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Detection of individual responses to clopidogrel: validation of a novel, rapid analysis using TEG 6s.

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Abstract

Introduction

There is potential value in testing individual response to P2Y12 inhibitors in order to predict ischaemic and bleeding risk in patients undergoing percutaneous coronary intervention. The aims of this study were:(a) to validate the ability of a novel point of care(POC) assay, TEG 6s, to detect changes in ADP-induced whole blood clotting in volunteers and patients given clopidogrel using TEG 5000 as a reference and (b) to compare a novel, rapid parameter, area under the curve at 15 minutes(AUC15), with the traditional maximum clot amplitude (MA) in TEG 6s.

Methods

25 participants were included in whom ADP-induced clotting was measured at 4 time points:a) 12 healthy volunteers given 600mg of clopidogrel;b) 12 patients with ACS given 600mg of clopidogrel c) 1 healthy volunteer given 600mg of clopidogrel on 5 separate occasions. All samples were tested using conventional TEG 5000 and the new point of care TEG 6S, and a new parameter called AUC15 was compared with MA in TEG6s.

Results

1.TEG 5000 and TEG 6s both detected changes in ADP-induced platelet activation. Bland Altman analysis demonstrated a good level of agreement between them.

2.For TEG 6S, correlation between MA and the novel AUC15 was strong for both thrombin and ADP channels ($R^2=0.867$, $R=0.936$, $p<0.001$), and the AUC15 result was available on average 13.3 mins earlier.

Conclusions

TEG 6s is a rapid, easy to use and accurate test of ADP-induced clotting using TEG 5000 as a reference. A novel parameter, AUC15, is a viable, time-saving option for this test and has potential value in personalised P2Y12 inhibitor therapy.

Keywords

clopidogrel, platelet function test, stents, thrombelastography, thrombosis

Introduction

Platelets play a key role in the pathophysiology of atherothrombotic events including myocardial infarction (MI) and stent thrombosis (ST). Conventional antiplatelet treatment after implantation of coronary drug eluting stent (DES) consists of aspirin in combination with a $P2Y_{12}$ inhibitor (often clopidogrel). It is well established that there is a variable individual response to clopidogrel, and that patients who have high on treatment platelet reactivity (HTPR) are at increased risk of subsequent ischaemic events, including ST. (1-6) Newer and stronger $P2Y_{12}$ inhibitors such as prasugrel and ticagrelor are, on a population basis, more effective at reducing risk of ischaemic complications but are also associated with increased risk of bleeding, although it is unclear whether this risk can be predicted in an individual by the level of response to these agents. (7, 8) In addition to this, there is also evidence that some patients do not have a therapeutic response to these agents. (9, 10)

Given the consequences of a subtherapeutic response to clopidogrel (or other $P2Y_{12}$ inhibitors), and the potential to establish a "therapeutic window" for these agents which minimises the risk of ischaemic or bleeding events, it may be suboptimal that there is no routine testing of the individual response to them. This is the case for two main reasons. Firstly, previous randomised trials of tailored $P2Y_{12}$ inhibitor therapy in DES patients exhibiting low response to clopidogrel using the VerifyNow point of care (POC) assay have been negative. (11-13) In fact, recent data call into question the appropriateness of VerifyNow for this task. (14, 15) Secondly, apart from VerifyNow there is no other cheap, quick, accurate and genuinely POC platelet function test (PFT). Specifically, no POC test has adequate clinical outcomes data to justify a robust and clinically meaningful cut off value that describes response in terms of risk of ischaemic or bleeding events.

Thrombeslatography (TEG) is an *ex vivo* whole blood clotting assay. (16) Using agonists that

predominantly stimulate platelet-induced clotting, TEG5000 represents a well established PFT, that correlates with aggregometry. (4, 17-23) However, TEG5000 requires specialist training and is not POC. The recently developed TEG 6s system (Haemonetics Corp, Massachusetts, USA) represents a genuinely near patient and user friendly test that has shown promise. (24, 25)

The aims of these experiments were as follows. Firstly, to validate the ability of TEG 6s to detect individualised response of patients and volunteers to clopidogrel, using TEG 5000 as a reference. Secondly, to test a novel parameter, area under the curve at 15 minutes (AUC15), in the TEG 6s assay in order to determine if it is possible to provide an accurate assessment of the response to clopidogrel significantly quicker than by conventional TEG 6s test.

Methods

Ethical approval

The study was sponsored by University Hospital Southampton NHS Foundation Trust, approved by National Research Ethics Service Committee Yorkshire & The Humber – Leeds West (REC reference: 14/YH/1278, IRAS project ID: 166264) and registered on the National Institute for Health Research portfolio database. Written informed consent was obtained from all study participants.

Study population

This was a single centre, prospective, observational study conducted at University Hospital Southampton NHS Foundation Trust. The following study groups were recruited: a) group 1- twelve healthy volunteers, who received clopidogrel 600 milligrams (mg) once and had blood sampling at baseline (pre drug) then 2 hours, 6 hours and 24 hours after clopidogrel ingestion; b) group 2 - twelve patients with suspected Non-ST Elevation Myocardial Infarction (NSTEMI), who received clopidogrel 600 mg and underwent blood sampling at baseline (pre

drug) then 2 hours, 6-18 hours and 24 hours after clopidogrel ingestion; c) one healthy volunteer, who received clopidogrel 600 mg on five separate occasions, at least two weeks apart, and underwent blood sampling at baseline (pre drug) and 6 hours after clopidogrel ingestion, in order to assess for intra-individual reproducibility. Inclusion and exclusion criteria are shown as supplementary Table 1.

Blood sampling

At each sampling interval, venesection was performed from the antecubital fossa using a BD Vacutainer® 21G needle and tourniquet. The first 2 ml of blood were discarded. Blood was then drawn firstly into a 2.7 ml Sodium Citrate BD Vacutainer® for thrombin channel analysis. For TEG 5000 platelet mapping channels and all TEG 6s channels blood was collected into 4.0 ml Sodium Heparin BD Vacutainers®. The blood bottles were inverted an appropriate number of times as per the manufacturer's instructions and each sample was analysed with the TEG5 000 and TEG 6s devices after fifteen minutes and within two hours of venesection. The time frame we used is consistent with the instructions provided by the manufacturer and included in the thrombelastography manual. The group has extensive experience of this methodology. (26, 27)

Thrombelastography

TEG 5000 system

The TEG 5000 device is a platelet function analyser which utilises the modified TEG platelet mapping system to determine individual response to APT. This method has been described in detail previously. (16) In brief, 3 agonist channels were run using: a) kaolin, b) activator F (a mixture of reptilase and factor XIIIa) and c) activator F and ADP (2 μM). 360 μL whole blood is pipetted into a disposable cup together with the appropriate reagents. The cup is heated to 37°C and oscillates by 4 degrees 45' at a frequency of 0.1 Hertz. A stationary

pin attached to a wire is suspended within it. As coagulation occurs, the strength of the resultant clot produces torsion on the pin which generates an electrical signal of varying magnitude. This is converted into a graphical trace by the device and displayed on a connected desktop computer.

TEG 6s system

TEG 6s is a new, automated PFT which utilises microfluidic cartridges to produce a value of the clot strength, using the same parameters as TEG 5000. To determine individual response to clopidogrel, the ADP platelet mapping microfluidic cartridge was used. This contains three different reagents: a) dried kaolin and heparinase (concentration >1800 IU/ml of blood), b) dried activator F and Reopro®, c) dried ADP and activator F.

TEG 6S determines clot viscoelasticity using the resonance method (Supplementary Figure 1). A cartridge is inserted into the device and an unmetered amount of heparinised blood (~300 μL) is added. The platelet mapping cartridges contain channels which are preloaded with the appropriate reagents. Following the initiation of sampling, the device draws a controlled volume of blood through to each channel which mixes with the reagents and excess blood then passes to a waste chamber. Each channel is then exposed to a fixed vibration frequency of between (20-500 Hz), the resultant motion of the blood meniscus is measured by an LED detector. The device determines the resonant frequencies of this movement using a Fast Fourier Transform, producing a TEG readout using a mapping function. Stronger clots have higher resonant frequencies and therefore produce a TEG trace of greater amplitude.

Area under the curve at 15 minutes and short thrombelastography

Traditionally, antiplatelet therapy response has been determined from the maximum amplitude (MA) of the TEG trace, which represents the strength of final clot. However, even using TEG 6s, the MA is often not reached until >25 minutes for adenosine diphosphate

(ADP) and >45 minutes for thrombin channels respectively. This group has developed and validated a TEG modification known as `Short-TEG` (sTEG) using TEG 5000 (28, 29). sTEG utilises a parameter called AUC15, which provides relevant and reproducible results at 15 minutes. It combines the strength of a clot and the rate of its formation, which reflects the complete picture of haemostasis. AUC15 is calculated using software written in Southampton: Areafinder 2:1 (National Instrument Labview 7.0), with two parameters needed for calculations – MA and corrected time (total time – R time). [Figure 1] R time is measured in minutes from the initiation of the specimen analysis to initiation of clot formation, as determined by the first separation of the TEG trace. It relates to an amplitude of 2 mm and represents the primary phase of plasma clotting and inhibitor activity. We have applied the AUC15 test to TEG 6s for the first time.

Statistical analysis

Continuous variables are presented as mean and standard deviation (SD) of the mean if normally distributed, or as median and interquartile range (IQR) if not. Normality of data was tested using the Shapiro-Wilk test. Categorical variables were presented as frequencies with percentage. Reproducibility of results was assessed by measuring the IQR/median ratio. Paired sample t-test was used to determine the difference between the devices at each time point and repeated-measures analysis of variance (ANOVA) was used to determine the difference in continuous variables over separate time points for each device. Statistical significance was considered at $p<0.05$ at all times, with Bonferroni's adjustment used for multiple comparisons. Outputs of the two TEG methods allowed direct comparison between devices. Correlation between continuous variables was assessed using Pearson`s correlation for parametric data and Spearman's rank correlation for non-parametric data. The level of agreement between the data derived from two devices was assessed using Bland Altman

analysis.

Results

Study participants and baseline characteristics

12 healthy volunteers [Group 1] and 12 patients [Group 2] were recruited, as well as one volunteer for the reproducibility experiment.

All subjects received a loading dose of 600 mg of clopidogrel (except 3 of Group 1 who received 450 mg due to dispensing error). One patient originally recruited to Group 2 was withdrawn due to split dose administration of clopidogrel. Baseline demographics for all three groups are presented in Table 1.

Validation of TEG6s

Precision testing

The MA results obtained from healthy volunteers and patients at different time points are expressed as median, IQR and IQR/median ratio to allow direct comparison. TEG 6s demonstrated very little inter-individual variation (groups 1 and 2) across a large number of samples and a high level of intra-individual reproducibility (one healthy volunteer). [Table 2]

The comparison of variation across all three groups and time points (means and 95% CI) is presented in Figure 2. The values in the thrombin channel for TEG5000 remain static, for TEG 6s the difference reach statistical significance ($p<0.05$) at 6 hours in group 1, at 24 hours in group 2 and at 6 hours for one healthy volunteer. The ADP channel show that both devices are able to detect platelet inhibitory effects of clopidogrel in a time-dependent manner. Details on comparison expressed as p values for TEG 5000 and TEG 6s between all time points for each device separately as well as a direct comparison between two devices at any time point are available as supplementary tables 2 and 3 respectively.

Correlation between TEG 5000 MA and TEG 6s MA

As assessed by Spearman`s rank correlation for all groups combined, MAs in the ADP channel showed the strongest correlation (N=100, correlation coefficient R=0.821, R^2=0.674, p<0.001), followed by thrombin (N=98, correlation coefficient R=0.494, R^2=0.244, p<0.001). [Figure 3] Details of correlation for each group and channel separately are available as a supplementary Figure 2.

Agreement between TEG 5000 MA and TEG 6s MA

The agreement between MAs produced by TEG 5000 and TEG 6s for individual agonist-activated channels for all groups was assessed using the Bland Altman analysis. [Figure 4] Data for groups 1,2 and 3 separately are available as a supplementary Figure 3.

The mean difference in the thrombin channel, measured at 98 time points was -2.84 with a SD of 6.19 and 95% CI between -14.97 and 9.29.

The mean difference in the ADP channel, measured at 100 time points was -6.31 with a SD of 9.23 and 95% CI between -24.39 and 11.77. Four data points do not lie within limits of agreement: 2 come from two separate healthy volunteers at 6 hours post clopidogrel ingestion. In addition to this, two further values from one patient do not agree at 2 hours (TEG 5000 MA 14.5 mm, TEG 6s MA 47.4 mm) and at 24 hours (TEG 5000 MA 11.6 mm, TEG 6s MA 49.3 mm).

AUC15 using TEG 6s

Ability of AUC15 to detect a change in clotting

Results of TEG 5000 AUC15 and TEG 6s AUC15 show similar pattern of change across all study groups [Supplementary Figure 4]. Values for both devices in thrombin channel as expected remain static and are higher than in ADP channel.

Time saved

As shown in Table 3 a mean of 5.5 minutes can be saved in thrombin channel and 13.32 minutes in ADP channel when using TEG 6s AUC15 to produce a result describing individual response using MA as the reference.

Correlation between TEG 6s MA and TEG 6s AUC15

For all groups and participants combined, correlation between TEG 6s MA and AUC15 in both thrombin (N=102, correlation coefficient R=0.867, R^2=0.752, p<0.001) and ADP channels (N=95, correlation coefficient R=0.936, R^2=0.876, p<0.001) were very strong. [Figure 5]

Correlation and agreement between TEG 5000 AUC15 and TEG 6s AUC15

Correlation and agreement between TEG 5000 AUC15 and TEG 6s AUC15 are presented in supplementary figures 5 and 6.

Discussion

There are two main findings presented here. Firstly, that TEG 6s has a close level of agreement with TEG 5000 in terms of detection of responses to clopidogrel in both healthy volunteers and patients. Secondly, that a novel parameter, AUC15, had a close correlation with the conventional MA parameter in the TEG6s test, and allows for a significantly quicker result regarding the response of an individual to clopidogrel.

The development of TEG 6s offers the potential for a genuinely POC platelet function assay with which to detect individual response to $P2Y_{12}$ inhibitor. It fulfils desirable criteria for this role, namely: ease of use, speed and accuracy. However, as a test of response of individuals to their $P2Y_{12}$ inhibitor therapy, it has required further validation with regard to reproducibility and accuracy using TEG 5000 as a reference. The current study has provided these data by making a systematic comparison between them. Previous data from Gurbel et al (24) has offered evidence of accuracy and reproducibility of the new assay. However, despite

a large number of comparative data points, the previous study did not provide a specifically designed simultaneous comparison of the ability of each assay to detect temporal responses of volunteers and patients to loading dose of clopidogrel. Our current data confirm good levels of agreement between the tests in the ADP channels.

This study has also confirmed the ability of TEG 6s AUC15 to assess individual *ex vivo* clotting responses to clopidogrel in healthy volunteers and patients with in 15 minutes. Combining the rapidity of this test and the truly POC nature of TEG 6s, this may have considerable clinical value in tailoring antiplatelet therapy to individual patients requiring $P2Y_{12}$ inhibitors. This new method has two main advantages. First and foremost, it delivers results in only 15 minutes, which in a demanding clinical environment is potentially advantageous. Secondly, calculation of AUC15 takes into account both speed and strength of clot formation, which in the future may be proven to be beneficial in terms of hard clinical end points than simply accounting for clot strength (i.e. MA).

So far only a few other studies have evaluated the use of TEG 6s in different clinical circumstances. A study by Bliden *et al* assessed the anticoagulant effect of non-vitamin K oral anticoagulants using TEG 6s (30). The authors showed that the presence of rivaroxaban or apixaban in 39 patients and dabigatran in 25 patients elongated R time in comparison to normal ranges obtained from healthy volunteers. Furthermore, in 50 patients undergoing cardiac surgery Kirmani *et al* compared the response to aspirin and $P2Y_{12}$ inhibitors using AUC from Multiplate assay to MA and percentage platelet inhibition (%PI) in TEG 6s (31). They found only modest correlation between the Multiplate AUC and TEG 6s in both AA and ADP channels, although the Bland Altman analysis showed good level of agreement between the tests for both agonists. Two other studies also investigated mechanistic features of TEG 6s. Gil M tested the performance of TEG 6s while exposed to motion and compared it to TEG 6s in a movement free environment (32). Nearly all parameters proved to be

significantly different, leading to the conclusion that TEG6s results are susceptible to movement. Finally, Dias *et al* assessed the reliability of either citrated multichannel cartridge or the PM cartridge in the TEG 6s or with kaolin in the TEG 5000 system in 20 volunteers (33) and found that reliable results can be obtained up to 4 hours in the citrated multichannel cartridge and up to 3 hours in the PM cartridge.

In the field of interventional cardiology, the concept of personalised antiplatelet therapy remains contentious. The awareness that clopidogrel and, more recently, prasugrel and ticagrelor, is/are subject to individual variability of response in terms of ADP-induced clotting renders the concept of tailored therapy of these agents both logical and attractive. Specifically, concern about the potential for some individuals to exhibit a "low" response, which would put them at risk of ischaemic events and some to exhibit an exaggerated response, that could result in bleeding, sponsors the notion of a therapeutic "sweet spot". Within this idealised theoretical target range of personalised $P2Y_{12}$ inhibitor therapy, patients would experience a low risk of both ischaemic and bleeding events. However, such a personalised therapy could only be achieved with the advent of an accurate, cheap, easy to use, POC test. Furthermore, more data are needed with regards to the possibility that a POC test could predict bleeding risk, particularly in populations treated with "stronger" $P2Y_{12}$ inhibitors such as prasugrel and ticagrelor. TEG6s may represent a candidate as a POC test that could be used routinely for personalised $P2Y_{12}$ therapy, subject to larger scale studies that are now in preparation. Previous randomised trials in PCI patients assessing personalised $P2Y_{12}$ inhibitor therapy have been negative. (11-13) However, VerifyNow was the test employed to determine response to clopidogrel in all of these randomised trials and important concerns about the appropriateness of this test for this function have been raised. (14, 15) Specifically, the VerifyNow assay assesses response to P2Y12 inhibitors using ADP-mediated clotting in the presence of prostaglandin E1 (PGE1). It was designed so that PGE1

increases specificity for P2Y12 blockade, by obstructing the involvement of P2Y1 to ADP-mediated clotting. However, the assumption that this has no overall effect upon the apparent level of ADP-induced clotting response appears flawed. This group has previously demonstrated that PGE1 in fact enhances P2Y12 blockade in some individuals via induction of adenylyl cyclase, which is negatively coupled to P2Y12, rather than P2Y1. This leads to potentiation of the apparent response to P2Y12 inhibitors in the VerifyNow assay as compared to simultaneously measured TEG 5000 responses in the same individuals. (14) Furthermore, addition of PGE1 to TEG-5000 enhances the inhibition of ADP-mediated clotting by in vitro P2Y12 inhibitors. (15) These two studies suggest that the VerifyNow assay overestimates the response to clopidogrel in some patients.

This study has several limitations. Firstly, this is a single centre study. Secondly, the number of participants in each group is low, although consistent with previous studies. Specifically, the TEG 5000 results described here are reproducible with other previous experiments, thus allowing a considerable degree of confidence that our reference is robust. Thirdly, although the results of this study are encouraging, comparing two devices is always associated with the uncertainty about interpretation regarding which is correct when they disagree. Finally, it is a mechanistic study with no clinical outcome data regarding potential ischaemic/bleeding benefits to individual participants.

In conclusion, TEG 6s is a rapid and easy to use POC test whose reproducibility and ability to detect responses of volunteers and patients to clopidogrel is satisfactory using TEG5000 as a reference. A novel parameter, AUC15, offers an even more rapid test for individual response to $P2Y_{12}$ therapy. TEG 6s may be a candidate as an appropriate test for personalised $P2Y_{12}$ strategy trials.

Compliance with ethical standards:

The study was sponsored by University Hospital Southampton NHS Foundation Trust Research and Development department, approved by National Research Ethics Service Committee Yorkshire & The Humber – Leeds West (REC reference: 14/YH/1278, IRAS project ID: 166264) and registered on the National Institute for Health research portfolio database.

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Author contributions:

- B Olechowski - had a substantial contribution to concept and design, analysis and/or interpretation of data; critical writing or revising the intellectual content; and final approval of the version to be published.
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Tables:

Table 1. Study cohorts baseline demographics.

Variable	Group 1 12 Healthy Volunteers Mean(SD)/Median(IQR)*	Group 2 12 Patients Mean(SD)/Median(IQR)*	1 Healthy Volunteer
Participants Demographics			
Gender, Male	8 (66%)	10 (84%)	1
Age	23 (7)*	64.5 (8)	22
Ethnicity, Caucasian	9 (75%)	12 (100%)	1
BMI (kg/m^2)	24.6 (4.1)	28.2 (5.4)	21.9
Risk Factors			
Hypertension	0	8 (67%)	0
Hyperlipidaemia	0	8 (67%)	0
Diabetes	0	1 (8%)	0
Previous or Current Smoker	1 (8%)	11 (92%)	0
Family History of premature CVD	1 (8%)	9 (75%)	0
Cerebrovascular disease	0	0	0
Ischaemic Heart Disease	0	2 (17%)	0
Medications			
Aspirin	0	3 (25%)	0
Beta Blocker	0	3 (25%)	0
Angiotensin Converting Enzyme inhibitor	0	1 (8%)	0
Calcium Channel Blocker	0	6 (50%)	0
Proton Pump Inhibitor	0	4 (33%)	0
Oral Hypoglycaemic agent	0	2 (17%)	0
Insulin	0	0	0
Statin	0	3 (25%)	0
Aldosterone antagonist	0	0	0
Diuretic	0	1 (8%)	0
Laboratory results			
Haemoglobin (g / litre)	143.5 (18.3)	140 (12)*	151
Platelet Count (x 10^9 / litre)	245.5 (88)*	252.5 (68.8)	273
MCV (fL)	87.4 (3.2)	89.7 (4.7)	85.1
Urea (mmol / litre)	4.9 (1.8)*	5 (1.6)*	5.7

Creatinine (µmol / litre)	76.2 (18.6)	76.4 (14.5)	67
Estimated Glomerular Filtration Rate (ml / min)	90 (0)*	90 (12)*	90
Total protein (g/L)	74.4 (4.5)	66.1 (5.4)	82
Albumin (g/L)	44.6 (2)	34.7 (3)	45
Bilirubin (µmol/L)	11.5 (8)*	10 (4)*	10
Alanine transaminase (U/L)	18.5 (5)*	22.4 (11.4)	21
Alkaline phosphatase (U/L)	81.4 (19)	102.4 (32.2)	85
Highly sensitive troponin (ng/L)	N/A	197 (15011)*	N/A

Table 2. MA results obtained from healthy volunteers and patients at different time points, expressed as median, IQR and IQR/median ratio.

Group	Time point	Agonist																	
		Thrombin						Fibrin						ADP					
		TEG5000			TEG6s			TEG5000			TEG6s			TEG5000			TEG6s		
		Median MA	IQR	IQR/Median Ratio	Median MA	IQR	IQR/Median Ratio	Median MA	IQR	IQR/Median Ratio	Median MA	IQR	IQR/Median Ratio	Median MA	IQR	Median/IQR Ratio	Median MA	IQR	IQR/Median Ratio
Group 1 12 HVs	Baseline	56.1	5.2	9%	60.1	3.6	6%	10	7.1	71%	6	4.3	72%	61.5	13.4	22%	60.9	2.4	4%
	2 hrs	59	5.4	9%	59.7	2.8	5%	11.2	5.3	47%	5.4	3.3	61%	49.2	9.1	18%	52.4	14.8	28%
	6 hrs	56.5	5.7	10%	60.2	3.5	6%	9.6	4.8	50%	5.7	3.9	68%	37.1	17.6	47%	43.4	21.2	49%
	24 hrs	55.5	8.8	16%	60.8	2.8	5%	10.8	6.5	60%	5.4	3.2	59%	40.7	13.1	32%	50.2	16	32%
Group 2 12 patients	Baseline	66	4.6	7%	63.3	11	17%	11.4	5.1	45%	15	6.1	41%	57.9	14.3	25%	58.1	9.5	16%
	2 hrs	65.8	4.8	7%	63.8	3.5	5%	13.4	7.4	55%	15.5	6	39%	33.7	27.3	81%	49.4	10.8	22%
	6/18hrs	64.4	5.8	9%	64.9	2.5	4%	10.8	10.2	94%	14.7	5.3	36%	39.1	31.2	80%	48.1	30.9	64%
	24 hrs	63.9	7.6	12%	64.8	3	5%	12.7	5.5	43%	15.2	5.7	38%	32.6	32.9	100%	47	21.6	46%
1 HV	Baseline	52.9	7.6	14%	64.1	2.3	4%	10.8	3.9	36%	8	5	62.5%	57.4	13.6	24%	64.7	1.4	2%
	6 hrs	55.5	8.5	15%	64.7	1.4	2%	9.1	8.3	91%	8.1	5.1	63%	46.3	23.3	50%	56.8	6.9	12%

HV- Healthy volunteer, Hrs- hours, IQR- Interquartile range

Table 3. R time, Total time, Time to reach MA and time saved for both thrombin and ADP channels expressed in minutes.

Agonist	R Time (min) Mean ± SD Max-Min	Total Time (min) Mean ± SD Max-Min	Time to reach MA (min) Mean ± SD Max-Min	Time saved (min)
Thrombin	6.21 ± 1.03 3.4 - 9.2	46 ± 11.38 26 - 61.65	26.74 ± 2.46 20.7 - 31.8	5.53
ADP	0.044 ± 0.101 0 - 0.5	28.36 ± 10.4 7 - 81.7	28.36 ± 10.4 7 - 81.7	13.316