International Journal of Cardiology 167 (2013) 430–435

Contents lists available at SciVerse ScienceDirect

International Journal of Cardiology

journal homepage: www.elsevier.com/locate/ijcard

Dual non-responsiveness to antiplatelet treatment is a stronger predictor of cardiac adverse events than isolated non-responsiveness to clopidogrel or aspirin

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ARTICLE INFO

Article history:
Received 24 August 2011
Received in revised form 1 January 2012
Accepted 6 January 2012
Available online 2 February 2012

Keywords:
Clopidogrel
Aspirin
MEA
Platelets
Cardiac adverse event
Diabetes mellitus

ABSTRACT

Background: High platelet reactivity (HPR) under treatment with clopidogrel or aspirin is associated with adverse outcome. We aimed to investigate whether high platelet reactivity (HPR) to both aspirin and clopidogrel is a stronger predictor of adverse events compared to isolated HPR to clopidogrel or aspirin.
Methods: In this prospective cohort study platelet reactivity to adenosine diphosphate (ADP) and arachidonic acid (AA) was assessed by Multiple Electrode Aggregometry (MEA) in 403 patients undergoing percutaneous coronary intervention. The rates of the composite of cardiac adverse events (acute coronary syndrome, stent thrombosis, stroke, death and revascularization) were recorded during 12-month follow-up.
Results: The composite endpoint of cardiovascular adverse events occurred more often in patients with high platelet reactivity (HPR) to both agonists ADP and AA (37.5%) than in those with isolated HPR to ADP (33.3%), AA (25.6%) or without any HPR (18.6%; $p = 0.003$). Classification tree analysis indicated that any HPR emerged as an independent predictor influencing outcome, which was associated with a 1.75 higher risk of cardiac adverse events (OR = 1.75: 95%CI = 1.1–2.9). Interestingly, the predictive value of HPR tended to be greater among patients with diabetes mellitus (OR = 2.18; 95%CI = 1.20–3.95). C-reactive protein and diabetes mellitus were independent predictors of high platelet reactivity to both agonists.
Conclusions: Dual low responsiveness to clopidogrel and aspirin is a strong predictor of cardiac adverse events, especially in patients with diabetes mellitus, which underlines the need for personalized antiplatelet treatment.

1. Introduction

The irreversible platelet inhibitors clopidogrel and aspirin represent the mainstay of treatment of coronary artery disease, especially in patients undergoing stent implantation [1,2]. Both clopidogrel and aspirin selectively inhibit a single pathway of platelet activation [3–5]. Clopidogrel is a potent and specific inhibitor of the platelet P2Y12 receptor. Aspirin inhibits the biosynthesis of thromboxane by inactivation of platelet cyclooxygenase-1 [6,7]. The effect of the antiplatelet drugs is, however, not uniform in all patients and some patients do not optimally benefit from them [8,9]. High platelet reactivity (HPR) to adenosine diphosphate (ADP) which mirrors an insufficient platelet inhibition by clopidogrel occurs in about 20–30% of patients [10,11]. Variable responses to clopidogrel can be explained by non-compliance, inappropriate dosing of the substance, variable absorption of the pro-drug or clearance of the active metabolite, polymorphisms of the metabolizing enzymes and drug–drug interactions [12–17]. High platelet reactivity (HPR) to arachidonic acid (AA) which reflects low responsiveness to aspirin occurs in 6–45% of patients, the frequency of which depends on the platelet function tests used [18,19]. Published data suggests that underdosing, non-compliance, poor absorption, drug–drug interference with non-steroidal anti-inflammatory drugs, stress-induced generation of COX-2 in platelets or polymorphisms of COX-1 might be responsible for the insufficient response to aspirin [6,20]. Patients with an isolated hyper-reactivity to arachidonic acid (AA) or adenosine diphosphate (ADP) have an increased risk of cardiac adverse events [21–23]. We hypothesized that high platelet reactivity (HPR) to both adenosine diphosphate (ADP) and arachidonic acid (AA) may better predict cardiac adverse events compared to isolated HPR to ADP or AA. Thus, we evaluated the impact of dual high platelet reactivity in patients undergoing stent implantation on adverse cardiac events during long term follow-up.

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doi:10.1016/j.ijcard.2012.01.016

2. Methods

2.1. Study design

The Ethics Committee of the Medical University of Vienna in accordance with the Declaration of Helsinki approved the study protocol. This was a prospective observational cohort study performed at the Medical University of Vienna. Participants were included into the study between March 2007 and September 2008, and followed-up until November 2009. Clinical follow-up information was obtained by contacting all patients by phone and/or mail at 3, 6, 9 and 12 months. Source documents of potential events were obtained. Additionally, information concerning the cause of death was obtained from national death registry (Statistics Austria). Data were collected until September 2010. Inclusion criteria were: written informed consent obtained before the study entry, stent implantation, clopidogrel loading with 600 mg at least 2 h pre PCI, aspirin intake (100 mg) at the day of PCI, age >18 years and planned treatment with clopidogrel (75 mg) and aspirin (100 mg) for 12 months. The only exclusion criterion was participation in interventional trials and use of GpIIbIIIa antagonists. The study population was a consecutive series of participants defined by the selection criteria. Four hundred three patients with coronary artery disease (CAD) undergoing PCI were consecutively enrolled. The majority of patients (99%) received a drug eluting stent. All interventions were performed according to current standard guidelines, and the type of stent implanted was at the discretion of the interventional cardiologist. All patients received unfractionated heparin 100 IU/kg before the procedure. All patients received a daily dose of 100 mg acetylsalicylic acid (aspirin) and additionally 250 mg acetylsalicylic acid i.v. directly before stent placement. Blood samples from patients were obtained from the arterial sheath (6 F) in the catheterization laboratory directly post PCI and at least 5 min after intravenous infusion of aspirin. The study is reported according to the STROBE (strengthening the reporting of observational studies in epidemiology) standards.

2.2. Impedance aggregometry

Whole blood aggregation was determined using Multiple Electrode Aggregometry (MEA) on a new generation impedance aggregometer (Multiplate Analyzer, Verum Diagnostica GmbH, Munich, Germany). The system detects the electrical impedance change due to the adhesion and aggregation of platelets on two independent electrode-set surfaces in the test cuvette [23]. We used hirudin as anticoagulant, which is recommended by the manufacturer. We used adenosine diphosphate (ADP) and arachidonic acid (AA) as agonists [24]. A 1:2 dilution of whole blood anticoagulated with hirudin and 0.9% NaCl was stirred at 37 °C for 3 min in the test cuvettes, ADP: 6.4 μM or AA: 0.5 mM were added and the increase in electrical impedance was recorded continuously for 6 min. The mean values of the 2 independent determinations are expressed as the area under the curve of the aggregation tracing (AUC). The MEA instrument allows two ways to express the AUC: as AU*min (arbitrary aggregation units) or as U (units). 10 AU*min correspond to 1 U. The recommendation to express the AUC as U was introduced by the manufacturer in order to simplify the expression of results by providing a more simple unit (U instead of AU*min) and also by providing smaller numbers. Admittedly, this is causing some confusion in the literature. We reported AUC in units (U) [25]. The cut-offs for high platelet reactivity (HPR) (ADP≥48U, AA≥14U) were chosen to provide the greatest sum of sensitivity and specificity. Platelet aggregometry was performed directly after blood sampling at the Department of Clinical Pharmacology at the Medical University of Vienna by trained laboratory technicians blinded to the results of outcomes. The tests were performed in each participant.

2.3. Study endpoints

The primary efficacy end point was the incidence of the composite of major adverse cardiovascular events (MACE: stent thrombosis, acute coronary syndrome, death, stroke, repeated revascularization: percutaneous coronary intervention or coronary artery bypass surgery) during a 12-month follow-up. Definite stent thrombosis was defined according to the Academic Research Consortium criteria as the occurrence of an acute coronary syndrome with either angiographic or pathological confirmation of thrombosis, whereas probable stent thrombosis was defined as any unexplained death within 30 days or target vessel MI without angiographic confirmation of thrombosis or other identified culprit lesion [26].

2.4. Statistical analysis

Based on a 30% rate of the composite of cardiac adverse events in the group with high platelet aggregation [27] we calculated that 366 patients would provide 85% power to detect a 100% higher event rate in patients with HPR than those without HPR (two sided alpha value of 0.016). To compensate for potential loss to follow-up, we included 37 additional patients. Normal distribution was tested with the Kolmogorov Smirnov test. Data are expressed as mean, standard deviation (SD), 95% confidence intervals (CI) median or interquartile range as appropriate. A receiver operating characteristic (ROC) curve analysis was used to determine the ability of the tests to distinguish between patients with or without cardiac adverse events. The optimal cut-off points were calculated to provide the greatest sum of sensitivity and specificity. Statistical comparisons were performed with the *t* test, the Mann Whitney *U* test and the X^2-test when applicable. Kaplan–Meier curves with the log rank test were used for survival analyses. The Bonferroni correction was used for multiple comparisons. Stepwise multivariable logistic regression analysis was used to estimate independent variables responsible for cardiac ischemic events as well as independent variables responsible for high platelet reactivity (HPR). The regression model for ischemic events included body mass index (BMI), C-reactive protein (CRP), diabetes mellitus, high platelet reactivity to AA or ADP, age > 65, renal failure (creatinine clearance <60 mg/ml), myocardial infarction (MI) at admission, prior myocardial infarction or PCI and sex. The regression model for HPR included body diabetes, C-reactive protein (CRP), sex, age > 65, renal failure (creatinine clearance <60 mg/ml), adipositas, myocardial infarction (MI) at admission, prior myocardial infarction or PCI. Classification tree analysis (Chi-squared Automatic Interaction Detection: CHAID) was used to detect discriminators of the risk of MACE. The analysis included high platelet reactivity to AA or ADP, common risk factors for coronary artery disease (cigarette smoking, diabetes mellitus, hypertension, family history of coronary artery disease, hyperlipidemia), past medical history (stroke, previous PCI, previous myocardial infarction), co-morbidities (renal failure, periphery or cerebral vascular disease), age, status at hospitalization (stable angina or acute coronary syndrome) and sex. All statistical calculations were performed using commercially available statistical software (SPSS Version 18.0; Chicago).

3. Results

3.1. Patient demographics

Patient demographics and co-medication are shown in Table 1. Most of the patients underwent non-emergent PCI due to stable angina. The majority of patients had high blood pressure and hyperlipidemia. Use of beta-blockers, proton pump inhibitors (PPIs) and statins was high. Interestingly, patients with high platelet reactivity (HPR) to adenosine diphosphate (ADP) had less frequently previous myocardial infarction or previous PCI compared to patients without HPR to ADP (19% vs. 35%; p = 0.006; 36% vs. 50%; p = 0.04; respectively; Table 1). Furthermore, patients with HPR to ADP had higher values of inflammation markers compared to patients without HPR to ADP: white blood cell count (8.6 G/L vs. 7.6 G/L; p = 0.003) and CRP (1.8 mg/dl vs. 1.1 mg/dl; p = 0.07; Table 1). In line, patients with HPR to ADP presented more frequently with acute coronary syndrome compared to patients without HPR to ADP (49% vs. 29%; p = 0.01 Table 1). Interestingly, there was no difference in the baseline characteristic between patients with or without HPR to arachidonic acid (AA; Table 1). None of the patients was lost to follow-up.

3.2. Predictive value of aggregometry for the composite of major adverse cardiac events (MACE)

Receiver operating characteristic (ROC) curve analysis demonstrated that adenosine diphosphate (ADP) and arachidonic acid (AA)-induced platelet aggregation distinguished between patients with or without subsequent cardiac adverse event (Fig. 1). The area under the curve (c-index) for the composite of MACE was 0.60 (95%CI: 0.53–0.66; p = 0.033) for ADP- and 0.58 (95%CI: 0.52–0.65; p = 0.015; Table 2) for AA-induced platelet aggregation.

3.3. Distribution and correlation of high platelet reactivity (HPR) to ADP or AA

Patients without high platelet reactivity to ADP or AA represented the majority (62%) of the study population (left lower quadrant; Fig. 2). High platelet reactivity to both agonists ADP and AA was found in 8% of study participants (right upper quadrant; Fig. 2). Isolated hyper-reactivity to ADP or AA was seen in 11% and 19% of patients, respectively (Fig. 2). The correlation between the ADP and AA-induced platelet aggregation was low (R = 0.34; p < 0.001).

3.4. Independent predictors of high platelet reactivity (HPR) to ADP or AA

Multivariable adjusted logistic regression analysis identified diabetes mellitus and heightened C-reactive protein (CRP) as independent predictors of high platelet reactivity to either ADP or AA (Table 3). Patients with diabetes had a 1.8-fold higher risk to have HPR than patients without diabetes (OR = 1.83; 95%CI = 1.13–2.98;