,7	14,7
9	27,1	33,2	10,5	13,8
10	27,7	33,8	10,7	13,5
11	27,3	32,9	10,6	13,3
12	26,9	33,5	10,5	13,7
13	27,9	33,1	10,7	13,2
14	27,5	32,4	10,7	13,3
15	27,1	34,2	10,5	13,4
16	27,1	33,3	11,2	13,9
17	27,5	33,1	10,7	13,7
18	27,5	32,9	10,6	12,9
19	28,9	33,5	11,1	13,5
20	28,9	32,5	10,9	12,9
21	28,9	33,3	11	13,5
Mean	27,69	33,0	11,2	13,5
SD	0,59	0,49	0,79	0,43
CV (%)	2,13	1,48	7,05	3,18

For the APTT parameter, out of 100 samples, 14 showed differences in interpretation between POCT and optical fully automatic methods. For PT, 28 samples demonstrated such differences, while for INR, 19 samples showed discrepancies. The mean differences between the two methods were 6.6 seconds for APTT, 4.98 seconds for PT, and 0.42 for INR. This study also performed a within-run precision test (table 5) using one normal-level sample, repeated 21 times for both aPTT and PT parameters, with the POCT and optical fully automatic methods. The precision results showed that, for POCT, the coefficient of variation (CV%) was 1.48% for aPTT and 3.18% for PT. In contrast, the optical fully automatic method yielded higher CV% values, namely 2.13% for aPTT and 7.05% for PT.

DISCUSSION

Haemostasis is the physiological process by which blood loss due to blood vessel injury is minimised through clotting, ensuring that blood flow within the vessels remains normal. Haemostasis involves mechanisms that form clots at the site of injury, prevent excessive clot formation, and dissolve clots. It is characterised by a series of reactions involving platelets, erythrocytes, coagulation factors, and anticoagulants, which occur in three stages. (Louka M & Kaliviotis E., 2021). The extrinsic and intrinsic pathways can be used for screening in vitro laboratory tests by extracting parameters such as PT and aPTT. PT and aPTT indicate the time required for blood clot formation in plasma involving calcium and thromboplastin; the magnitude of this time depends on device-specific parameters. (Pagana KD et al., 2019)

Laboratory testing has a long turnaround time and is expensive, thus increasing healthcare costs. To meet the need for comprehensive POCT, viscoelastic testing provides a rapid alternative to routine laboratory testing, enabling real-time assessment of global haemostasis. (Tshikudi DM et al., 2017) A key benefit of POCT devices is that examinations take 25–45 minutes, compared to 40–60 minutes for conventional coagulation testing. (Aria MM et al., 2019) Various laboratory equipment and POCT devices have been developed over the years for assessing blood coagulation using different blood properties. (Louka M & Kaliviotis E., 2021). This study assessed differences in PT and INR parameters using the POCT method compared to a optical fully automatic method. The median PT and INR parameter value was higher using the POCT method (15.45 seconds; 1.37) than the optical fully automatic method (11.9 seconds; 1.09). Consistent with previous research by Nam et al., the INR value obtained using the POCT Coagucheck Pro II method was higher than the INR value obtained using laboratory equipment with STA-R Max. Notably, the average difference in INR values between the Coagucheck Pro II and STA-R Max methods increased as the INR value increased. (Nam M et al., 2021) However, this finding contrasts with the results of a study by Kim et al., which compared POCT using the CoaguCheck XS Plus device with laboratory coagulometry using the ACL TOP 750 device, the results showed no difference in the average INR value between the two devices in the INR ≤3 group. (Kim, Y. S., et al., 2022) (2007).

Although the INR was introduced to reduce inter-laboratory variability, this variability remains clinically significant due to variations in instrumentation and reagent composition. INR values obtained using human rTF thromboplastin, which is more sensitive to protein-induced vitamin K antagonists (PIVKA), are higher than those obtained using rabbit thromboplastin, which is less sensitive to PIVKA. (Nam M et al., 2021). In this study, the median aPTT value obtained using the POCT method was 32.65 seconds, which was higher than the value obtained using the optical fully automatic method (26.4 seconds). However, this study contradicts the findings of Surman et al., who obtained a lower median aPTT POCT value of 55.90 seconds with intravenous samples, compared to the intravenous laboratory aPTT median value of 75 seconds. (Surman TL et al., 2018) This discrepancy is due to the lack of standardisation between different aPTT tests. Therefore, it is necessary to evaluate the concordance analysis approach used to verify whether the same clinical judgement can be achieved with the POCT aPTT examination as with the laboratory aPTT examination when used as a reference method.

The Mann-Whitney test results comparing the POCT and optical fully automatic methods showed significant differences in PT and aPTT values for both methods ($p < 0.001$). The Bland-Altman test showed no agreement between POCT and optical fully automatic measurements for the aPTT and INR parameters. However, Niederdöckl et al. found that POCT PT and aPTT showed concordance with laboratory PT and aPTT, respectively. The relative difference in results between capillary and venous blood was 0.2% for POCT PT and 8.4% for POCT aPTT. The coefficient of variation for repeat POCT PT using whole blood was found to be between 2% and 3.6%. (Niederdöckl J et al., 2016). The Spearman correlation test revealed a positive correlation between the aPTT, PT and INR values obtained using the POCT method and the optical fully automatic method, with respective results of 0.728 and 0.886. Thabet et al. also demonstrated a strong correlation between INR measurements obtained using POCT and those obtained using standard laboratory tests ($r = 0.95$). Therefore, POCT can be considered an effective alternative to standard laboratory tests for assessing INR within the therapeutic and supra-therapeutic ranges. (Thabet A et al., 2021)

Baker et al. demonstrated that the CoaguChek XS and the Stago, which utilise recombinant human (ISI = 1.01) and rabbit brain (ISI = 1.25) thromboplastins, respectively, exhibited good agreement for INRs below 3.0 ($k = 0.62$), but significant discrepancies for INRs above 3.0 ($k = 0.10$). In contrast, CoaguChek XS and Siemens BCS XP, which both use human recombinant thromboplastin (ISI = 1.02), showed greater concordance for INR values (INR <3.0: $k = 0.84$; INR ≥3.0: $k = 0.70$). (Baker WS et al., 2017). Karigowda et al. found that the mean turnaround time for POCT aPTT

results was significantly shorter than that for laboratory aPTT results (5.0 ± 0.2 minutes vs. 64.6 ± 2.7 minutes, $p < 0.001$). When POCT aPTT results obtained in under 90 seconds using whole blood were compared with laboratory aPTT results, the limits of agreement narrowed (-23.243 to 28.419) and the percentage bias decreased to 5%. Agreement between clinical decisions regarding heparin dosing based on the two methods was not optimal with either plain or citrated blood (κ values of 0.35 and 0.11, respectively). The POCT aPTT test produced faster results than standard laboratory tests. However, these results are not accurate enough for use with critically ill patients receiving heparin infusions. (Arachchillage DRJ et al., 2019)

Based on the CLSI H47, 3rd edition, the total daily coefficient of variation (CV%) for PT and aPTT from the analytical system should be less than 5% in both normal and abnormal control plasma. (CLSI Guideline, 2023) In this study, a within-run precision test was carried out using one sample of normal levels, repeated 21 times for the aPTT and PT parameters on each POCT and optical fully automatic device method. The results of the POCT method precision test showed a CV% of 1.48% for aPTT and 3.18% for PT. The optical fully automatic method had a higher CV%: aPTT 2.13% and PT 7.05%. According to Dorn-Beineke et al., the electromechanical method has the advantage of being unaffected by turbid samples in icteric, haemolytic, and lipemic cases. However, another study by Lefkowitz et al. showed that the photo-optical method has advantages in cases of dysfibrinogenaemia due to familial mutations. (Yunensie PA et al., 2021).

Conventional coagulation tests generally assess a patient's coagulation status by measuring aPTT, PT, platelet count, and fibrinogen levels. This process requires a lot of time to separate plasma, platelets, and other components from the patient's blood. (Gong J et al., 2021) Coagulation parameter analysis devices have been used as part of laboratory automation in central laboratories to reduce total turnaround time, thereby saving costs and labour. (Hörber S et al., 2020) In addition, point-of-care testing (POCT) devices allow patients to self-monitor using capillary blood. Although POCT testing is more expensive than laboratory testing, it remains cost-effective when completion time and labor savings are considered. (Şahingöz GE et al., 2021).

CONCLUSION

The PT and aPTT comparison test results showed significant differences between the point-of-care testing (POCT) method and the optical fully automatic method. However, the POCT method demonstrated good precision, suggesting that it can serve as an alternative for coagulation testing.

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