International Journal of Cardiology 167 (2013) 2882–2888

Contents lists available at ScienceDirect

International Journal of Cardiology

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

Value of platelet pharmacogenetics in common clinical practice of patients with ST-segment elevation myocardial infarction ☆

Jeffrey J.W. Verschuren [a], Helèn Boden [a], Judith A.M. Wessels [b], Bas L. van der Hoeven [a], Stella Trompet [a,c], Bastiaan T. Heijmans [d,f], Hein Putter [e], Henk-Jan Guchelaar [b], Martin J. Schalij [a], J. Wouter Jukema [a,f,g,h,*]

[a] Department of Cardiology, Leiden University Medical Center, Leiden, The Netherlands
[b] Department of Clinical Pharmacy and Toxicology, Leiden University Medical Center, Leiden, The Netherlands
[c] Department of Geriatry and Gerontology, Leiden University Medical Center, Leiden, The Netherlands
[d] Department of Molecular Epidemiology, Leiden University Medical Center, Leiden, The Netherlands
[e] Department of Medical Statistics, Leiden University Medical Center, Leiden, The Netherlands
[f] The Netherlands Consortium of Healthy Ageing, The Netherlands
[g] Interuniversity Cardiology Institute of the Netherlands, Utrecht, The Netherlands
[h] Durrer Center for Cardiogenetic Research, Amsterdam, The Netherlands

ARTICLE INFO

Article history:
Received 8 February 2012
Received in revised form 13 June 2012
Accepted 21 July 2012
Available online 29 August 2012

Keywords:
Pharmacogenetics
Antiplatelet therapy
ST-segment elevation myocardial infarction
Treatment outcome
Cytochrome P450 2C19
Cyclooxygenase 1

ABSTRACT

Background: Antiplatelet drug resistance is a well-known problem, causing recurrent cardiovascular events. Multiple genetic polymorphisms have been related to antiplatelet resistance by several large trials, however data from common clinical practice is limited. We examined the influence of previously described polymorphisms, related to aspirin and clopidogrel resistance, on treatment outcome in a real life unselected population of patients presenting with ST-segment elevation myocardial infarction (STEMI) treated with percutaneous coronary intervention.

Methods and results: This cohort study consisted of 1327 patients with STEMI. Patients were treated according to a standardized guideline-based protocol. Nine polymorphisms, COX1 (−842A>G), $P2Y_1$ (893C>T), GPIa (807C>T), GPIIIa (PlA1/A2), CYP2C19 (*2, *3 and *17), ABCB1 (3435T>C) and PON1 (576A>G), were genotyped. During 1 year of follow up the primary endpoint, a composite of cardiac death or recurrent myocardial infarction, was reached in 86 patients. The COX1 and CYP2C19*2 polymorphisms were associated with the primary endpoint, HR 2.55 (95% CI 1.48–4.40), P = 0.001 and HR 2.03 (1.34–3.09) P = 0.001, respectively. The combined analysis demonstrated a 2.5-fold increased risk for individuals with ≥2 risk alleles, $P = 6.9 \times 10^{-9}$. The association of COX1 was driven by mortality related events whereas that of CYP2C19*2 was mainly attributed to myocardial infarction and stent thrombosis.

Conclusion: In this unselected, real life population of STEMI patient on dual-antiplatelet therapy, the polymorphisms COX1 −842A>G and CYP2C19*2 were determinants of thrombotic complications during follow-up. We show that in a clinical setting, testing for these polymorphisms could be of value in the identification of STEMI patients at risk for recurrent cardiovascular events.

1. Introduction

Insufficient platelet inhibition during adequate guideline antiplatelet therapy is a well-known problem in the secondary prevention of coronary artery disease, causing a considerable amount of patients to suffer from recurrent thrombotic events [1]. Individual differences in the intrinsic rate of platelet reactivity and variability in response to antiplatelet therapy are the main underlying mechanisms responsible for this so-called antiplatelet therapy resistance. For both aspirin and clopidogrel this problem has been recognized. Several factors play a role in this inadequate response to antiplatelet agents. Clinical factors like increased body mass index (BMI), diabetes mellitus and drug–drug interactions have been implicated [1]. Moreover, genetic polymorphisms causing individual variability in drug absorption, metabolism and availability of biological targets, like platelet receptors, influence the platelet inhibition during therapy in each individual [1].

Pharmacogenetics is the upcoming field of research exploring the influence of genetic variation on response on drug therapy, to pursue

☆ Grant support: This work was funded by grants from the Interuniversity Cardiology Institute of The Netherlands (ICIN), the European Community Framework KP7 Programme under grant agreement [no. HEALTH-F2-2009-223004], the Center for Medical Systems Biology (CMSB), The Netherlands Consortium for Healthy Ageing (NCHA), The Netherlands Heart Foundation, the Netherlands Society of Cardiology and an unrestricted research grant from Guidant, Nieuwegein, The Netherlands.

* Corresponding author at: Department of Cardiology, Leiden University Medical Center, PO Box 9600, 2300 RC Leiden, The Netherlands. Tel.: +31 71 526 20 20; fax: +31 71 526 68 85.
E-mail address: j.w.jukema@lumc.nl (J.W. Jukema).

0167-5273/$ – see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.ijcard.2012.07.020

achievement of individualized therapy. There is growing evidence that aspirin- and especially clopidogrel resistance are partially determined by carrying risk alleles of several single nucleotide polymorphisms (SNPs) in genes resulting in altered drug efficacy [1]. In comparison with the largely inconsistent pharmacogenetic evidence on aspirin resistance due to high variability in laboratory tests and the small sample size of most studies [2], clopidogrel pharmacogenetics has become a well-recognized risk factor for resistance to treatment. Over the last years, multiple studies consistently reported an association of the reduced-function alleles of CYP2C19 and clopidogrel resistance and occurrence of thrombotic events [3–5]. However, the most recent meta-analyses did not demonstrate this association [6,7]. The studies included in these analyses comprise several patient groups, including patients with stable and unstable angina and the spectrum acute coronary syndromes. Specific data on patients with ST-segment elevation myocardial infarction (STEMI), a subpopulation specifically at high risk for thrombotic events, is limited. Furthermore, data on the generalizability of this evidence to the patients seen in the daily clinical practices is largely lacking.

The only large population based study on this subject is that of Simon et al. in the French Registry of Acute ST-Elevation and Non-ST-Elevation Myocardial Infarction (FAST-MI) population (n = 2208) [8]. Although this well-designed study investigated probably a good representative population, they still applied several exclusion criteria such as pre-specified levels of biomarkers and duration of complaints. Moreover, of the total included population, only 53% were STEMI patients and only 70% of the patients were treated with percutaneous coronary intervention (PCI), making it a quite heterogeneous population. Also the genotypic analysis was limited to 4 candidate genes. All other cohort studies were smaller and had even less patients with STEMI [9–11].

To obtain answers on the role of pharmacogenetics in the outcome of patients suffering from STEMI, our study investigated the effect of the best described polymorphisms implicated in antiplatelet therapy efficacy on thrombotic complications in a prospectively gathered real life unselected population of patients presenting with STEMI. The goal was to evaluate whether the described effects of these polymorphisms were detectable in this high risk population and could therefore possibly be of value for application in the common clinical practice.

2. Methods

2.1. Study population

The population of the current study consists of patients who were admitted with the diagnosis STEMI to the Leiden University Medical Center (LUMC), Leiden, The Netherlands, between February 2004 and January 2010. All patients underwent primary PCI and were treated according to the previously described standardized guideline-based MISSION! AMI care program [12]. In brief, the protocol includes a pre-hospital triage system based on 12-lead electrocardiography and when eligible pre-hospital administration of aspirin (300 mg), clopidogrel (600 mg) and abciximab (25 μg/kg bolus, followed by 10 μg/kg/min for 12 h) was performed to pursue early reperfusion. Patients were directly transferred to the catheterization laboratory for primary PCI. Beta-blockers and angiotensin-converting enzyme (ACE) inhibitors were titrated to achieve an optimal heart rate and blood pressure control. Moreover statin treatment was started in all patients. Patients without complications were discharged at day 3 after education on lifestyle changes and drug compliance. Aspirin (100 mg/day) was prescribed indefinitely, and clopidogrel (75 mg/day) for 12 months, irrespective of implanted stent type. All patients were offered an outpatient rehabilitation program.

2.2. Follow-up and study endpoints

During the first year, 4 outpatient clinic visits were scheduled. During this 1 year of follow-up all thrombotic cardiovascular events were recorded. In this study, the primary endpoint was the composite of cardiac death and recurrent myocardial infarction (MI). Secondary endpoints included cardiac death and MI separately, as well as definite stent thrombosis, repeat revascularization and all-cause mortality. All deaths were defined as cardiac, unless clearly proven non-cardiac. Myocardial infarction was defined as a troponin T level above the upper limit in the presence of ischemic complaints or PCI or a re-rise of >25% after recent MI in the presence of symptoms or PCI. Stent thrombosis was defined as angiographic or pathological confirmation of a partial or total thrombotic occlusion within the stent or 5 mm proximally or distally to the stent [13]. Finally, data on revascularization of any coronary artery, irrespective of the treatment modality (PCI or CABG) was collected. Target vessel revascularizations were all clinically driven.

2.3. Genotyping

EDTA blood was prospectively collected on admission and DNA was extracted following standard procedures. DNA was available from 1370 of the 1674 consecutive patients, reasons for missing DNA were death before the collection of blood or failure of the DNA extraction.

The most well replicated genetic polymorphisms related to aspirin and clopidogrel resistance were selected after a systematic search of literature, as described previously [1]. In brief, relevant articles were identified by searching MEDLINE using keywords and Medical Subject Headings (MeSH) terms including the following: pharmacogenetics, single nucleotide polymorphism, treatment outcome, adverse effects, drug therapy and cardiovascular disease (CVD). All available literature until May 2011 was included and reviews, editorials, and articles in languages other than English were excluded. A multiplex assay was designed using Assay designer software. When a SNP did not fit the multiplex, a proxy of that SNP was selected with the highest R^2 value. The final set included cyclooxygenase 1 (*COX1*) −842A>G (rs10306114), P2Y purinoceptor 1 (*P2Y$_1$*) 893C>T (rs1065776), *P2Y$_{12}$* 52G>T (rs6809699), glycoprotein (GP) Ia 807C>T (rs1126643), *GPIIIa* rs8069732 (in complete linkage disequilibrium (LD) ($R^2 = 1.0$) with PlA1/A2 [rs5918]), cytochrome P450 (CYP) 2C19*2 (rs4244285), CYP2C19*3 (rs4986893) and rs11188072 (in complete LD ($R^2 = 1.0$) with CYP2C19*17 [rs12248560]), ATP-binding cassette sub-family B member 1 (*ABCB1*) rs2235048 (as a proxy for 3435T>C [rs1045642], in complete LD with $R^2 = 1.0$) and paraoxonase-1 (*PON1*) 576A>G (rs662) (Supplementary Table 1).

These SNPs, except the SNP in *PON1*, were genotyped by MALDI-TOF mass spectrometry, using the MassARRAY™ methodology (Sequenom Inc., San Diego, CA, USA), following the manufacturer's instructions. The *PON1* 576A>G (rs662) SNP was genotyped using a TaqMan drug metabolism genotyping assay (Assay ID C_2548962_20; Applied Biosystems, Foster City, CA, USA). As quality control, 5% of the samples were genotyped in duplo. No inconsistencies were observed. All the negative controls (2%) were negative. Call rate of all SNPs was above 98%. Two SNPs deviated significantly from Hardy–Weinberg (HW) equilibrium. $P2Y_{12}$ 52G>T (rs6809699) (HW ChiSq 362.7, $P = 7.4 \times 10^{-81}$) was excluded from further analysis. The CYP2C19*3 polymorphism (HW ChiSq 64.0, $P = 1.3 \times 10^{-15}$) was included, since the deviation was caused by 1 homozygote of the minor allele of this low frequency SNP (0.3% in the current population). This minor allele frequency was lower than the reported frequency in HapMap CEU reference panel (1.7%) (http://www.hapmap.org). All other SNPs matched with the reported frequencies. Finally, individuals with 50% or more failed SNPs were excluded (3%). The final analysis included 1327 patients.

3. Statistics

To compare the group with and the group without a primary outcome event, categorical parameters are compared with Pearson chi-square or Fisher's exact test when appropriate. Continuous data were compared with unpaired 2-sided Student's t-test. In the case of non-Gaussian distribution, variables were compared with the Mann–Whitney test. All SNPs were tested for deviation from Hardy–Weinberg equilibrium using chi-square analysis.

Associations of SNPs with outcome events were calculated using multivariable, stepwise, forward Cox analyses assuming an additive genetic model. This multivariable model included the clinical variables that were significantly different between the primary event group and the group without. For the polymorphisms associated with $P < 0.05$, recessive and dominant models were tested. Statistical analysis was performed using SPSS software (SPSS Inc., Chicago, IL, USA). The authors of this manuscript have certified that they comply with the Principles of Ethical Publishing in the International Journal of Cardiology.

4. Results

4.1. Baseline characteristics

All patients were treated with a primary PCI and received dual antiplatelet therapy with aspirin and clopidogrel. In-hospital mortality of the final study population was 1.8% (24 patients). At discharge from the hospital admission following the primary intervention, aspirin and clopidogrel were prescribed to 96.0% and 99.7% of the