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Galectin-3 binding protein plasma levels are associated with long-term mortality in coronary artery disease independent of plaque morphology

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ABSTRACT

Background and aims: Galectin-3 binding protein (Gal-3BP) is a secreted protein associated with inflammation and carotid atherosclerosis. We hypothesized that high Gal-3BP levels may indicate unfavorable plaque morphology and outcome in coronary artery disease (CAD).
Methods: Gal-3BP plasma levels were measured by ELISA in 233 patients (63 ± 10 years, 50.2% male) undergoing computed coronary angiography tomography (CCTA).
Results: In 149 patients, CCTA confirmed CAD (stenosis grade >20%). Mean Gal-3BP plasma levels were 5.9 ± 2.7 μg/mL and did not differ between patients with or without CAD. Over a follow-up time of up to 4.4 years (median 2.5 years), there were 17 cases of revascularization, five cases of myocardial infarction, and five deaths (four non-cardiac, one fatal myocardial infarction). Kaplan-Meier analysis revealed that high Gal-3BP levels were significantly associated with long-term mortality ($p < 0.001$). Cox proportional hazards regression analysis showed that this association was independent of cardiovascular risk factors (HR 1.238, 95%-CI 1.012–1.514, $p = 0.038$). After adjustment for troponin T and C-reactive protein (hs-CRP) levels, significance was lost ($p = 0.123$). Further analysis revealed that Gal-3BP levels were significantly related to body mass index and hs-CRP levels indicating an association with metabolic and inflammatory distress. There was no correlation between Gal-3BP and calcium score, plaque volume, or vascular remodeling.
Conclusions: While high Gal-3BP plasma levels are associated with long-term mortality, we could not confirm it as a marker of cardiac mortality or unstable plaque morphology.

1. Introduction

Atherosclerosis and its clinical consequences such as myocardial infarction and stroke are the major cause of morbidity and mortality worldwide [1]. Over the past years, it has become evident that atherosclerosis is an inflammatory disease of the arterial wall [2]. Adverse events such as myocardial infarction are in most cases the consequence of plaque rupture leading to atherothrombosis and occlusion of the coronary artery [3]. Plaque rupture is seen in so-called unstable plaques, which are characterized by a thin fibrous cap and a high prevalence of inflammatory cells [3]. Surprisingly, plaque rupture is oftentimes seen in plaques associated with mild or moderate stenosis [4]. Thus, recognizing coronary plaques prone to rupture and developing strategies leading to plaque stabilization would be highly desirable in order to prevent adverse cardiovascular events.

It has recently been demonstrated that Galectin-3 binding protein (Gal-3BP, also known as 90K) is expressed in coronary

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artery plaque macrophages [5]. Gal-3BP is a secreted 585 amino acid protein [6]. It is expressed in hematopoietic and epithelial cells [6,7], but is also present in many other tissues including the colon, duodenum, stomach, and lung [7]. Furthermore, Gal-3BP is detectable in many body fluids like semen, saliva, urine, tears [6], human milk [8,9], and plasma, where it is associated with microparticles [10]. Its function is largely unknown.

In the past, Gal-3BP plasma levels have been associated with infectious and autoimmune diseases like hepatitis C [11], human immunodeficiency virus infection [12,13], or bronchial asthma [14]. *In vitro* data suggest a pro-inflammatory role of human Gal-3BP as demonstrated in peripheral blood mononuclear cells (increased IL-2 production [7]) or bone marrow stroma cells (IL-6 production [15]). Additionally, we have previously found that Gal-3BP plasma levels are associated with carotid intima media thickness independent of risk factors such as age, race, smoking status, body mass index, or high-sensitivity C-reactive protein [5]. Based on these considerations, we hypothesized that Gal-3BP plasma levels may be a biomarker of coronary atherosclerosis.

Coronary computed tomography angiography (CCTA) is an excellent non-invasive tool providing detailed insights into coronary artery plaque morphology in patients with coronary artery disease [16,17]. Other than invasive coronary angiography, CCTA may not only describe narrowing of the vessel lumen, but also give insight into plaque morphology, the degree of calcification, and vascular remodeling, which are of prognostic relevance [17,18].

We hypothesized that Gal-3BP plasma levels may be associated with features of plaque instability as determined by CCTA. Several previous CCTA studies have suggested that non-calcified so-called 'low-attenuation' plaques are responsible for future acute coronary syndromes in patients with presumably stable coronary artery disease [17–19]. In a monocentric retrospective approach, we therefore sought to test whether (a) Gal-3BP levels may predict outcome in coronary artery disease (CAD) and whether (b) high Gal-3BP levels represent an indicator of unfavorable plaque morphology.

2. Materials and methods

2.1. Study population

Blood samples were obtained with approval from the local ethics committee (S-317/2008). All participants provided their written and verbal informed consent to participate in this study. All studies have been performed in accordance with the ethical standards laid down in the 1975 Declaration of Helsinki and its later amendments as reflected in a priori approval by the institution's human research committee.

The study population comprised 233 consecutive patients with preserved left ventricular function undergoing coronary computed tomography angiography (CCTA) for suspected coronary artery disease (CAD). Cardiovascular risk factors including arterial hypertension (blood pressure $\geq$ 140/90 mm Hg or antihypertensive therapy) [20], hyperlipidemia (low-density lipoprotein cholesterol (LDL-C) $\geq$130 mg/dL or statin therapy) [21], current or prior smoking, diabetes mellitus, and a family history of CAD were available for all participants. Furthermore, a set of routine laboratory parameters was obtained for all patients.

2.2. Coronary computed tomography angiography (CCTA) methodology

The CCTA protocol and quantitative assessment of atherosclerotic plaque components is described in detail elsewhere [22,23]. CCTA was performed using a 256-slice Brilliance iCT scanner (Philips Healthcare, Hamburg/Germany).

Patient preparation included intravenous administration of metoprolol (Lopresor®, Novartis Pharma) if baseline heart rate was $\geq$60 beats per minute. All patients received 0.8 mg of sublingual glyceryl nitrate 3–5 min prior to the CT scan. During a single breath-hold, CCTA was performed with 65–80 mL (6 mL/s) of nonionic contrast agent (Ultravist® 370, Bayer Schering Pharma) followed by 30–50 mL (5 mL/s) of saline.

2.2.1. Assessment of plaque composition and luminal narrowing

Assessment of Agatston score, luminal narrowing, and coronary plaque volume and composition was performed using the dedicated software (Extended Brilliance Workspace 4.0, Philips Medical Systems) as described previously [24,25]. Coronary CT angiograms and Agatston score were analyzed independently by two experienced readers (S.J.B. and G.K.) both with >5 years of experience in coronary CTA equivalent to the clinical competence statement training level 3 of the American College of Cardiology Foundation/American Heart Association (AHA) [26].

2.2.2. Coronary plaque volume and composition

For each coronary artery segment the vessel lumen and wall were automatically registered, and after identification of each lesion the boundaries were manually edited by S.J.B. or G.K. if necessary. Subsequently, the semi-automatically identified plaques were marked. These findings were then evaluated in corresponding axial, cross-sectional multiplanar and longitudinal images in order to differentiate real findings from artifacts. Care was taken to correctly discriminate between iodinated blood (300–600 HU) and calcified plaque, and Gaussian algorithms were used to distinguish between components of low to intermediate attenuation (0–150 HU) and calcified plaque components with higher attenuation values [18,25,27]. This model separates components with different densities within the plaque using a Gaussian mixture model. Finally, plaque composition results from a linear combination of the resulting 1–3 Gaussians curves. For each lesion the following were assessed: non-calcified plaque volume, coronary lumen narrowing, plaque composition, and vascular remodeling. According to the volumetric calcium content, plaques were classified into *non-calcified* (calcium content < 20%), *partially calcified* (calcium content between 20% and 80%) and *calcified* (calcium content > 80%). Non-calcified and partially calcified plaques are expected to contain substantial amount of lipid cores or fibrotic tissues [18,22] apart from calcified tissue. The non-calcified plaque volume for each individual lesion and each patient was obtained by summing the individual volumes of soft or mixed plaques in all three coronary vessels.

2.2.3. Agatston score

For the assessment of coronary calcification prospective ECG-gated non-contrast scans were performed at 75% of the cardiac cycle, and using 120 kV tube voltage and 364 mA tube current, and resulting images with a 3 mm slice thickness were used for the calculation of the Agatston score.

2.2.4. Luminal narrowing

Coronary lumen narrowing (maximum diameter reduction) was analyzed using longitudinal reconstructions by dividing the minimal diameter in the diseased segment through the diameter in the adjacent proximal disease-free section. If stenosis was present at the ostium of a coronary artery or a vessel bifurcation point, the distal or the proximal reference vessel point were used, respectively. When multiple lesions were present in a segment, the most severe lesion was considered.