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# Platelet expression of transforming growth factor beta 1 is enhanced and associated with cardiovascular prognosis in patients with acute coronary syndrome

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ABSTRACT

**Background**. Functional recovery and prognosis after acute coronary syndromes (ACS) are mainly driven by the extent of reperfusion injury and myocardial repair mechanisms. Transforming growth factor-beta 1 (TGF-β1) is critically involved in cardiac injury, repair and remodeling. In this study, we investigated the prognostic role of platelet TGF-β1 surface expression and circulating TGF-β1 levels in patients with coronary artery disease (CAD). **Methods and results**. Expression of TGF-β1 in platelets and circulating TGF-β1 levels were investigated by flow cytometry and ELISA, respectively, among patients with ACS and stable CAD undergoing percutaneous coronary intervention (PCI). In a cohort study, platelet and circulating TGF-β1 was measured in 299 patients with symptomatic CAD (stable CAD = 145, ACS = 154) at the time of PCI. The primary combined endpoint was defined as death and/or STEMI during 12-month follow-up. Platelets expressed TGF-β1 and circulating TGF-β1 showed a weak, but significant negative correlation. TGF-β1 surface expression was significantly elevated on platelets in ACS patients compared to patients with stable CAD (median MFI 13.4 vs. median MFI 11.7, $p = 0.003$). During follow-up, lower platelet expression of TGF-β1 was associated with all-cause mortality (median MFI 11.0 vs. median MFI 13.9, $p = 0.011$) as well as for the combined endpoint of death and/or STEMI, (median MFI 10.8 vs. median MFI 13.9, $p = 0.006$). In multivariate analysis platelet TGF-β1 expression was independently associated with the combined primary endpoint in the overall cohort (Hazard Ratio 0.31, 95% Confidence Interval 0.11–0.89, $p = 0.029$) and was strongly associated with prognosis in ACS patients. There was no significant association of circulating TGF-β1 levels neither with the presence of ACS nor the occurrence of the primary endpoint. **Conclusion**. These findings highlight a potential role of platelet expressed TGF-β1 in ACS and indicate a prognostic value of TGF-β1 on clinical outcomes in patients with acute coronary syndromes. Large scale studies are warranted to further evaluate the regulatory mechanisms of platelet TGF-β1 expression- and its prognostic impact in CAD.



## 1. Introduction

Functional recovery and prognosis after acute coronary syndromes (ACS) are mainly driven by the extent of reperfusion injury and myocardial repair mechanisms. Transforming growth factor beta (TGF-β) is critically involved in cardiac injury, repair and remodeling [1,2]. TGF-β is a pleiotropic cytokine and is implicated in a variety of cell functions, in regulation of inflammation, extracellular matrix deposition, cell proliferation-, differentiation- and growth [3]. TGF-β consists of 3 isoforms, TGF-β1, TGF-β2 and TGF-β3 [2]. TGF-β1 is found almost ubiquitously whereas expression of TGF-β2 and TGF-β3 is more limited to certain tissues [1]. TGF-β1 is secreted to a large extend by platelets [4]. Platelets contain up to

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100 times as much TGF-β as other cells and immediately release it upon activation [5,6]. Effects of TGF-β1 in myocardial infarction and heart failure are widely studied in experimental settings and animal models. In myocardial infarction, TGF-β1 might play a critical role in cardiac remodeling by effects on inflammation and reparation [2]. Experimental studies suggest TGF-β1 to be some kind of "master switch" in mediating the transition from inflammation to scar formation [1]. Furthermore, TGF-β shows an up-regulation in animal models of heart failure. Elevated levels of TGF-β1 are associated with cardiac hypertrophy- and fibrosis [4,5]. TGF-β1 overexpressing mice develop significant cardiac hypertrophy in addition to interstitial fibrosis [7]. On the contrary, heterozygous TGF-β1 ± mice are protected against age-associated cardiac fibrosis and diastolic dysfunction [8]. In a mouse model of pressure overload, mice with specific deletion of TGF-β1 showed less cardiac interstitial- and perivascular fibrosis than wild-type mice. Mice with deletion of TGF-β1 had a lower expression of myofibroblasts in their hearts compared with wild-type mice [9]. Myofibroblasts are probably key elements of cardiac tissue fibrosis since they produce collagen with a higher activity than their progenitors, fibroblasts, and TGF-β1 is capable of inducing the differentiation of cardiac fibroblasts to myofibroblasts [10–13]. To date, there are no data regarding the prognostic impact of TGF-β in cardiovascular patients. Hence, the aim of the present study was to investigate differential regulation of TGF-β on the surface of platelets, which are considered one of the richest sources of TGF-β, in patients with symptomatic coronary artery disease (CAD).

## 2. Subjects and methods

### 2.1. Patient characteristics and blood sampling

Blood samples were collected during PCI and immediately analyzed for surface expression of TGF-β1 and GPIb by flow cytometry. All subjects gave written informed consent. Patients were admitted to the department of cardiology at the university of Tübingen, Germany. The study was approved by the institutional ethics committee (270/2011BO1) and complies with the declaration of Helsinki and the good clinical practice guidelines [14]. We included 299 consecutive patients with symptomatic CAD (stable CAD $n = 145$, ACS $n = 154$). ACS was defined as worsening of angina or acute myocardial infarction. An acute myocardial infarction was diagnosed by a rise and/or fall of cardiac biomarker values [preferably cardiac troponin (cTn)] with at least one value above the 99th percentile upper reference limit and with at least one of the following: Symptoms of ischemia, new or presumed new significant ST-segment–T wave (ST–T) changes or new left bundle branch block (LBBB), development of pathological Q waves in the ECG, imaging evidence of new loss of viable myocardium or new regional wall motion abnormality, identification of an intracoronary thrombus by angiography [15].

### 2.2. Surface expression by whole blood flow cytometry

Platelets in whole blood collected in CPDA anticoagulant were analyzed for the surface expression of TGF-β1 specifically gating for platelets as the GPIb positive population. Blood was diluted 1:50 with PBS (Gibco) and incubated with the respective fluorochrome conjugated antibodies-mouse monoclonal anti-human TGF-β1-PE (R&D systems, clone 9016, catalog number: IC240P, Ig class: IgG1) and mouse anti human GPIb-FITC (Beckman Coulter, clone SZ2) under resting condition, for 30 min at room temperature. After staining, the samples were fixed with 0.5% paraformaldehyde and analyzed by flow cytometry (FACS-Calibur flow cytometer Becton–Dickinson, Heidelberg, Germany).

### 2.3. Circulating levels of TGF-β1 measured by ELISA

Serum levels of TGF-β1 were evaluated in samples from patients with stable CAD and ACS using specific TGF-β1 ELISA kit from Enzo Life Sciences having a sensitivity range of (31.25–1000) pg/ml. Blood was collected from patients in CPDA anticoagulant. Serum samples were processed as per manufacturer's instructions and levels of TGF-β1 are reported as pg/ml.

### 2.4. Follow-up

All patients were tracked after initial PCI for clinical events including all cause death and STEMI. Follow-up was performed by telephone interview and/or review of patients' charts on readmission by investigators blinded to the results of laboratory testing. The day after PCI we investigated LVEF% by transthoracic echocardiography. Course of LVEF% was evaluated in ACS patients using transthoracic echocardiography at a median 3-months follow-up. 2D echo LVEF% was assessed using the Simpson's biplane method of discs by manual planimetry of the endocardial border in end-diastolic and end-systolic frames [16].

### 2.5. Statistical analysis

All statistical analysis was performed using SPSS version 20.0 (SPSS Inc., Chicago IL). The primary endpoint of this study was the combination of all-cause death and/or ST-elevation myocardial infarction during follow-up. With a probability of 90% that the study will detect a minimal risk ratio of 1.5% for the primary endpoint between high vs. low TGF-β1 levels (e.g. > and ≤median) at a two-sided 5.0% significance level we estimated a total sample size of 256 patients [17]. Normally distributed data were compared

**Table 1**
Baseline patient characteristics in patients with acute coronary syndrome compared to patients with stable CAD.

| Characteristics | All ($n = 299$) | Acute coronary syndrome ($n = 154$) | Stable CAD ($n = 145$) | $p$ |
|---|---|---|---|---|
| $n$ male | 219 (73.2%) | 116 (75.3%) | 103 (71.0%) | 0.458 |
| $n$ female | 80 (26.8%) | 38 (24.7%) | 42 (29.0%) | |
| Age years (mean ± SD) | 68 (±12) | 69 (±12) | 68 (±11) | 0.906 |
| **CVRF** | | | | |
| Arterial hypertension | 262 (87.6%) | 126 (81.8%) | 136 (93,8%) | 0.002 |
| Hyperlipidemia | 195 (65.2%) | 90 (58.4%) | 105 (72.4%) | 0.015 |
| Diabetes | 85 (28.4%) | 45 (29.2%) | 40 (27.6%) | 0.702 |
| Smoking | 56 (18.7%) | 27 (17.5%) | 29 (20.0%) | 0.620 |
| Ex-smoking (>6 months) | 55 (18.4%) | 26 (16.9%) | 29 (20.0%) | 0.519 |
| Atrial fibrillation | 65 (21.7%) | 34 (22.1%) | 31 (21.4%) | 0.937 |
| LV function (LVEF%) (mean ± SD) | 49.7 (±11.1) | 48.2 (±11.0) | 51.2 (±11.2) | 0.192 |
| LVEF% normal | 128 (42.8%) | 52 (33.8%) | 76 (52.4%) | 0.009 |
| LVEF% mild impairment | 68 (22.7%) | 41 (26.6%) | 27 (18.6%) | |
| LVEF% moderate impairment | 55 (18.4%) | 35 (22.7%) | 20 (13.8%) | |
| LVEF% severe impairment | 45 (15.1%) | 24 (15.6%) | 21 (14.5%) | |
| LVEF% unknown | 3 (1.0%) | 2 (1.3%) | 1 (0.7%) | |
| Renal function (GFR) (Mean ± SD) | 75.2 (±24.8) | 75.3 (±26.9) | 75.1 (±21.6) | 0.426 |
| **Medication on admission** | | | | |
| Acetyl Salicylic Acid | 178 (59.5%) | 83 (53.9%) | 95 (65.5%) | 0.118 |
| Clopidogrel | 64 (21.4%) | 18 (11.7%) | 46 (31.7%) | <0.001 |
| Prasugrel | 9 (3.9%) | 2 (1.3%) | 7 (4.8%) | 0.090 |
| Ticagrelor | 16 (5.4%) | 9 (5.8%) | 7 (4.8%) | 0.606 |
| Oral anticoagulants | 33 (11.0%) | 13 (8.4%) | 20 (13.8%) | 0.201 |
| ACE inhibitors | 144 (48.2%) | 63 (40.9%) | 81 (55.9%) | 0.027 |
| AT1-receptor antagonists | 48 (16.1%) | 23 (14.9%) | 25 (17.2%) | 0.750 |
| Beta blockers | 173 (57.9%) | 74 (48.1%) | 99 (68.3%) | 0.001 |
| Statins | 169 (56.5%) | 72 (46.8%) | 97 (66.9%) | 0.001 |