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“Vitamin K-dependent GLA-proteins – new biomarkers for cardiovascular calcification”

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One of the serious complications of cardiovascular disease (CVD) is vascular calcification. At present, there are no laboratory parameters to measure vascular calcification.

The **aim** of the project is to clarify the role of extrahepatic vitamin-K-dependent Gla-proteins (matrix Gla-protein, Gla-rich protein, and osteocalcin) in ectopic calcification and the changes of the soluble ST2 as a marker for inflammation and fibrosis in CVD.

The main tasks to be performed are: to determine the levels of circulating Gla-proteins and sST2 in different groups of patients with CVD with a different treatment regimen; determining gene expression of selected Gla-proteins in PBMC; research of interrelations between the tested laboratory parameters and the calcium score.

Patients: The study will include 150 hospitalized patients with proven CVD such as paroxysmal or persistent atrial fibrillation, heart failure, and ambulatory patients at high risk for CVD.

Methods: In addition to routine noninvasive methods used in cardiology, innovative and highly specialized diagnostic methods such as computerized tomography for determining calcium scores will also be used. Circulating levels of Gla-proteins will be investigated by immunochemical methods. Gene expression of selected Gla-proteins will be performed with real-time polymerase chain reaction. The results will be analyzed with appropriate statistical methods.

Expected results: Circulating levels and gene expression of extrahepatic Gla-proteins will be determined for the first time. With the help of modern highly specialized diagnostic methods, the relationship between the calcium score, the studied Gla-proteins and sST2 will be established. With statistical modeling tools, the reliability of the investigated Gla-proteins as potential biomarkers and predictors for vascular calcification will be assessed. The relationship between the changes in Gla-protein levels and vitamin K status will be evaluated.

Results:

1. **Circulating ucMGP**

1.1. Twice higher levels in patients with heart failure compared to controls.

1.2. Clear tendency to increase ucMGP with CACS

1.3. Statistically significant negative correlation between ucMGP and Castelli's index (TC/HDL-C); positive correlation with HDL-C levels; negative correlation with uric acid levels; correlation with decarboxylated (inactive)/ carboxylated (active) osteocalcin ratio – poor vitamin K status is associated with elevated ucMGP levels and ineffective carboxylation; significant relationships between ucMGP, as a dependent variable, and plasma uric acid, TC/HDL-C, and LDL-C/HDL-C in patients with CACS.

1.4. Significantly reduced ucMGP levels in male patients, overweight and with proven arterial hypertension; significant relationships between ucMGP as a dependent variable, BMI and WC in patients with CACS.

2. **Gla-rich protein (t-GRP)**

2.1. Reduced circulating t-GRP in patients with CVD compared to controls; lower t-GRP levels in patients with heart failure vs. those with atrial fibrillation.

2.2. Significantly lower GRP levels in patients with CACS>0 compared to those with CACS=0.

2.3. Negative associations of t-GRP with total cholesterol and triglycerides in the CVD group.

3. **Circulating sST2**

3.1. Significant increase in sST2 levels with the severity of CVD.

3.2. Clear trend for an increase of sST2 with CACS; positive relationship between sST2 and CACS in patients with and without coronary calcium.

3.3. In patients with heart failure: higher sST2 levels in women vs. men and in patients with abdominal obesity.

3.4. Significant positive association between sST2 and ucMGP for the whole cohort and for CVD patients.

3.5. Serum sST2 levels could be used as a predictor for the development of heart failure in patients with atrial fibrillation.

4. **Gene expression of MGP in peripheral mononuclear cells (PBMC)**

4.1. Gradual increase in ucMGP gene expression with CACS.

4.2. A clear trend for a decreased ucMGP expression with age, in women, with the increase of both BMI and waist circumference, with the presence of hypertension and dyslipidemia.

4.3. ucMGP expression is significantly lower in patients with LDL-and total cholesterol above their median values.

5. **Effect of statin therapy**

5.1. Significantly higher CACS in statin users than those in non-statin users.

5.2. Significantly elevated ucOC levels and ucOC/cOC (vitamin K status marker) in statin users.

6. Role of asymptomatic hyperuricemia

6.1. Significant increase in uric acid levels with BMI, waist circumference, and with hypertension.

6.2. Gradual increase in uric acid levels with CACS; significant association between serum uric acid as a dependent variable and CACS for the whole studied cohort.

6.3. Low predictive power of uric acid when assessed alone, and much higher predictive power when uric acid is added to conventional risk factors for CVD.

7. Vitamin D status

7.1. Significantly reduced levels of 25OHD3 in patients with CVD compared to controls.

7.2. Vitamin D status worsens with the severity of CV pathology.

7.3. As CACS increases, 25HD3 levels decrease significantly; significant negative association between 25OHD3 and CACS.

7.4. Decreased gene expression of 1-alfa hydroxylase (CYP27B1) in patients with CVD and in those with the highest coronary calcium.

7.5. Significant positive correlation between 25OHD3 and CYP27B1 gene expression.

7.6. Reduction in both 25OHD3 and CYP27B1 gene expression in statin users.