Thrombosis Research 125 (2010) e184–e189

Contents lists available at ScienceDirect

Thrombosis Research

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

Regular Article

Platelet glycoprotein VI (GPVI) for early identification of acute coronary syndrome in patients with chest pain

Boris Bigalke [a,*], Michael Haap [b], Konstantinos Stellos [a], Tobias Geisler [a], Peter Seizer [a], Elisabeth Kremmer [c], Dietrich Overkamp [d], Meinrad Gawaz [a]

[a] Medizinische Klinik III, Kardiologie und Kreislauferkrankungen, Eberhard-Karls-Universität Tübingen, Germany
[b] Intensive Care Unit, Medizinische Klinik, Eberhard-Karls-Universität Tübingen, Germany
[c] Institute for Molecular Immunology, Helmholtz Center Munich, Germany
[d] Emergency Room, Medizinische Klinik, Eberhard-Karls-Universität Tübingen, Germany

ARTICLE INFO

Article history:
Received 22 October 2009
Received in revised form 14 December 2009
Accepted 11 January 2010
Available online 1 February 2010

Keywords:
Platelets
Glycoprotein VI
Chest Pain
Acute Coronary Syndrome

ABSTRACT

Background: Platelet glycoprotein VI (GPVI) is elevated in patients with acute coronary syndrome (ACS), stroke and associated with acute coronary events. GPVI may be helpful to distinguish an imminent ACS from non-coronary (NC) causes in patients with chest pain who were transferred to chest pain unit, before the myocardial necrosis is evident with classical biomarkers.
Methods: Based on the findings of our previous studies, we consecutively examined 1004 patients with chest pain in a prospective study design. ACS was found in 416 (41.4%), stable angina pectoris (SAP) in 233 (23.2%), and NC causes of chest pain (hypertension, musculoskeletal disease, pulmonary embolism, myocarditis, cardiophobia) in 355 patients (35.4%). Platelet surface expression of GPVI was measured by flow cytometry.
Results: Patients with ACS showed significantly enhanced GPVI expression levels compared to patients with SAP or NC causes of chest pain (ACSvs.SAP(mean fluorescence intensity (MFI) $\pm$ SD):18.9 $\pm$ 7.4vs.17.9 $\pm$ 9.5; $P = 0.028$;ACSvs.NC:15.4 $\pm$ 6.9;$P = 0.002$). Elevated GPVI expression was associated with ACS independent of markers of myocardial necrosis like troponin and creatine kinase-MB. Patients with an elevated GPVI expression (MFI $\geq$ 18.6) had a poorer clinical outcome than patients with baseline GPVI expression in regard to composite cumulative survival that included myocardial infarction, stroke, and cardiovascular death at three months (Log rank;$P = 0.025$).
Discussion: Platelet GPVI surface expression is enhanced in patients at risk for an ACS and is an early marker for imminent acute coronary events in patients with chest pain.

Introduction

Distinguishing patients that present to the hospital with chest pain have an acute coronary syndrome (ACS) or a non-coronary (NC) problem is often difficult, time-consuming, and expensive [1]. Usually the electrocardiogram (ECG) is the first specific diagnostic test that is rapidly available [2]. Most patients with ACS do not provide clear ECG changes or even show normal ECG tracings and biomarkers represent an important diagnostic value in these patients [3,4].

Several cardiac biomarkers have emerged as strong predictors of risk among patients presenting with suspicion of an ACS and are now routinely available to clinicians [5]. Particularly, the determination of elevated biomarker levels upstream from markers of myocardial necrosis (creatine kinase-MB, troponins-T and -I), such as markers of platelet activation (P-Selectin) and inflammation (C-reactive protein, CD40L), or myocardial stretch (BNP) has been considered as diagnostic targets [6–8]. The biomarkers have been associated with poor prognosis in patients with ACS when myocardial damage has already occurred [9].

Platelets play a key role in arterial thrombosis and in ACS. Platelet collagen receptor glycoprotein VI (GPVI) has proved to be the initial trigger of platelet activation and aggregation at the site of vascular injury where collagen is exposed [10,11].

In recent studies, we could show that the surface expression of platelet GPVI is elevated in patients with ACS, stroke and associated with acute coronary events [12–16]. Furthermore, an increased

Abbreviations: ACE, angiotensin converting enzyme; ACS, acute coronary syndrome; BNP, brain natriuretic peptide; CAD, coronary artery disease; CV, cardiovascular; ECG, electrocardiogram; GPVI, glycoprotein VI; MFI, mean fluorescence intensity; MI, myocardial infarction; NC, non-coronary; ROC, receiver operating characteristic; SAP, stable angina pectoris; SD, standard deviation.

* Corresponding author. Medizinische Klinik III,Eberhard-Karls-Universität Tübingen,Otfried-Müller-Str.10, D-72076 Tübingen, Germany. Tel.: +49 70712983688; fax: +49 7071 295749.
E-mail address: boris.bigalke@med.uni-tuebingen.de (B. Bigalke).

0049-3848/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.thromres.2010.01.005

surface expression of platelet GPVI was associated with a poor clinical outcome in patients with ACS and stroke [13,14].

The question of the present prospective study was if the determination of platelet GPVI may be helpful to distinguish an imminent ACS from NC causes in patients with chest pain who were transferred to chest pain unit, before the myocardial necrosis is evident with classical markers such as creatine kinase-MB and troponin.

Methods

Study population and design

1004 (12.8%) of 7845 patients who presented to our emergency unit have been transferred to the chest pain unit under suspicion of ACS. According to the AHA/ACC guidelines, a diagnostic coronary angiography has been performed in 716 (71.3%) of 1004 consecutive patients with chest pain [2]. Thus, we evaluated patients by coronary angiography according to an urgent (e.g., ST-elevation myocardial infarction, hemodynamic or electrical instability), early (e.g., Non-ST-elevation myocardial infarction, Troponin positive ACS), or selective invasive strategy (e.g., failure of medical therapy with refractory angina, objective evidence of ischemia with dynamic ECG changes, high-risk stress test), whereas complied with the recommendations not to perform in patients with serious comorbitities or contraindications such as liver, renal, pulmonary failure and cancer [2]. Angioplasty has been performed in 655 of 716 patients (91.5%) with significant stenoses. The prospective study was approved by the ethics committee at the University Hospital Tübingen, Germany. All patients included have given informed consent.

The exclusion criteria comprised patients with an age below 18 years, lack of informed consent, cardiogenic shock, ischemic stroke, a history of malignancy, psychiatric disease, disease of the connective tissue, thyroid gland, kidneys, anorexia, administration of steroids or hormone replacement therapy or pregnancy in women.

Clinical outcome was assessed by doctor's letters, rehospitalization, or structured, predefined telephone interviews after a three-month follow-up (91.7%: 921 of 1004 patients).

Definitions of ACS

ACS definition followed according to the AHA/ACC guidelines [2].

ACS: episodes of >20 min of angina pectoris with progress over time or with recurrent episodes at rest or with minimal physical strain within the last 24 h. The ECG shows at least one of the following criteria: new ST-depression of at least 0.1 mV, or negative T of at least 0.3 mV in two or more leads of standard ECG. CK and troponin-I levels may be increased, but can be normal as well. Diagnostic thresholds based on previously published studies for for creatine kinase-MB (≥24 U/L) and troponin-I (≥0.04 μg/L) [17,18].

SAP: stable angina pectoris, symptoms without progression and stable intensity, with evidence of coronary artery disease in coronary angiography presenting non-elevated biomarkers and no dynamic ECG changes.

Sample collection

Blood was drawn from the anticubital vein on time of admission to the hospital. The median time between chest pain onset and hospital admission was 135 minutes in our study population. The blood was filled into 5 mL citrate phosphate dextrose adenine vials, processed and analyzed immediately by flow cytometry as described [12,13]. Since the time course of GPVI expression has been analyzed before, we did not perform serial level checks [13].

FACS analysis

The surface expression of the platelet receptors GPVI and GPIb was determined by two-color whole blood flow cytometry. Mean fluorescence intensity (MFI) was used as the index of receptor expression. Phycoerythrin-conjucated anti-CD42b (clone SZ2) monoclonal antibodies (mAb) was purchased from Immunotec, Beckman Coulter, Inc., USA. The fluorescein-conjugated anti-GPVI mAb 4C9 was generated and characterized, as described previously [12,13].

Statistical Analysis

A probability value of less than 0.05 was considered as statistical significant and evaluted with appropriate non-parametric tests. For correction of multiple tresting, a Bonferroni-Holm correction was performed. Adjustment by possible confounders was performed by the multifactorial analysis of covariance for the decadic logarithm of GPVI. Association of the platelet GPVI surface expression was found to be independent of medical treatment at the time of admission, classical cardiovascular risk factors, and laboratory markers. The association of GPVI after adjustment for conventional laboratory markers was assessed by the logistic regression analysis in patients under suspicion for ACS. In order to predict the individual risk for ACS by using the levels of GPVI, we calculated the receiver operating characteristic (ROC) curve. A log-rank test (Mantel-Cox) was applied for the evaluation of associations between event and variables. All statistical analyses were performed using SPSS version 15.

Results

We consecutively investigated the platelet surface expression of collagen receptor GPVI in a total of 1004 patients with chest pain, who were admitted to the chest pain unit under suspicion of ACS. The demographic details are given in Table 1.

Among these patients 716 (71.3%) underwent coronary angiography which showed a coronary cause in 649 patients (90.6%) and an NC cause of chest pain in 67 patients (9.4%) such as hypertension (n = 30), musculoskeletal disease (n = 11), pulmonary embolism (n = 15), and myocarditis (n = 11). The platelet surface expression of GPVI was significantly enhanced in patients with coronary cause compared to patients with NC (Coronary vs.NC (mean fluorescence intensity (MFI) ± SD): 18.5 ± 8.2vs.10.8 ± 6.2;P = 0.002) (Fig. 1A).

Table 1
Baseline Patients` Characteristics and Medication on Hospital Admission.

Characteristics	All (n = 1004)	SAP (n = 233)	ACS (n = 416)	Non-Coronary (n = 355)
Age – years	65.6 ± 14.6	69.4 ± 11.5	67.7 ± 13	60.8 ± 16.7
Sex – no. (%)				
Female	331 (33)	73 (31.3)	102 (24.5)	156 (43.9)
Male	673 (67)	160 (68.7)	314 (75.5)	199 (56.1)
Cardiovascular Risk Factors – no. (%)				
Arterial Hypertension	766 (76.3)	207 (88.8)	352 (84.6)	207 (58.3)
Hyperlipidemia	543 (54.1)	164 (70.4)	252 (60.6)	127 (35.8)
Diabetes	274 (27.3)	83 (35.6)	113 (27.2)	78 (22)
Family History of CAD	167 (16.6)	51 (21.9)	71 (17.1)	45 (12.7)
Smoking	345 (34.4)	90 (38.6)	181 (43.5)	74 (20.8)
Atrial Fibrillation	77 (7.7)	19 (8.2)	38 (9.1)	20 (5.6)
Medication – no. (%)				
ACE Inhibitors	267 (26.6)	92 (39.5)	103 (24.8)	72 (20)
Angiotensin Receptor Blockers	63 (6.3)	20 (8.6)	26 (6.3)	17 (4.8)
Beta Blockers	406 (40.4)	124 (53.2)	163 (39.2)	119 (33.5)
Statins	328 (32.7)	109 (46.7)	98 (23.6)	121 (34.1)
Aspirin	433 (43.1)	129 (55.4)	161 (38.7)	143 (40.3)
Clopidogrel	102 (10.2)	63 (27)	27 (8.5)	12 (3.4)
Vitamin K Antagonist	84 (8.4)	27 (11.6)	32 (7.7)	27 (7)

CAD denotes coronary artery disease, ACE angiotensin converting enzyme.