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Full Length Article

Comparison of Multiplate and VerifyNow platelet function tests in predicting clinical outcome in patients with acute coronary syndromes

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ABSTRACT

Introduction: This study examined the ability of two widely used "point of care" platelet function assays, VerifyNow and Multiplate, to predict adverse outcomes in patients with acute coronary syndromes (ACS).
Methods: We examined platelet reactivity using VerifyNow and Multiplate $P2Y_{12}$ assays in patients with ACS and the relationship between platelet reactivity and both MACE (defined as a composite of death, myocardial infarction, stroke, stent thrombosis and unplanned revascularisation) and TIMI major bleeding at 1 year.
Results: In 619 ACS patients, 65 patients (10.5%) had experienced MACE at 1 year and 6 patients (1%) had TIMI major bleeding events. The two measures of platelet reactivity were only moderately correlated (Rho = 0.43, $p = 0.0001$). Both measures demonstrated a statistically significant relationship with MACE, with area under the curve for VerifyNow of 0.632 (0.001) and for Multiplate of 0.577 ($p = 0.04$), and neither measure showed a significant relationship with bleeding. Logistic regression analysis found that only VerifyNow was a statistical predictor of MACE ($p = 0.01$). MACE occurred in 16% of those classified as having HPR using VerifyNow compared to 7% in those without HPR (odds ratio of 2.6 (95% CI 1.5–4.4, $p = 0.001$). In those classified as having HPR by the Multiplate assay, MACE occurred in 13% compared to 9% of those without HPR (Odds ratio 1.5 95% CI 0.9–2.5, $p = 0.11$).
Conclusion: The two points of care platelet function tests examined in this study were only moderately correlated. The VerifyNow assay demonstrated a stronger relationship to MACE than the Multiplate assay.

1. Introduction

There is considerable variability in the level of platelet inhibition observed in patients treated with clopidogrel and aspirin following acute coronary syndromes (ACS) [1–3]. Those patients with high platelet reactivity (HPR) on treatment clopidogrel have been shown to have an increased risk of death, myocardial infarction and stent thrombosis [4,5]. Switching of patients with HPR to a more potent agent, such as ticagrelor or prasugrel, has been shown to be an effective strategy to overcome HPR [2,6], and a recent meta-analysis suggests that this approach lowers the rate of stent thrombosis and cardiovascular death [7].

There are a number of different approaches that may be used to examine platelet reactivity on clopidogrel. These include using flow cytometry and light transmission aggregometry, as well as using point of care tests based on aggregation of platelets [8]. Each test measures different aspects of platelet reactivity and in turn, this will lead to different classifications of HPR [9,10]. The two most widely used "point of care" assays, VerifyNow and Multiplate, are both recommended by experts [11], but to date there is no head to head comparison examining the predictive value of these assays in patients with ACS.

This study was undertaken to compare two different point of care assays, to examine the extent of reclassification that would occur depending on which test was used and to determine whether one assay was superior to the other in terms of the relationship between high levels of platelet reactivity and adverse outcomes in ACS.

2. Methods

2.1. Patient population

Patients presenting to Wellington Regional Hospital with ACS between January 2012 and June 2014 were eligible for inclusion in the study if there was an invasive approach (coronary angiography ± PCI) planned. All participants were appropriately pre-treated with aspirin and clopidogrel. Exclusion criteria included a platelet count $<100 \times 10^9$/L, known platelet function disorder, administration of a fibrinolytic agent within 24 h of enrolment, use of a glycoprotein IIb/IIIa receptor antagonist within 7 days or administration of an oral

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antiplatelet agent other than aspirin or clopidogrel within 2 weeks of enrolment. The study was reviewed and approved by the Lower Regional South Ethics Committee (LRS/11/09/035). All patients provided written informed consent.

2.2. Definitions

An ACS was defined as symptoms suggestive of myocardial ischaemia lasting >10 min and either troponin elevation or ≥1 mm of new ST segment deviation or T wave inversion on an electrocardiogram in at least 2 contiguous leads [12]. Adequate pre-treatment was defined as chronic therapy (≥7 days) with aspirin (≥75 mg) and clopidogrel (≥75 mg) and/or loading with aspirin ≥300 mg at least 2 h and clopidogrel ≥300 mg at least 6 h prior to enrolment.

Clinical follow-up was collected by telephone and accessing case notes, hospital admission databases and death registry at 1 year. The primary endpoint of major adverse cardiovascular events (MACE) was a hierarchical composite endpoint of all-cause mortality, spontaneous myocardial infarction, stroke, stent thrombosis and unplanned revascularisation. Bleeding was defined as non-Coronary artery bypass graft (CABG) related Thrombolysis in Myocardial Infarction (TIMI) major bleeding constituting intracranial bleeding, overt bleeding with a decrease in haemoglobin ≥5 g/dL or decrease in haematocrit ≥15% [13].

2.3. Platelet function testing

The level of on treatment platelet reactivity was quantified using the VerifyNow $P2Y_{12}$ assay (Accumetrics, San Diego, CA, USA) and the Multiplate analyser (Roche Diagnostics, Rotkreuz, Switzerland), both of which have been shown to be predictive of clinical outcomes [4,5]. The VerifyNow $P2Y_{12}$ assay, a turbidimetric-based optical detection system, was used according to the manufacturer's instructions. This device uses fibrinogen-coated microbeads, an agonist of adenosine diphosphate (20 mM ADP), and light transmittance through whole blood, to measure platelet agglutination. An optical signal, reported as $P2Y_{12}$ reaction units (PRU), was recorded. The Multiplate analyser is a multiple electrode impedance aggregometer that assesses platelet function in whole blood as previously described [14]. Briefly, whole blood was added to the test cuvettes, diluted (1:2 with 0.9% NaCl solution), stirred and warmed to 37 °C. ADP was added to a final concentration of 6.4 mM and aggregation was then continuously recorded for 6 min. Aggregation values are quantified as area under the aggregation curve expressed as aggregation units × minutes (AU). All material used for platelet function testing was obtained from the manufacturer (Roche Diagnostics, Rotkreuz, Switzerland). Thresholds for high on treatment platelet reactivity (HPR) were defined as >208 PRU for VerifyNow and >46 AU for Multiplate measurement [15].

It has recently been demonstrated that platelet reactivity, particularly as measured by VerifyNow, may be sensitive to renal dysfunction, as patients with renal impairment will have lower levels of haemoglobin and VerifyNow and not Multiplate is sensitive to this [16–18]. To ensure our results were not being driven by this potential bias, we examined the relationship between platelet reactivity and MACE and high on treatment platelet reactivity and MACE excluding those patients with renal dysfunction (eGFR <45 mL/min/1.73m^2).

2.4. Statistical analysis

We compared continuous variables between those subjects with and without MACE using either an unpaired students *t*-test (where the variable was normally distributed) or a Mann Whitney *U* test (where the variable did not have a normal distribution). Categorical variables were compared using a Chi-Squared test. The relationship of platelet reactivity with MACE and bleeding was also examined using a receiver operator curve (ROC) and logistic regression. p values < 0.05 were considered statistically significant. Statistical analysis was performed using SPSS v.20 (IBM, New York, USA).

3. Results

We measured platelet reactivity prior to angiography in 676 patients thought to have ACS who had been adequately pre-treated with clopidogrel and aspirin. Fifty-seven of these patients were reclassified following angiography with an alternative diagnosis (myocarditis, pericarditis, Takotsubo etc.), leaving 619 patients with confirmed ACS in the cohort. Demographic data for this group are given in Table 1.

The most common clinical presentation was NSTEMI (78%), followed by STEMI (19%) and UA (3%). The cohort was predominantly male (70%), of European ethnicity (83%) having a mean age of 63 ± 11 years. A past medial history of hypertension (63%) and dyslipidaemia (67%) were common. In addition, 22% were diabetic, 23% current smokers and 27% had at least one prior MI. Management of the coronary disease was percutaneous coronary intervention in 376 (61%), coronary bypass graft surgery in 81 (13%) and medical management in 162 (26%) patients.

At one year after the index admission, 65 patients (10.5%) had experienced MACE (Table 2). There were 23 deaths, 20 myocardial infarctions, 6 cases of stent thrombosis, 12 strokes, and 4 unplanned revascularisations. The demographic variables that were statistically associated with MACE are shown in Table 1. Prior medical history of dyslipidaemia, renal dysfunction, prior heart failure, and diabetes were all more common in those with MACE. Measures of platelet reactivity using the Multiplate and VerifyNow assays were significantly higher in those patients with MACE. At one year there were 6 cases

Table 1
Demographics and clinical variables of MACE vs no MACE patients.

Demographics	ACS patients $n = 619$	No MACE $n = 554$	MACE $n = 65$	p value
Male	435 (70.3)	390 (70.4)	45 (69.2)	0.846[a]
Age (mean ± SD)	63.3 ± 11	63 ± 10.5	66 ± 13.2	0.076[b]
BMI (mean ± SD)	29.5 ± 5.7	29.4 ± 5.6	29.8 ± 6.6	0.67[b]
Ethnicity				
European	514 (83)	461 (83.2)	53 (81.5)	0.77[a]
Maori + Pacific Islander	80 (13)	70 (12.6)	10 (15.4)	
Other	25 (4)	23 (4.2)	2 (3.1)	
Risk Factors				
Hypertension	391 (63.2)	343 (62)	48 (73.8)	0.059[a]
Dyslipidaemia	414 (66.9)	361 (65.2)	53 (81.5)	0.008[a]
Renal dysfunction	36 (5.8)	25 (4.5)	11 (16.9)	<0.0001[a]
Congested heart failure	19 (3.1)	12 (2.2)	7 (10.8)	<0.0001[a]
Prior myocardial infarction	168 (27.1)	146 (26.4)	22 (33.8)	0.202[a]
Diabetes	140 (22.6)	116 (21)	24 (36.9)	0.004[a]
Smoking				
Current	144 (23.3)	126 (22.7)	18 (27.7)	0.32[a]
Former	252 (40.7)	223 (40.3)	29 (44.6)	
Never	223 (36)	205 (37)	18 (27.7)	
Clinical presentation				
STEMI	117 (19)	109 (19.7)	8 (12.3)	0.089[a]
NSTEMI	485 (78.3)	432 (78)	53 (81.5)	
Unstable angina	17 (2.7)	13 (2.3)	4 (6.2)	
Platelet reactivity				
Multiplate (median, IQR)	36 (24–55)	36 (24–55)	46 (25–63.5)	0.041[c]
VerifyNow (median, IQR)	178 (104.5–241)	169.5 (101–237)	228 (153–270.5)	<0.0001[c]

[a] Chi-square test, categorical data.
[b] Student's unpaired *t*-test, continuous data.
[c] Mann Whitney *U* test, continuous data.