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Impact of point-of-care testing for *CYP2C19* on platelet inhibition in patients with acute coronary syndrome and early dual antiplatelet therapy in the emergency setting ☆

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ABSTRACT

Aims: Only limited data exist about the role of point of care *CYP2C19* testing in the acute setting in the early phase of acute coronary syndromes (ACS). Therefore, the present study was designed to investigate the impact of *CYP2C19* loss-of–function point-of-care (POC) genotyping in patients presenting with acute coronary syndromes (ACS) and treated with dual antiplatelet therapy in the emergency setting.
Methods and Results: 137 subjects with ACS scheduled for percutaneous coronary intervention were consecutively enrolled. Pre- and on-treatment platelet aggregation was assessed by multiple electrode aggregometry (MEA) after stimulation with adenosine diphosphate (ADP). Patients were loaded according to current guideline adherent indications and contraindications for use of $P2Y_{12}$ inhibitors in ACS. POC genotyping for *CYP2C19*2* was performed in the emergency room after obtaining a buccal swab using the Spartan RX CYP2C19 system and obtaining patient's informed consent. Prasugrel and ticagrelor treated patients had significantly lower PR compared to clopidogrel-treated patients. The benefits of prasugrel and ticagrelor compared to clopidogrel treated patients in terms of platelet inhibition were more pronounced in *CYP2C19*2* carriers. Non-carriers showed similar inhibition regardless of particular $P2Y_{12}$ inhibitor treatment. Statistical analyses adjusting for factors associated with response (e.g. smoking) revealed that *CYP2C19*2* allele carrier status and loading with different type of P2Y12 receptor blockers were significant predictors of on-treatment platelet reactivity in the early phase of ACS.
Conclusion: The results of this pilot study of treatment of patients in the early phase of ACS indicate that *CYP2C19*2* POC genotyping might help to identify patients at risk with poor response to clopidogrel treatment, thereby benefiting from reloading and switching to alternative $P2Y_{12}$ receptor inhibition.

Introduction

The novel $P2Y_{12}$ inhibitors prasugrel and ticagrelor are more potent platelet inhibitors compared to clopidogrel due to markedly decreased variability of ADP-induced platelet inhibition [1]. The cytochrome P450 (CYP) 2C19 enzyme is majorly involved in the conversion of clopidogrel into its active metabolite [2] and the variability of clopidogrel response has been associated with impaired CYP2C19 enzyme function. *CYP2C19* is genetically polymorphic and loss-of-function alleles (e.g. *CYP2C19 *2, *3*) as well as the ultra-rapid metabolizer *CYP2C19*17* allele have been reported (http://www.cypalleles.ki.se/cyp2c19.htm). Homozygous subjects carrying the reference allele for *CYP2C19* (**1/*1*, so-called extensive metabolizers, EM) are able to efficiently bioactivate clopidogrel resulting in highest systemic exposure of active clopidogrel metabolites and subsequently in highest levels of platelet inhibition [2]. In contrast, individuals carrying the *CYP2C19*2* allele reveal decreased levels of platelet aggregation inhibition following clopidogrel administration at standard dosage. The potential of genetic testing to guide individualized patients' response to drug therapy is a matter of discussion since 15 years [3] but hitherto only limited examples are available to demonstrate clinical utility of genetic testing in clinical practice [4,5]. The Spartan RX CYP2C19 system is a polymerase chain reaction (PCR)-based "bedside" solution to genotype for *CYP2C19* variant alleles using a buccal swab of the patient.

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This device is able to extract DNA and analyses the candidate alleles within approximately one hour. As proof of concept the RAPID GENE (Reassessment of Antiplatelet Therapy Using an Individualized Strategy Based on Genetic Evaluation) study [6] was performed and 200 patients undergoing percutaneous coronary intervention (PCI) for acute coronary syndrome (ACS) or stable angina were randomly assigned to rapid point-of-care (POC) genotyping using Spartan RX CYP2C19 testing vs. standard of care treatment without *CYP2C19* genotyping. *CYP2C19*2* allele carriers were given 10 mg prasugrel daily, and non-carriers as well as patients in the standard treatment arm were treated with 75 mg clopidogrel daily. The primary endpoint representing the proportion of *CYP2C19*2* carriers with high on-treatment platelet reactivity ($P2Y_{12}$ reactivity unit value higher than 234) was significantly reduced in the genotype-guided study arm. Currently, little data exist of the impact of *CYP2C19* genotyping in the early phase after ACS. Although preferred use of novel $P2Y_{12}$ is implemented in the guidelines [7,8], the use of clopidogrel in the prehospital setting of ACS patients is still relevant particularly due to local treatment patterns [9] and poorly defined criteria and strategies how to switch patients to novel antiplatelet agents. The aim of the present study was to elucidate the impact of the loss-of-function *CYP2C19*2* allele by the use of POC-genotyping on pre-treatment platelet reactivity as well as on early platelet inhibition in the real-world ACS setting.

Methods

Study Population

From October 2011 till May 2013, 137 patients treated with coronary stenting for ACS were consecutively enrolled at the Department of Cardiology, University Hospital, Tübingen, Germany. Inclusion criteria were age older than 18 years, ACS and planned coronary angiography, as well as, informed consent. Exclusion criteria were any indication for oral anticoagulation and planned use of GPIIb-IIIa inhibitors. Patients were treated with $P2Y_{12}$ inhibitors according to current guideline adherent indications and contraindications for use of $P2Y_{12}$ inhibitors in ACS [5,6]. The study was conducted in accordance with the principles of good clinical practice and the Declaration of Helsinki and was approved by the ethical committee of the University Tübingen (270/2011BO1). All patients gave written informed consent.

Point-of-care DNA Testing

The Spartan RX™ sample-to-result POC DNA testing system (Spartan Bioscience, Ottawa, Canada) was used to identify carriers of the *CYP2C19*2* allele. Genotyping for *CYP2C19*3* and *CYP2C19*17* was not performed since at initiation of study in 2011 only the Spartan POC *CYP2C19*2* test system was commercially available. A buccal swab was acquired and inserted into an assay cartridge. After insertion of the reaction solution into the genetic testing device, *CYP2C19*2* genotype analysis was started. Materials and instrument were kindly provided by Spartan Bioscience. The company had no influence on the development, interpretation and dissemination of study results.

Platelet Function Analysis

Before loading and earliest 2 h after loading (median 12 hours) after a 600 mg loading dose of clopidogrel (300 mg in case of prior clopidogrel treatment), 180 mg of ticagrelor or 60 mg of prasugrel was administered, adenosine diphosphate-induced platelet aggregation was assessed on whole blood samples by impedance aggregometry using a Multiplate® analyzer (ROCHE). Blood samples were obtained after venous puncture and was placed in 4.5-ml plastic tubes containing the anticoagulant lepirudin (25 μg/ml; Refludan, Dynabyte, Munich, Germany). Details of this method have been reported elsewhere [10–13]. Reproducibility of the Multiplate assay has been previously demonstrated by low intra-assay variability in simultaneous measurement in independent donors [14]. Aggregation measured on the Multiplate device was quantified as area under the curve of aggregation units (AU) (area under the curve = AU × min).

Statistical Analysis

All statistical analyses were performed with SPSS Statistics software, version 21.0 (IBM/SPSS, Inc., Chicago, IL, USA) and R-3.0.2 (www.r-project.org) with additional package pwr-1.1.1.

Continuous data are presented as mean ± SD. Categorical variables are expressed as number (%).Observed and expected allele and genotype frequencies were compared using Hardy-Weinberg equilibrium calculation.

Equality of distribution between subgroups was analyzed by chi-squared test for categorical variables and by one way ANOVA for continuous variables. Platelet function parameters (pre- and on-treatment platelet reactivity) were tested for normality with the help of the Kolmogorov-Smirnov test. Since normality hypothesis was rejected, Box-Cox transformation was applied to achieve normal distribution with a lambda of 0.35 and 0.34 for pre- and on-treatment platelet reactivity, respectively). Differences between platelet function parameters according to genotype and treatment group were analyzed using Welch´s t-test. In each of the six groups defined by antiplatelet therapy and *CYP2C19*2* genotype, a power calculation for the paired t-test between pre- and on-treatment platelet reactivity values (Box-Cox transformed) was performed using a significance level of 5% (two-sided test).Statistical power for pairwise comparison between pre- and on-treatment platelet aggregation among P2Y12 receptor blocker treatment groups ranged from 94.2 to 97.9% in non-carriers of the *CYP2C19*2* allele and from 17.5 to 40.9% in *CYP2C19*2* carriers.

A linear regression model was established to show independent associations of on-treatment platelet reactivity. Variables entered into the model included clinical risk factors, relevant com-medication on admission, pre-treatment platelet reactivity and platelet count. A power calculation for this model was performed with function pwr.f2.test in R-library pwr (using a significance level of 5%), resulting in a power of 98.6% to detect a significant effect of at least one of the independent variables in the model on on-treatment platelet reactivity.

Results

Baseline Characteristics

137 patients with ACS were consecutively enrolled in the study. Coronary artery disease was known in 61 Patients (46.6%), 47 (41.6%) had been previously treated with PCI and coronary stenting and CABG surgery had been performed in 9 patients (8.0%). 54 (46.2%) had a history of smoking and 40 (34.2%) patients suffered from diabetes type II. Prior to admission to hospital, 58 (42.3%) patients were treated with 100 mg of acetylsalicylic acid (ASA) and 12 (8.8%) with clopidogrel. 42 (30.66%) of the patients were identified as carriers of the *CYP2C19*2* allele by Spartan RX testing. We found 38 heterozygous variant carriers (27% of all patients) and 4 homozygous variant carriers (3% of all patients). The *CYP2C19*2* allele frequency was in the range of previous reported frequencies in Caucasian populations [10,15] and the genotype distribution was in Hardy-Weinberg equilibrium ($p = 0.93$). Due to the low number of 4 homozygous variant *CYP2C19*2* carriers for statistical analyses heterozygous and homozygous variant carriers were merged into one group (CYP2C19 AC) and compared to *CYP2C19*1/*1* subjects (CYP2C19 WT). Baseline patients´ characteristics are given stratified according to *CYP2C19* genotypes (Table 1). No differences regarding clinical risk factors for ACS (e.g. diabetes type II, smoking status, hypercholesterinemia) were observed between both groups. Patients´