Thrombosis Research 131 (2013) 333–337

Contents lists available at SciVerse ScienceDirect

Thrombosis Research

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

Regular Article

# Intrinsic platelet reactivity and thrombus burden in patients with ST-elevation myocardial infarction

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ARTICLE INFO

*Article history:*
Received 5 December 2012
Received in revised form 6 February 2013
Accepted 11 February 2013
Available online 6 March 2013

*Keywords:*
Platelets
Platelet reactivity
ST elevation myocardial infarction
Intracoronary thrombus

ABSTRACT

*Introduction:* In patients with ST elevation myocardial infarction (STEMI) increased platelet reactivity has been described to affect the primary percutanuous coronary intervention (PPCI) outcome. We aimed to evaluate the predictive accuracy of intrinsic platelet reactivity for intracoronary thrombus burden in P2Y12 inhibitor- naïve STEMI patients.

*Patients and Methods:* In a prospective, observational, cohort study we enrolled 94 consecutive STEMI patients undergoing PPCI, subjected to platelet reactivity assessment prior to any P2Y12 blockade, with visible angiographic thrombus in the infarct related artery (stratified as Grade A, B and C). Platelet-function testing was performed with the VerifyNow point-of-care P2Y12 assay immediately prior to intervention.

*Results:* Intrinsic platelet reactivity was higher with greater thrombus burden: Grade A 158.8 ± 51.1 PRU, Grade B 217.4 ± 62.1 PRU and Grade C 243.4 ± 58.6 PRU, p = 0.009 and Spearman r = 0.32 (0.12-0.49 95% CI), p = 0.002. ROC analysis revealed an AUC = 0.7 (Standard error 0.07, p = 0.03). An intrinsic platelet reactivity value of > 220 PRU had 65% sensitivity (53–76 95%CI), 76% specificity (55–91 95%CI), 88% positive predictive value (76–96 95%CI) and 44% negative predictive value (29–60 95%CI) for detection of high thrombus burden. In multivariate analysis intrinsic platelet reactivity > 220 PRU emerged as an independent predictor of high thrombus burden (RR = 1.5, 1.15-2.07 95% CI, p = 0.004).

*Conclusions:* In patients admitted with STEMI the intrinsic platelet reactivity -as assessed by a point-of-care assay- is positively associated with the degree of intracoronary thrombus, while having a moderate accuracy in predicting high thrombus burden.



## Introduction

The importance of thrombus formation in the pathogenesis of ST elevation myocardial infarction (STEMI) is well appreciated, with platelets having a central role in this process [1]. Platelet activation has been described in patients with STEMI [2,3]. In patients undergoing primary percutaneous coronary intervention (PPCI) an increased intrinsic platelet reactivity (PR) before P2Y12 blockade has been reported to impact on the angiographic success, the degree of ST-segment resolution, the extent of myocardial necrosis, and the short- and mid-term clinical outcome [3–5]. In acute coronary syndrome patients also platelet hyperfunction has been found to predict long term events [6]. On the other hand, the intracoronary thrombus burden is predictive of major adverse cardiac events, infarct-related artery patency and stent thrombosis [7]. In patients pre-treated with clopidogrel and delayed PPCI an increased PR predicts a larger thrombus burden and a worse post-PCI myocardial flow and perfusion [8]. However, to our knowledge, there has been no study of any possible relationship between PR and thrombus burden in the very early phase of STEMI before any interfering intervention.

We aimed, therefore, to evaluate in P2Y12 inhibitor-naïve STEMI patients the association of intrinsic PR, as assessed by a point-of-care assay, and the almost simultaneously obtained intracoronary thrombus angiographic grading.

## Methods

In this prospective, observational, cohort study we enrolled consecutive STEMI patients undergoing PPCI between Feb 2012 and July 2012 in our University Hospital catheterization laboratory who had intrinsic -prior to any P2Y12 blockade- PR assessment. STEMI was defined as ST-segment elevation of 0.1 mV in 2 or more limb leads, or 0.2 mV in 2 or more contiguous pre-cordial leads of the 12-lead

*Abbreviations:* AUC, area under the curve; BARC, Bleeding Academic Research Consortium; CI, Confidence interval; HPR, high platelet reactivity; HTB, high thrombus burden; LTB, low thrombus burden; PPCI, primary percutaneous coronary intervention; PR, platelet reactivity; ROC, Receiver Operating Characteristic; STEMI, ST elevation myocardial infarction.

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http://dx.doi.org/10.1016/j.thromres.2013.02.010

electrocardiogram and with persistent symptoms of chest pain between 30 minutes and 12 hours after symptom onset. All patients received oral aspirin 325 mg (Unipharma AE, Greece) and 70 U/kg of unfractionated heparin intravenously at first medical contact. All patients participated in concomitantly performed pharmacodynamic studies involving ticagrelor or prasugrel administration, we therefore excluded from intrinsic PR assessment those with contraindications to these drugs. Specifically, we excluded patients with a history of stroke/transient ischemic attack, bleeding diathesis, chronic oral anticoagulation treatment, previous antiplatelet treatment, contraindications to antiplatelet therapy, PCI or coronary artery bypass grafting <3 months, hemodynamic instability, platelet count <100000/μL, hematocrit <30%, creatinine clearance <30 ml/min, severe hepatic dysfunction, use of strong CYP3A inhibitors or inducers, increased risk of bradycardia, severe chronic obstructive pulmonary disease or periprocedural IIb/IIIa inhibitors administration. All patients underwent standard coronary angiography and PPCI with stent implantation. Manual aspiration of intracoronary thrombus and the type of stent implanted was left at the discretion of the operator.

Analysis of the angiogram obtained prior to PPCI was performed blindly to platelet function analysis by a consensus opinion of 2 experienced interventional cardiologists. In case of disagreement, the expert opinion of a third independent interventional cardiologist was decisive. The culprit lesion was determined by angiographic and electrocardiographic features. The angiographic view that best demonstrated the thrombus was selected for analysis. Grading for thrombus classification was performed as previously described [7,8] (1) No detectable thrombus; (2) Grade A: definite thrombus with greatest dimensions ≤1/2 the vessel diameter; (3) Grade B: definite thrombus with greatest linear dimension >1/2 but <2 vessel diameters; and (4) Grade C: definite thrombus with greatest linear dimension ≥2 vessel diameters. In cases with an occluded infarct related artery thrombus was assessed after flow achievement with either guidewire crossing or a small (diameter 1.5 mm) balloon dilation. We further stratified patients into 2 groups: small thrombus burden (Grade A and B) and high thrombus burden (Grade C). TIMI-flow in the infarct related artery was also assessed prior to PPCI.

*Platelet Function Analysis*

Peripheral venous blood sample was drawn with a loose tourniquet through a short venous catheter inserted into a forearm vein exactly following coronary angiography and immediately before PPCI and administration of P2Y12 inhibitors. The first 2 to 4 ml of blood was discarded to avoid spontaneous platelet activation and blood was collected in 3.2% citrate (1.8 mL draw plastic Vacuette tubes; Greiner, Monroe, NC, USA). Platelet-function testing was performed with the VerifyNow (Accumetrics Inc., San Diego, CA, USA) point-of-care P2Y12 assay as previously reported [9]. The results are reported in P2Y12 reaction units (PRU), BASE and % inhibition. The % inhibition is calculated as: ([BASE-PRU]/BASE) X 100. Two definitions of high PR (HPR) were used, HPR1 for PR≥230 PRU and HPR2 for PR≥208 PRU based on previous investigations linking the cut-off point to post-PCI ischemic risk [10,11].

We aimed to evaluate the accuracy and the optimal cut-off of intrinsic PR in predicting high thrombus burden. We also examined the association between intrinsic PR and intracoronary thrombus angiographic grades. Potential risk factors for high thrombus burden and PR predictive accuracy for TIMI 0 flow were also assessed.

*Clinical Follow Up*

Clinical follow up was performed either by a clinical visit or by telephone contact to the patient. The occurrence of death, myocardial infarction, stroke, urgent revascularization, as well as any other adverse event was recorded.

*Statistical Analysis*

The predictive accuracy of intrinsic PR for the presence of high thrombus burden was assessed by Receiver Operating Characteristic (ROC) analysis. Power analysis was performed with a two-sided z-test [12]. A sample of 59 patients from the high thrombus burden (HTB) group and 24 patients from the low thrombus burden (LTB) group (assuming an allocation ratio HTB group/LTB group of 2.5:1, based on our own preliminary data) would suffice for a 85% power to detect a difference of 0.2 between the area under the curve (AUC), under the null hypothesis of AUC = 0.5 (no diagnostic accuracy) and the alternative hypothesis of AUC = 0.7 (moderate diagnostic accuracy), at a significance level of 0.05.

Categorical data are presented as frequencies and group percentages. Continuous data with normal and skewed distribution are presented as means ± standard deviation (SD) and medians (range) respectively. The Kolmogorov-Smirnov test was used to examine data distribution normality. Fisher's exact test was used for comparison of categorical data. Two-sample t-test and 1-way ANOVA were used for comparison of normally distributed continuous data. The Mann–Whitney U test and the Kruskal-Wallis test were used for comparison of skewed continuous data. The association between intrinsic PR and the degree of intracoronary thrombus was examined by Spearman rank correlation analysis and thrombus was rated as 1 (Grade A), 2 (Grade B) and 3 (Grade C). To assess potential risk factors for high thrombus burden we used modified Poisson regression models for binary data (log-Poisson regression with robust estimate of variance) [13], since odds ratios produced by logistic regression may overestimate the magnitude of association between covariates and the outcome of interest when the outcome variable is not rare [14]. We first assessed the unadjusted relative risks of high thrombus burden for various patients' characteristics and then a final multivariate model was fitted including simultaneously (enter method) all the factors found to be related to high thrombus burden status at the level of $p<0.1$ in the univariate analyses. All tests were 2-tailed and statistical significance was considered for p-values<0.05. Statistical analyses were performed using SPSS for Windows (version 16.0 SPSS Inc.Chicago Il USA) and MedCalc statistical software (Version 11, Mariakerke, Belgium).

All patients gave informed consent for participation in the study, which was approved by the Ethics Committee of the University Hospital of Patras, Greece.

## Results

Patients with no detectable thrombus (n = 2) were excluded from further analysis. In the remaining 94 patients there were no significant differences in the demographic and clinical characteristics according to thrombus burden (Table 1). Intrinsic PR was higher with increasing thrombus burden (Table 2). There were no differences in BASE or % inhibition between groups. HPR1 and HPR2 rates were higher with increasing thrombus grade (Table 2). Individual values of intrinsic PR stratified by thrombus burden are shown in Fig. 1. Spearman rank analysis indicated a significant positive correlation of moderate strength between intrinsic PR and thrombus grades, r = 0.32 (0.12-0.49 95% CI), p = 0.002. ROC analysis showed that intrinsic PR had an AUC of 0.7 (Standard error 0.07, p = 0.03, Fig. 2) for predicting high thrombus burden. An intrinsic PR value of >220 PRU has a sensitivity of 65% (53–76 95%CI), a specificity of 76% (55–91 95%CI), a positive predictive value of 88% (76–96 95%CI) and a negative predictive value of 44% (29–60 95%CI) for detection of high thrombus burden. Unadjusted relative risks of high thrombus burden for patients' demographic and clinical characteristics are shown in Table 3. In multivariate analysis (Fig. 3) intrinsic PR >220 PRU emerged as an independent predictor of high thrombus burden (RR = 1.5, 1.15-2.07 95% CIs, p = 0.004). Intrinsic PR was not found to be predictive of TIMI flow 0 (observed in 63.8% of