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Full Length Article

Association between high on-treatment platelet reactivity and occurrence of cerebral ischemic events in patients undergoing percutaneous coronary intervention☆

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ABSTRACT

Introduction: Percutaneous coronary angioplasty (PCI) has become a routine treatment in symptomatic patients with coronary artery disease. The use of new generation drug eluting stents (DES) and dual antiplatelet therapy has significantly improved treatment outcomes and increased patients' safety by reducing the risk of stent thrombosis.

Aims: The goal of this study was to assess whether high on treatment platelet reactivity (HTPR), despite clopidogrel treatment, measured with Multiplate Electrode Aggregometer (MEA) is associated with the risk of adverse ischemic cerebral events.

Methods: Symptomatic patients with coronary artery disease admitted for coronary angiography and angioplasty (PCI) were consecutively enrolled in this study. 249 consecutive patients underwent coronary artery stenting for stable angina (n = 215) or non-ST-elevation acute coronary syndrome (n = 34). Inhibition of platelet aggregation was assessed by MEA. Genetic polymorphism of CYP2C19 was tested by HRM Real-Time PCR method in 150 patients.

Results: Patients with HTPR were more frequently diagnosed with ischemic stroke (p = 0.0351, OR = 16.818, 95% CI [1.464–193.23]) and other ischemic cerebral events (stroke or TIA, p = 0.0339, OR = 6.5, 95% CI [1.36–31.07]). Cumulative assessment of all ischemic and hemorrhagic events showed no statistical significance. Cerebral ischemic event was the only adverse event that correlated with CYP2C19 (*2/*2) allele (p = 0.0489, OR = 10; 95% CI [1.39–71.80]).

Conclusions: HTPR assessed by MEA, in patients treated with clopidogrel after coronary artery stenting was found to be an important risk factor of ischemic cerebral events. In concordance, the carriers of CYP2C19*2/*2 allele showed an increased rate of ischemic cerebral events.

1. Introduction

Percutaneous coronary angioplasty (PCI) has become a routine treatment in symptomatic patients with coronary artery disease. New generation drug eluting stents (DES) have significantly improved treatment outcomes and increased patients' safety reducing the risk of stent thrombosis. Several publications have already proven that high on treatment platelet reactivity (HTPR) increased risk of ischemic cardiovascular events (including stent thrombosis) in patients who underwent PCI [1,2,3]. However, there is a lack of data whether HTPR under dual antiplatelet therapy (DAPT) predicts ischemic events after implantation of last generation DES. Similarly, it is unknown whether HTPR in patients treated with clopidogrel may affect the long-term cerebral outcome following the implantation of new generation DES.

Platelet reactivity is determined by a variety of genetic markers and no single marker itself can be a target of anti-platelet therapies. Antiplatelet therapies can also fail to suppress platelet function due to high on-treatment systemic inflammation which can be a target of anti-inflammatory agents [4,5].

The CYP2C19 enzyme is of critical importance in a process of clopidogrel bioactivation. Among the several polymorphisms, the

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CYP2C19*2 loss-of-function allele, was found by Hulot et al. to be associated with impaired antiplatelet response to clopidogrel [6]. There are several studies that have confirmed a correlation between the HTPR on clopidogrel, induced by the expression of CYP2C19*2, and the occurrence of adverse ischemic events, especially stent thrombosis and cerebral ischemic events [7,8]. Bhatt et al. however, have proven no correlation between CYP2C19 genotype and ischemic events [9]. The clinical impact of CYP2C19*2 has to be thoroughly investigated.

The goal of this prospective study was to assess whether HTPR, despite clopidogrel treatment, measured with Multiplate Electrode Aggregometer (Multiplate) is associated with the risk of adverse ischemic cerebral events.

2. Methods

This was a single-center study conducted at 1st Cardiology Department, Poznan University of Medical Sciences, Poland. Symptomatic patients with coronary artery disease admitted for coronary angiography and angioplasty (PCI) were consecutively enrolled and platelet function analysis and genetic CYP2C19 polymorphism testing was performed in each patient. The study design was approved by the local ethics committee (Policy no. 86/11). Signed informed consent was obtained from all patients.

2.1. Study population

A total of 249 consecutive patients underwent coronary artery stenting for stable angina (n = 215; 61 females and 154 males) or non-ST-elevation acute coronary syndrome (NSTE-ACS) (n = 34; 12 females and 22 males) from March 2011 to January 2012. Among the NSTE-ACS patients, positive markers of myocardial necrosis (troponin or CK-MB) were found in 28 persons.

The patients pretreated or on clopidogrel therapy for at least 5 days prior to PCI did not receive any additional loading dose of the drug. Clopidogrel naive patients received a loading dose of 300 mg one day prior to elective PCI, followed by a maintenance dose of 75 mg. A loading dose of 600 mg was given to all patients with acute coronary syndrome (ACS) prior to PCI, followed by a maintenance dose of 75 mg. All patients were already on acetylsalicylic acid (ASA) treatment; 75-mg daily dose. Unfractionated heparin was periprocedurally administered to all patients at the dosage of 70 U/kg. None of them required additional GP IIb/IIIa inhibitor infusion. PCI procedure was performed and other medications were administered in accordance with the current guidelines [10,11].

2.2. Platelet aggregation assessment

Blood samples were obtained from all 249 patients in order to determine the inhibition of platelet aggregation using Multiple Electrode Aggregometry (MEA) — Multiplate Analyzer (Dynabyte).

Peripheral venous blood was collected from antecubital vein — 5 ml (the first 5 ml were discarded to avoid spontaneous platelet activation) and shot into blood collecting tubes containing hirudin. The tests were performed 30–60 min after blood drawing. In all blood samples platelet aggregation was determined using MEA — Multiplate Analyzer (Dynabyte) using ADP-test (20 μmol/l solution of ADP) as agonist [12]. The mean value of two independent tests is expressed as the area under the curve (AUC) of the aggregation tracing determined as AU/min units. A mean value is calculated by the device, if the two results don't show a significantly different results. Only if the two results significantly vary, the test must be repeated. This ensures a satisfactory intra-assay variability. The inter-assay variability has been reported to be low (<6%) [13].

The patients undergoing elective PCI had their blood samples drawn on the next day after angioplasty (shortly before the administration of a 75-mg daily dose). In ACS patients' blood samples were taken on the third day after PCI (just before the administration of the next 75-mg daily dose).

According to Bonello et al. consensus on definition of HTPR, patients with values higher than 468 AU/min were classified as HTPR and below this cut-off value — as clopidogrel responders: non-HTPR [14].

2.3. Genotyping

Genetic polymorphism of CYP2C19 was tested using the HRM Real-Time PCR method (HRM, high resolution melting; PCR, polymerase chain reaction) and it was performed in 150 patients. Due to certain technical problems reliable results were obtained in 147 patients. Single nucleotide polymorphism (SNP) was used in order to identify polymorphisms. All genotyping procedures were performed in DNA Center, Poznan, Poland.

Genomic DNA was extracted from peripheral blood leukocytes with a commercial Dneasy Blood Mini Kit for genomic DNA isolation. Blood samples (5 ml) were drawn into EDTA-K_2 tubes and stored at 20 °C until DNA isolation. The following functional polymorphisms were selected: CYP2C19*2 (c. G681A; rs4244285) and CYP2C19*3 (c G636A; rs4986893). Genotyping of polymorphisms was performed using a high resolution melting analysis — PCR followed by HRM analysis [15] (HRM; Rotor Gene 6000®, Qiagen, Courtaboeuf, France) using specific primers: 5′ TGCAATAATTTTCCCACTATCA 3′ and 5′ CCTTGCTTTTATGG AAAGTGA 3′ and 5′ CCTTGCTTTTATGGAAAGTGA 3′ and 5′ TTTTTGCT TCCTGAGAAACCA 3′.

2.4. Study end points and clinical observation

The patients' outcomes were assessed at discharge and 12 months later at follow-up visits or by phone. The data regarding deaths were obtained from Polish national identification number registry.

The primary end point of this study was the cumulative incidence of ischemic events such as cardiovascular death, myocardial infarction (MI) and cerebral events (transient ischemic attack (TIA) ischemic stroke). The diagnosis of MI was established according to Thrombolysis in Myocardial Infarction (TIMI) criteria [16] and based on new abnormal Q-wave on the electrocardiogram and/or an increase in CK-MB value of three or more times above the normal limit. TIA was defined as an episode of neurologic dysfunction lasting less than 24 h. Stroke was defined as a sudden neurological deficit persisting over 24 h, confirmed by a computed tomography scan [17].

2.5. Statistics

Calculations were determined as an arithmetic mean and standard deviation (SD). The qualitative features are presented as the number and frequency (percentage) of the observed cases. Previously, we checked if data follows normal distribution (Shapiro–Wilks test). The distribution of blood platelet aggregation values did not follow the normal distribution and the comparison of more than 2 groups was performed by Kruskal–Wallis test with Dunn's post-hoc test. Nominal data were compared using chi-square test of independence. The risk of adverse events depended on platelet aggregation tertiles (T1 + T2 vs. T3) was assessed by odds ratio and 95% confidence interval. Differences in frequencies of adverse events in the studied tertiles were analyzed using the Chi-square for dependence or in case of small observed frequencies Fisher exact test was used [18,19,20]. Genotype frequencies were investigated for compatibility with Hardy–Weinberg equilibrium. Survival function was assessed by Kaplan–Meier estimator. The comparison between survival time in the studied groups was performed by log-rank test. The data were analyzed using Statistica 10.0 software (StatSoft, Inc.). The results were considered significant at $p < 0.05$.