Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.elsevier.com/locate/crvasa

Original research article

Platelet function testing after acute myocardial infarction: The correlation among various assays is insufficient

Radomir Nykl [a,*], *Jiri Plasek* [b], *Tomas Fürst* [c], *Jana Fürstova* [d], *Jaromir Gumulec* [e], *Karin Licha* [e], *Miroslav Homza* [b]

[a] *Cardiovascular Department, University Hospital Ostrava, Czech Republic*
[b] *Cardiovascular Department, University Hospital Ostrava and Faculty of Medicine, University of Ostrava, Czech Republic*
[c] *Department of Mathematical Analysis and Applications of Mathematics, Faculty of Science, Palacky University Olomouc, Czech Republic*
[d] *First Faculty of Medicine, Charles University in Prague, Czech Republic*
[e] *Institute of Clinical Haematology, University Hospital Ostrava, Czech Republic*

ARTICLE INFO

Article history:
Received 30 September 2015
Received in revised form
9 November 2015
Accepted 13 November 2015
Available online 24 December 2015

Keywords:
Dual antiplatelet therapy
Clopidogrel
Acetylsalicylic acid
High on-treatment platelet reactivity
Reproducibility
Myocardial infarction

ABSTRACT

Aim: High on-treatment platelet reactivity (HPR) after Acetylsalicylic acid (ASA) and/or Clopidogrel was identified by four different platelet function assays in high risk acute myocardial infarction patients. Correlation among the methods was examined and variability in the measurement of each platelet function assay was evaluated.

Methods: Each of the 53 patients was sampled twice for LTA, PFA-100/200, Multiplate®, and VerifyNow® platelet function assays in the range of 3–5 days after a myocardial infarction. Non-parametric correlation and linear regression were used to assess the variability in the data.

Results: All HPR platelet function assays for Clopidogrel are significantly correlated, however, the correlation values are only moderate. The correlation among the four assays for ASA is generally low and insignificant. Low reproducibility of HPR measurements and no significant correlation between Troponin I value and HPR were observed, both for ASA and Clopidogrel. No significant correlation between the type of acute coronary syndrome and HPR was observed, both for ASA and Clopidogrel.

Conclusion: At least two methods to identify HPR are recommended. The average of at least two measurements is recommended for any assay.

* *Corresponding author at*: Cardiovascular Department, University Hospital Ostrava, 17 listopadu 1790, Ostrava-Poruba, Czech Republic. Tel.: +420 603105762.
E-mail address: nyky@post.cz (R. Nykl).

Abbreviation: ACS, acute coronary syndrome; ADP, adenosine diphosphate; APTT, activated partial thromboplastin time; ASA, Acetylsalicylic acid; AUC, area under curve; CABG, a history of cardiovascular surgery; DAPT, dual antiplatelet therapy; DM, diabetes mellitus; HPR, high on-treatment platelet reactivity; LMWH, low molecular weight heparin; LTA, Light Transmittance Aggregometry; NSTEMI, non-ST-segment elevation acute coronary syndrome; PCI, percutaneous coronary intervention; POCT, point of care testing; SIRS, systemic inflammatory response syndrome; STEMI, ST-segment elevation acute coronary syndrome; TnI, Troponin I; UFH, unfractionated heparine; vWF, von Willebrand factor.

http://dx.doi.org/10.1016/j.crvasa.2015.11.004

Introduction

The dual antiplatelet therapy (DAPT) is crucial for pharmacological treatment after PCI (percutaneous coronary intervention). The main reason for DAPT is to reduce the occurrence of thrombotic events. Acetylsalicylic acid (ASA) and Clopidogrel are substances with proven clinical efficacy: ASA reduces the risk of ischemic vascular events in the primary prevention by 20–25% [1], in the secondary prevention by up to 40% [2]. Clopidogrel can reduce the relative risk of vascular complications by 8.7% [3] compared to ASA.

There is no doubt that the dual antiplatelet therapy is the cornerstone of treatment after a PCI. However, its efficacy may differ from patient to patient. This variability might be caused by high on-treatment platelet reactivity (HPR), in other words, lower platelet inhibition. The most appropriate definition of HPR was given by Hankey in 2004 [4]. HPR can be tested by platelet function assays. There are a lot of unanswered questions related to this topic: Does HPR predict ischemic episodes? Which platelet function assay should be used? Do the results of different assays correlate well? Are we able to tailor the treatment according to the HPR status?

Clopidogrel resistance is presumably multifactorial [5,6]. Clopidogrel is a prodrug with two-step activation to the active form, with dependence on the cytochrome P-450 polymorphism. The prevalence of HPR is between 5 and 40% [5,6]. The variability of the ASA treatment response has also been observed but its relation to thrombotic events has not been fully explained [7].

The aim of this analysis is to assess the agreement among various platelet function assays for ASA and Clopidogrel HPR, and to examine the reproducibility of each of the assays. Further, the possible dependence is examined of the HPR status and the Troponin I level and the type of myocardial infarction (ST-elevation or non-ST-elevation). At the site of vascular damage, many matrix proteins (e.g. von Willebrand factor and collagen) are exposed to the blood which leads to platelet activation [8]. It can be assumed that the larger the area of the myocardium is damaged, the greater number of platelets are involved, which may hinder their effective inhibition.

The following four assays were examined: Light Transmittance Aggregometry (LTA), the PFA-100/200 system, VerifyNow® and the Multiplate® impedance aggregometry. All these assays are used in clinical practice and clinical trials [5]. The Methods section contains more details about the four assays.

This paper presents a subanalysis of the REACT-MI trial (the design of REACT-MI is available at: clinicaltrials.gov NCT01381185).

Methods

Study population

The study cohort consists of 53 patients with acute coronary syndrome (ACS) who were admitted to the Coronary Unit of the Cardiovascular Department, University Hospital in Ostrava (STEMI in 59%). From the 478 STEMI and 518 NSTEMI patients treated between 2011 and 2014 at our institution. The most frequent reason for exclusion from the cohort of screened patients was de novo myocardial infarction (without ASA in the previous medication) and pre-treatment with novel antiplatelet agents.

Only 65 patients fulfilled the demanding inclusion criteria of the REACT-MI trial (see the details at clinicaltrials.gov NCT01381185). In order to avoid any interference with the platelet function assays, strict exclusion criteria were applied in terms of the hematocrit levels (<0.25 and >0.55), anemia (<80 g/l), polyglobulia (>160 g/l), SIRS, renal insufficiency, etc. Five of the patients did not sign the informed consent (otherwise complying with the inclusion criteria), two were lost to follow-up, and in one patient a violation of the study protocol occurred. In several patients, various problems with blood sampling and aggregation measurement occurred. Due to the amount of blood sampling, some patients refused to be sampled for the second time. For more details about the number of assays performed, see the descriptive statistics in the Results section. Each of the patients underwent PCI in 24 h after admission. The acute coronary syndrome occurred although the patients had taken ASA, Clopidogrel or both of them, i.e. the patients were in the secondary prevention, a high-risk population. If a patient had not taken Clopidogrel before the admission, a loading dose of 600 mg was administered. According to the study protocol, the dose of ASA (or Clopidogrel or both) was doubled (200 mg of Aspirin and 75 mg of Clopidogrel bid) if HPR to ASA (or Clopidogrel or both) was found by at least two platelet function assays. The descriptive statistics of the study cohort are summarized in Table 1. See the list of abbreviations.

Blood sampling

On the second day after PCI (LMWH/UFH discontinued for 12 h) and then on the third to fifth day after PCI, five tubes of blood were taken from each patient:

1. a tube with Na-citrate 3.2% (Quick, APTT, Fibrinogen, von Willebrand factor, Light Transmittance Aggregometry),
2. a tube with 3.8% buffered citrate for PFA analysis according to the manufacturer's protocol (PFA-100®; Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany),
3. a greiner tube for VerifyNow® analysis (Accumetrics, San Diego, CA),

Table 1 – The study cohort characteristics given as mean ± standard deviation, or percentage. See the list of abbreviations.

Age	66 ± 11.29
Height	171 ± 7.32
Weight	82.38 ± 13.94
BMI	29.04 ± 4.09
Waist circumference	90.45 ± 14.17
Male sex	75%
STE-MI	59%
Hypertension	92%
Dyslipidemia	67%
DM	51%
CABG	15%
Heart failure	51%
Smokers	35%