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Association of serum sclerostin concentrations and atherosclerosis advancement in patients referred to invasive coronary angiography

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Short title: Sclerostin in coronary artery disease

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INTRODUCTION

Sclerostin is a soluble glycoprotein secreted by osteocytes and has been identified as an important regulator of bone formation and an inhibitor of the Wnt/β-catenin signaling pathway [1]. The Wnt/β-catenin signaling pathway plays a vital role in the regulation of endothelial inflammation, vascular calcification, and mesenchymal stem cell differentiation and therefore contributes to atherosclerosis disease [2]. As a result, it is suspected that sclerostin might play a role in patients with atherosclerosis [3, 4].

Few studies focus on the pathophysiologic effects between sclerostin and atherosclerosis process in the population without severe chronic kidney disease (CKD) [5, 6]. Thus, our study aimed to analyze the profile of serum concentrations as well as correlations between traditional parameters and new indicators of bone turnover in a group of patients referred to coronary angiography.

METHODS

Participants and study design

Consecutive patients undergoing coronary angiography between June 29, 2011, and November 17, 2011, and fulfilling the inclusion criteria were enrolled. The inclusion criteria were as follows: age ≥ 40 years and below 80 years, eligibility for coronary angiography due to stable coronary artery disease (CAD) or acute coronary syndrome, serum creatinine concentration before the procedure ≤ 1.2 mg/dL, ejection fraction in echocardiography > 30%, available data on weight and height as well as willingness to participate in the study and sign a written informed consent form. An independent ethics committee of the University of Warmia and Mazury in Olsztyn approved the study protocol.

CAD severity
The CAD severity was assessed as 1-vessel disease (VD), 2VD, 3VD or left main stem (LM) as well as \( \leq 22 \) points (low risk), 23 – 32 points (intermediate risk) and \( \geq 32 \) points (high risk) according to European Society of Cardiology guidelines [7]. The non-obstructive disease was defined as the lack of lesions over 40% diameter stenosis.

**Biochemical analysis**

The following biochemical parameters were analyzed: triglyceride (TG), total cholesterol (TC), LDL-cholesterol (LDL-c), HDL-cholesterol (HDL-c), high sensitive C-reactive protein (hs-CRP), glycated hemoglobin (HbA1c), creatinine and bone turnover markers (such as levels of intact parathormone [iPTH]). eGFR was calculated according to the simplified MDRD formula. Serum sclerostin levels were measured by ELISA (DY1406, R&D SYSTEMS, USA). The limit of sclerostin detection was 41.5 pg/mL. The intact form of FGF-23 protein (60-6600, Immunotopics, Inc., USA) was detected at 1.5 pg/mL. The sensitivity of the ELISA for Klotho protein (CSB-E13235h, CUSABIO, China) was 39 pg/mL. The intra- and inter-assay variability was less than 10% for all proteins tested.

**Statistical analysis**

R software version 3.6.1 for Mac, and GraphPad Prism 6 were used to analyze the data. The Kolmogorov–Smirnov test was applied to check the normality of continuous variables. Student’s t-test compared variables between two groups if normally distributed. ANOVA test was applied in multi-group comparison. The Mann–Whitney U test was used in case of skewed variables. Data are presented as counts with percentages for qualitative values and as the means (standard error of the mean [SEM]) or medians with interquartile ranges (IQR) for quantitative values. The \( \chi^2 \) test or Fisher’s exact test was used to compare categorical variables presented as percentages. The Pearson’s correlation was used to assess the association between serum sclerostin and other clinical parameters. Differences were considered statistically significant at a p-value of less than 0.05.
RESULTS and DISCUSSION

In total, we enrolled 205 patients with the mean (SEM) age of 62.9 (0.6) years, and males stand for 70.2% (n = 144). Patients were classified into five subgroups: no obstructive disease (23.9%), 1-VD (24.9%), 2-VD (24.4%), 3-VD (20.9%) and LM disease (5.9%). No differences were observed between groups except for SYNTAX score value ($P < 0.001$), fasting plasma glucose ($P = 0.046$), HDL-c level ($P < 0.01$) and potassium concentration ($P = 0.03$) (Suppl. Table 1).

No significant differences were observed in mean serum sclerostin, iPTH, Klotho protein and FGF-23 concentrations between patient subgroups depending on the CAD advancement (Table 1).

Median serum sclerostin concentration was 133.22 pg/mL with interquartile range of 64.0 – 276.17 pg/mL. In patients with higher serum sclerostin concentrations (> median) we observed higher mean (SEM) BMI value (26.9 [0.3] kg/m$^2$ vs. 28.3 [0.5] kg/m$^2$, $P = 0.049$) and lower mean (SEM) eGFR value (89.9 [2.2] mL/min/1.73m$^2$ vs. 83.7 [2.4] ml/min/1.73m$^2$, $P = 0.01$) as well as mean (SEM) fibrinogen concentration (406.9 [7.22] mg/dL vs. 390.1 [10.8] mg/dL, $P = 0.04$) (Suppl. Table 3, Suppl. Figure 1).

The most reproducible relationship in the whole group and in different subgroups was found between sclerostin and iPTH being strongest among patients with SYNTAX score 23 – 32 points ($r = 0.6671$, $P < 0.001$). Correlation for the whole study group was $r = 0.513$, $P < 0.001$ (Suppl. Table 3).

Vascular calcification and remodeling are involved in the development and progression of atherosclerosis. PTH takes part in vascular dysfunction via direct PTH receptor interaction on the vessel as well as indirectly via the inflammation process [8]. Low-grade inflammation contributes to the development of atherosclerosis, and both CRP and fibrinogen are inflammatory markers, linked to atherosclerosis and cardiovascular disease [9, 10].
Sclerostin levels correlated strongly and negatively with serum iPTH. It is noteworthy since we also found a strong but positive association between these two bone turnover regulating proteins. This may point to the different nature of the interaction between these hormones in patients with normal kidney function and those with advanced CKD. The positive association between sclerostin and serum calcium found by Qureshi et al. was also observed in one of the subgroups investigated in our study. Interestingly, cited authors did not observe the relationship between sclerostin levels and CRP nor interleukin 6; they, however, found a positive association with tumor necrosis factor alpha. We were able to show the positive association between sclerostin and CRP in a whole study group and specific subgroups. However, we are unable to define the relation between sclerostin and inflammation as we did not check the level of inflammatory cytokines. Moreover, this kind of association is still the subject of debate [11].

Our study has several limitations. First, this is an observational, single-center study with a limited number of elderly participants enrolled, and the possibility of bias one cannot exclude. No formal sample size calculation was performed, and the only criterium for not having clinically significant chronic kidney disease was serum concentration < 1.2 mg/dL.

Our study suggests that there was no direct relationship between sclerostin level and coronary artery advancement, but to some extent sclerostin concentration correlated with hsCRP, intact parathormone, and Klotho protein.
CONFLICT OF INTEREST

None declared.

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References:


Table 1. Sclerostin concentration depending on the coronary artery disease advancement

<table>
<thead>
<tr>
<th>Parameter (mean [SEM])</th>
<th>Patients</th>
<th>Coronary artery disease advancement</th>
<th>SYNTAX Score</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N = 205</td>
<td>N = 49</td>
<td>N = 51</td>
<td>N = 50</td>
</tr>
<tr>
<td>SCLEROSTIN (pg/mL), median (IQR)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>iPTH (pg/mL)</td>
<td>36.1 (2.1)</td>
<td>35.01 (4.5)</td>
<td>38.1 (4.31)</td>
<td>37.8 (5.23)</td>
</tr>
<tr>
<td>KLOTHO (pg/mL)</td>
<td>232.1 (15.0)</td>
<td>243.7 (34.3)</td>
<td>253.7 (29.9)</td>
<td>225.0 (32.0)</td>
</tr>
<tr>
<td>FGF23 (pg/mL)</td>
<td>1.37 (0.05)</td>
<td>1.37 (0.09)</td>
<td>1.52 (0.08)</td>
<td>1.17 (0.1)</td>
</tr>
</tbody>
</table>

The results are presented as means (SEM), except for sclerostin – median (IQR). 1VD – one vessel disease; 2VD – two vessel disease; 3VD – three vessel disease; iPTH – intact parathormone; FGF23 - fibroblast growth factor 23; LM – left main