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Impact of counterbalance between macrophage migration inhibitory factor and its inhibitor Gremlin-1 in patients with coronary artery disease

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ABSTRACT

Objective: Monocyte infiltration is a critical step in the pathophysiology of plaque instability in coronary artery disease (CAD). Macrophage migration inhibitory factor (MIF) is involved in atherosclerotic plaque progression and instability leading to intracoronary thrombosis. Gremlin-1 (Grem1) has been recently identified as endogenous inhibitor of MIF. To date there are no data on the clinical impact of this interaction in cardiovascular patients. **Methods and results**: Plasma levels of MIF and Grem1 were determined by enzyme-linked immunoassay in patients with acute coronary syndromes (ACS, $n = 120$; stable CAD, $n = 166$ and healthy control subjects, $n = 25$). MIF levels were significantly increased in ACS compared to stable CAD and healthy control (ACS: median 2.85; IQR 3.52 ng/ml; versus SAP: median 1.22; IQR 2.99 ng/ml; versus healthy control: median 0.10; IQR 0.09 ng/ml, $p < 0.001$). Grem1 levels were significantly higher in ACS and stable CAD patients compared to healthy control (ACS: median 211.00; IQR 130.47 ng/ml; SAP: median 220.20; IQR 120.93 ng/ml, versus healthy control: median 90.57; IQR 97.68 ng/ml, $p < 0.001$). Grem1/MIF ratio was independently associated with ACS, whereas the single parameters were not associated with the presence of ACS. Furthermore, Grem1/MIF ratio was associated with angiographic signs of intracoronary thrombi and severity of thrombus burden. **Conclusion**: These novel findings suggest a potential role of Grem1/MIF ratio to indicate acuity of CAD and the grade of plaque stability. Prospective angiographic cohort studies involving plaque imaging techniques are warranted to further characterize the prognostic role of this novel risk marker in CAD patients.

1. Introduction

Macrophage migration inhibitory factor (MIF) is a pro-inflammatory protein critically involved in plaque progression in coronary artery disease (CAD) by regulating monocyte recruitment towards atherosclerotic lesions [1]. It was recently shown that up-regulation of MIF is associated with adhesion and accumulation of macrophages and foam cell transformation and that MIF plays a decisive role in plaque cellularity, vulnerability and rupture [2–4] complicated by subsequent coronary thrombosis. Enhanced plasma expression of MIF has been previously found in patients with cardiovascular disease [5]. Its role as an independent risk factor to develop CAD and acute coronary syndromes (ACS) has been controversially discussed [6]. Gremlin-1 (Grem1) belongs to the DAN/Cerberus protein family, a subdivision of the “cysteine knot” protein superfamily, which includes several other cytokines, such as Transforming Growth Factor beta (TGF-beta) or Vascular Endothelial Growth Factor (VEGF) [7]. It is expressed in and secreted by monocytes/macrophages [8] and endothelial cells [9] exposed to disturbed flow e.g. in human coronary arteries suggesting a role in inflammation and atherosclerosis. Recently, we could characterize Grem1 as an endogenous inhibitor of MIF, which

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attenuates atherosclerotic plaque growth in ApoE deficient mice suggesting a potential plaque stabilizing role [8]. Currently, there are no clinical data describing the potential relationship between MIF and Grem1 in cardiovascular patients and investigating their relation with regard to instability of CAD. Therefore, the aim of the present cohort study was to characterize the role of Grem1 and MIF and its ratio in cardiovascular patients compared to healthy volunteers.

2. Subjects and methods

2.1. Patient characteristics and blood sampling

Blood samples were collected during coronary angiography and/or percutaneous coronary intervention (PCI). A standardized method in timing of the blood samples was used. Patients received a clopidogrel loading dose (LD) of 600 mg and at least 6 h after LD (median 24 h) blood samples were collected. Blood samples were collected as soon as possible after PCI. All subjects gave written informed consent. Patients were admitted to the department of Cardiology of the University of Tuebingen, Germany. We included 286 consecutive patients with symptomatic CAD (stable CAD $n = 166$, ACS $n = 120$). ACS was defined as worsening of angina pectoris, acute myocardial infarction or sudden cardiac death. An acute myocardial infarction was diagnosed by a rise and/or fall of cardiac biomarker values, e.g. cardiac troponin I (TnI) 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 or identification of an intracoronary thrombus by angiography [10]. During PCI we investigated LVEF % by levo-cardiography.

25 healthy young adults with no history of any kind of diseases, hospitalization or medication in the age between 20 and 35 years served as healthy control subjects. 14 of them were female, 11 were male. They did not undergo coronary angiography. Blood samples were collected in the morning between 8 and 10 a.m.

The study was approved by the institutional ethics committee (270/2011BO1) and complies with the declaration of Helsinki and the good clinical practice guidelines [11–13].

Table 1
Demographics of the patient cohort.

Characteristics	All ($n = 286$)	Stable CAD ($n = 166$)	Acute coronary syndrome ($n = 120$)	P
n male	202 (70.6)	111 (66.9)	91 (75.8)	0.1
n female	84 (29.4)	55 (33.1)	29 (24.2)	
Age years (mean ± SD)	66.6 ± 12.8	66.2 ± 13.5	70.2 ± 11.9	**0.01**
CVRF				
Arterial hypertension	221 (77.3)	117 (70.5)	104 (86.7)	**0.001**
Hyperlipidemia	168 (58.7)	89 (53.6)	79 (65.8)	**0.04**
Diabetes	88 (30.8)	53 (31.9)	35 (29.2)	0.62
Active smoking/ ex-smoking	105 (36.7)	53 (31.9)	52 (42.3)	0.05
Renal failure	75 (26.2)	49 (29.5)	26 (21.7)	0.14
GFR (MDRD)	34.2 ± 17.1	32.8 ± 16.9	38.2 ± 17.6	**0.01**
LV function (EF%) (mean ± SD)	46.6 ± 14.0	45.7 ± 15.4	49.7 ± 11.2	**0.03**
LV function normal	110 (38.7)	61 (36.7)	49 (41.5)	**0.03**
LV function mild impairment	70 (24.6)	38 (22.9)	32 (27.1)	
LV function moderate impairment	55 (19.4)	29 (17.5)	26 (22.0)	
LV function severe impairment	49 (17.3)	38 (22.9)	11 (9.3)	
Prior myocardial infarction	87 (30.4)	47 (28.3)	40 (33.3)	0.36
Medication on admission				
Acetyl salicylic acid	189 (66.1)	103 (62.0)	86 (71.7)	0.09
Clopidogrel	87 (30.4)	44 (26.5)	43 (35.8)	0.89
Oral anticoagulants	20 (7.0)	13 (7.8)	7 (5.8)	0.513
Ca-channel blockers	48 (16.8)	24 (14.5)	24 (20.0)	0.22
ACE inhibitors	177 (61.9)	116 (69.9)	61 (50.8)	**0.001**
AT1-receptor antagonists	36 (12.6)	24 (14.5)	12 (10.0)	0.26
Beta blockers	214 (14.8)	130 (78.3)	84 (70.0)	0.11
Statins	140 (49.0)	76 (45.8)	64 (53.3)	0.21
Atherogenic risk factors				
Cholesterol	193.4 ± 77.1	193.2 ± 49.8	188.9 ± 106.1	0.75
C-reactive protein	1.65 ± 3.21	1.32 ± 2.39	3.56 ± 4.47	**<0.001**

p values <0.05 were considered significant as marked in bold.

2.2. Classification of coronary thrombus burden

Presence and grade of thrombus containing lesions in ACS patients were classified by two independent investigators according to TIMI classification i.e. grade 0 = no angiographic evidence of thrombus is present; grade 1 = possible thrombus, with reduced contrast density, haziness, irregular lesion contour, or a smooth convex (meniscus) at the site of total occlusion suggestive but not diagnostic of thrombus; grade 2 = definitive thrombus, with greatest linear dimensions ≤ half of vessel diameter; grade 3 = definitive thrombus, with greatest linear dimensions > half of vessel diameter, but < 2 vessel diameters half of vessel diameter; grade 4 = definitive thrombus, with greatest linear dimension ≥ 2 vessel diameter; grade 5 = total occlusion [14]. In case there was a deviation of thrombus classifications between both investigators, thrombus grade was adjudicated by a third experienced investigator.

2.3. Enzyme-linked immunosorbent assay

Plasma levels of MIF and Grem1 were determined in all patients ($n = 286$ patients and $n = 25$ healthy control subjects) using a commercially available enzyme-linked immunosorbent assay kit according to the manufacturer's guidelines (R&D Systems, Minneapolis, MN, USA). Ethylenediamine tetraacetic acid plasma probes were centrifuged for 15 min at 10.000 g within 30 min of collection. Probes were aliquoted and stored at −80 °C until analysis.

2.4. Statistical analysis

All statistical analysis was performed using SPSS version 18.0 (SPSS Inc., Chicago IL). Normal distribution of the data was examined with the help of the Kolmogorov–Smirnov test. Plasma concentrations of MIF and Grem1 were not normally distributed. Therefore we used Kruskal–Wallis – Test for comparison across groups or Mann–Whitney U Test for comparison between individual groups. Normally distributed demographic data were compared using independent student's T-test. Correlations were assessed by Spearman's rank and Pearson correlation coefficient (ρ). Multivariate analysis was performed using logistic regression analysis with ACS compared to stable CAD as categorical dependent variable. Independent covariates entered into the model included those that were tested with a statistical significance of $p \leq 0.1$ in univariate analysis (Table 1), namely clinical risk factors (hypertension, hyperlipidemia, smoking), age, gender, glomerular filtration rate, LVEF% and cardiovascular co-medication (Acetyl Salicylic Acid and ACE inhibitors). Histograms were plotted and the Kolmogorov–Smirnov test was performed to check normality of the residuals of the regression model.