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Dual antiplatelet response during PCI: VerifyNow P2Y12 predicts myocardial necrosis and thromboxane B2 generation confirms wide variation in aspirin response

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ABSTRACT

Introduction: There remains concern that the antiplatelet effects of aspirin and clopidogrel vary between patients and poor responders may be at increased risk of adverse events. However, the optimal method of measuring aspirin and/or clopidogrel response remains unresolved. We compared three methods of measuring clopidogrel response recommended by a recent consensus statement for the European Society of Cardiology, and investigated a novel approach to measuring aspirin response in patients established on both aspirin and clopidogrel. In addition, we investigated whether any of these assays predict peri-procedural myocardial necrosis following percutaneous coronary intervention (PCI).

Methods: A cross-section of 323 patients attending for PCI was tested for clopidogrel response using VerifyNow P2Y12, VASP Platelet Reactivity Index (VASP-PRI) and whole blood impedance aggregometry (WBPA). Aspirin response was assessed by measuring the residual ability of platelets to generate thromboxane, calculated as the difference between thromboxane B2 levels in serum and plasma, $[TxB2]_{S-P}$. Peri-procedural myocardial necrosis was determined by a change in troponin I >0.2 μmol/l.

Results: Patients demonstrated wide variation in response to both aspirin and clopidogrel. Correlation between VerifyNow P2Y12 and VASP-PRI was good ($r = 0.702$, $p < 0.001$). Correlation was moderate between WBPA and VerifyNow P2Y12 ($r = 0.639$, $p < 0.001$) and weak for WBPA and VASP-PRI ($r = 0.353$, $p < 0.001$). Only VerifyNow P2Y12 predicted peri-procedural myocardial necrosis.

Conclusions: The three methods of measuring response to clopidogrel identify different patients as poor responders. Poor response to clopidogrel assessed by VerifyNow P2Y12 predicts myocardial necrosis. Measurement of $[TxB2]_{S-P}$ demonstrates a wide variation in aspirin response in patients taking dual antiplatelet therapy.

Introduction

Dual antiplatelet therapy (DAPT) with aspirin and a $P2Y_{12}$ antagonist is critical for patients undergoing percutaneous coronary intervention (PCI) and plays a central role in the management of acute coronary syndromes (ACS) [1,2]. Although newer, more potent $P2Y_{12}$ inhibitors (ticagrelor and prasugrel) have emerged [3,4], concerns regarding bleeding and financial constraints are likely to maintain the global use of clopidogrel. Variability in response to both aspirin and clopidogrel has been demonstrated [5,6] and there is evidence that poor response to these agents is associated with increased cardiovascular risk [7,8]. This has been addressed by a recent guideline publication from the European Society of Cardiology (ESC) [9]. Whilst acknowledging that variation in response to aspirin may exist, a lack of correlation between platelet function assays of aspirin response and a paucity of clinical outcomes data means that no single test is recommended for clinical practice. This is further complicated by the influence of clopidogrel on aggregation-based methods of measuring aspirin response as, for example, in the landmark study by Gum et al. [10], that used platelet aggregation following stimulation with arachidonic acid and/or ADP to define aspirin 'resistance'. In contrast, the ESC consensus statement suggests that the assessment of clopidogrel response is more robust and recommends either the flow cytometry assay of vasodilator-stimulated phosphoprotein platelet reactivity index (VASP-PRI), or two aggregation-based assays; VerifyNow P2Y12 and the Multiplate

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analyser (impedance aggregometry) for this purpose [9]. Data supporting VerifyNow P2Y12 as a marker of risk has emerged from the ADAPT-DES registry [11]. However, a number of issues remain; there is no large data registry to confirm the prognostic importance of VASP-PRI or the Multiplate assay, agreement between all 3 assays is uncertain and the threshold of 'poor' response for each assay is not robustly established [12–14]. In addition, the clinical circumstances in which platelet function should be measured have not been defined, and no study has yet demonstrated that alterations to antiplatelet therapy on the basis of testing for platelet response to ADP are of clinical benefit [14–16].

In this study, we assessed the response to clopidogrel in a cohort of patients attending for coronary angiography +/− PCI who were already established on DAPT with aspirin and clopidogrel. We compared VerifyNow P2Y12, VASP-PRI and whole blood impedance platelet aggregometry (WBPA) in response to ADP for the assessment of clopidogrel response and we assessed the response to aspirin by measuring the residual ability of platelets to generate thromboxane, calculated as the difference between thromboxane B2 levels in serum and plasma, $[TxB2]_{S-P}$. We have previously shown that this method is independent of the effect of clopidogrel [17]. Finally, in those patients undergoing PCI, we investigated whether any of these assays predicted the incidence of peri-procedural myocardial necrosis.

Methods

Patient Selection

Sequential patients attending the cardiac catheterisation laboratory, in the Western Infirmary in Glasgow, for coronary angiography with a view to intervention were recruited (Fig. 1). All patients had received 75 mg of aspirin for at least 5 days and clopidogrel according to one of the following recognised regimens; 75 mg/day for at least 5 days, a 300 mg loading dose >24 hours previously followed by 75 mg/day, a 600 mg loading dose >2 hours prior to sampling followed by 75 mg/day. The additional inclusion criteria for all patients were that they were aged between 18 and 85 years and were able to provide written informed consent. Patients were excluded if they had received a GPIIbIIIa antagonist, dipyridamole or NSAID therapy within the preceding 2 weeks, were pregnant, anaemic (Hb < 10 g/dl), had a platelet count <100 or >500 x10^9/l, or had a personal or family history of a bleeding disorder. Patients were also excluded if they were non-compliant with aspirin or clopidogrel therapy, as assessed by direct questioning at the time of recruitment. Ethical approval was granted by the West Research Ethics Committee, Glasgow.

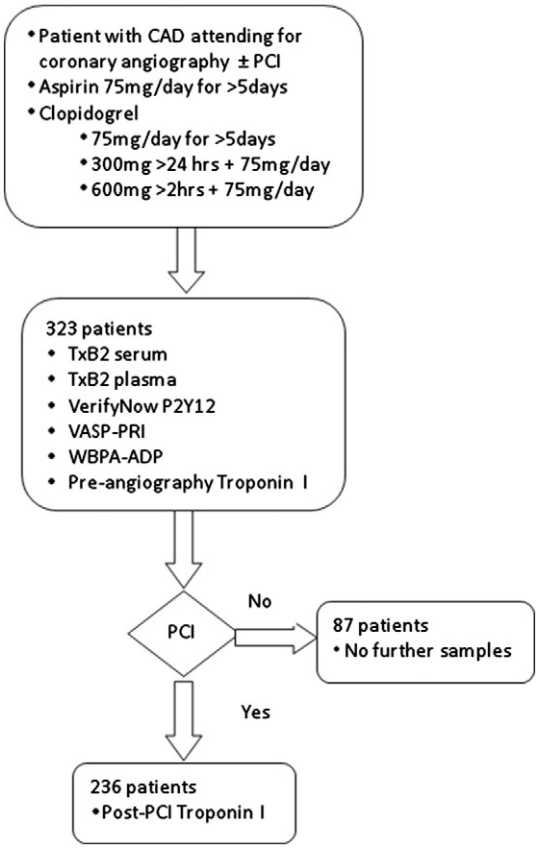

Fig. 1. Flow diagram of patient recruitment and sampling.

Sample Collection

Samples from patients established on DAPT were collected immediately prior to angiography. Where possible, samples were collected by venepuncture from a large calibre vein in the antecubital fossa using a 21G butterfly needle and vacutainer system (BD Biosciences). Patients were asked to sit or lie for 10 minutes prior to sampling and use of a tourniquet was kept to a minimum. The utmost care was taken to avoid unnecessary trauma and agitation to the sample during and after collection. A 3.5 ml 3.2% sodium citrate tube was collected and then discarded followed by 3 further 3.5 ml 3.2% sodium citrate tubes, a 2 ml 3.2% sodium citrate tube, a 3 ml glass vacutainer tube containing thrombin, and a tube containing EDTA in strict sequential order. Patients without a suitable calibre vein had blood samples taken directly from the arterial sheath by the cardiac catheterization laboratory medical staff. Immediately following insertion of a 6 F sheath into either the femoral or radial artery, 5mls of blood was aspirated and discarded following which blood was gently collected into a 20 ml syringe and immediately transferred to the appropriate vacutainer tubes in the sequential order described above. Samples were immediately mixed by gently inverting 5 times.

Thromboxane B2 Assay

Within 5 minutes of sample acquisition, a single 3.2% sodium citrate whole blood tube was centrifuged for 30 minutes at 1500 g (3000 rpm) and the plasma supernatant stored in 0.5 ml aliquots at −80 °C until use. The thrombin containing tube was left at room temperature for 1 hour after which it was centrifuged at 1500 g (3000 rpm) for 10 minutes and the serum supernatant collected in 0.5 ml aliquots and immediately stored at −80 °C. TxB2 was analysed by ELISA (R&D Systems Europe; cat. no. KGE011) following the manufacturer's instructions. Thromboxane generation by platelets was calculated by subtracting the levels in plasma from the paired thrombin-serum samples, as described previously [17].

VerifyNow P2Y12 Assay

The Ultegra rapid platelet function analyser (RPFA) and VerifyNow P2Y12 assay cartridges were purchased from Accumetrics Inc (USA) and used according to the manufacturer's instructions. VerifyNow P2Y12 results are expressed as platelet reaction units (PRU) or %inhibition relative to a control channel containing thrombin receptor activating peptide. As recommended by the manufacturer's guidelines, the 2 ml 3.2% sodium citrate samples were stored at room temperature for at least 30 minutes before assaying and all samples were analysed between 30 minutes and 2 hours of acquisition.

VASP-PRI Assay

Measurement of phosphorylated VASP in platelets was performed by flow cytometry using a commercially available assay (VASP/$P2Y_{12}$, Biocytex, France). Reagents were prepared as instructed by the manufacturer and each box was used within 1 month of opening. The samples