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The relationship between antibeta 2 glycoprotein antibodies and SYNTAX score in patients undergoing coronary artery by-pass graft surgery

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Abstract. – OBJECTIVE: The SYNTAX Score was recently developed to characterize the coronary vasculature with respect to the number of lesion's location, complexity, and functional impact and it is a quantitative scoring system to assist with patient selection for optimal revascularization strategy between percutaneous coronary intervention (PCI) and coronary artery by-pass surgery (CABG). β_2-glycoprotein I (β2GPI), a plasma protein that binds cardiolipin, acts as a modulator of platelet aggregation and coagulation. Antibodies to β2GPI may have a role in atherosclerosis by inducing endothelial cell activation. We investigated the relationship between anti beta 2 GPI and severity of coronary artery stenosis by calculating the SYNTAX Score among patients undergoing CABG surgery.

PATIENTS AND METHODS: We prospectively investigate 612 patients who undergo elective coronary angiography between September 2012 and June 2013. Patients were evaluated for blood chemistry and anti-β2GPI IgA, IgM and IgG. Ninety seven patients with complete biochemical analysis including anti Beta 2 GPI antibodies and undergone CABG have been enrolled in this study. We divided patients in to 2 groups according to the SYNTAX scores. Group 1 included 48 patients with low SYNTAX scores (<23) and group 2 included 49 patients with intermediate and high SYNTAX scores (>23).

RESULTS: There was significant correlation between elevated anti β2GPI IgG levels and higher SYNTAX score which indicate advanced and complex CAD. In this study, lesion complexity increased progressively with increasing anti-β2GPI-IgG type of antibody levels. According to this findings, anti-β2GPI-IgG is a strong predictor of higher SYNTAX score.

CONCLUSIONS: In addition to the traditional risk factors for atherosclerosis, the proinflammatory and procoagulant activities of antiphospholipid antibodies appear to be important risk factors for atherosclerotic occlusive disease.

Key Words:
Anti β2-glycoprotein I antibody, SYNTAX score, Atherosclerosis, Coronary artery disease.

Introduction

The SYNTAX score was recently developed to characterize the coronary vasculature with respect to the number of lesion's location, complexity, and functional impact[1]. Higher SYNTAX score indicates a complex condition, a bigger therapeutic challenge, and a potentially worse prognosis in patients undergoing revascularization. SYNTAX score is a useful and quantitative scoring system to assist with patient selection for optimal revascularization strategy between percutaneous coronary intervention (PCI) and coronary artery by-pass surgery (CABG)[2].

β_2-glycoprotein I (β2GPI), a multifunctional plasma protein that binds cardiolipin, acts as a modulator of platelet aggregation and coagulation[3,4]. β2GPI is the main target antigen involved in the binding of antiphospholipid antibodies to anionic phospholipids[3-5].

The relationship of anti-ß2GPI antibodies with atherosclerosis is intriguing, since ß2GPI has been found in atheroma[6]. An association of IgG anti β 2GPI with coronary heart disease was found in patients with or without a history of previous myocardial infarction, which suggests that the antibodies are not induced by tissue necrosis[7]. Although controversial, aPL may have a role in accelerated atherosclerosis by inducing endothelial cell activation[7-9]. There are studies demonstrating that aPL is an independent risk factor for atherosclerotic disease in systemic lupus erythematosus (SLE)[7-10].

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We investigated the relationship between anti beta 2 GPI and severity of coronary artery stenosis by calculating the SYNTAX score among patients undergoing CABG surgery.

Patients and Methods

We prospectively investigate 612 consecutive patients who undergo elective coronary angiography between September 2012 and June 2013. Indications for coronary angiography were commonly chest pain or non-invasive tests in which myocardial ischemia was suspected. Patients with any acute illness including acute coronary syndromes (ACS), with a history of malignancy within the past 5 years and with any predominant non-cardiac disease were excluded.

At the time of hospitalization, key demographic and clinical characteristics were collected including age, gender, ethanol intake and presence of traditional risk factors for atherosclerosis (hypertension, diabetes mellitus, smoking, hyperlipidemia, positive family history). Patients were evaluated for blood chemistry and anti-β2GPI IgA, IgM and IgG.

In SYNTAX study, the extent of coronary artery disease (CAD) was assessed by using the SYNTAX score, which is based on the location, severity, and extent of coronary stenoses, with a low score indicating less complicated anatomic CAD. In post hoc analyses, a low score was defined as 22; intermediate, 23 to 32; and high, 33. Of 612 patients undergoing coronary angiography, 137 patients were decided to proceed with CABG by heart team (including experienced interventional cardiologists and cardiovascular surgeons). Finally 97 patients with complete biochemical analysis including anti Beta 2 GPI antibodies and undergone CABG have been enrolled in this study (Figure 1). We divided patients in to 2 groups according to the SYNTAX scores. Group 1 included 48 patients with low SYNTAX scores (<23) ang group 2 included 49 patients with intermediate and high SYNTAX scores (>23).

Routine laboratory measurements were performed as previously described. In brief, venous blood sampling was performed in the morning before coronary angiography and routine laboratory parameters were immediately determined, whereas remaining blood samples were snap frozen for further determinations and stored at –80°C until analysis. Anti β2GPI antibodies were measured by ELISA following the minimal requirements proposed by the European Forum on antiphospholipid antibodies[11-14]. Data for anti β2GPI antibodies were expressed as IgG and IgM home units using a reference plasma for each isotype (considered containing 100 home units) of two strongly positive patients. Cut-off values for medium titer (99th percentile) were 17 and 20 for IgG and IgM, respectively.

Study was given approval by an Institutional Review Committee and that informed consent was given by the subjects.

Statistical Analysis

Statistical analyses were performed using SPSS 15.0 (SPSS Inc., Chicago, IL, USA) software. The data are presented as mean ± SD with 95% confidence intervals (CI). The Student *t*-test was used for continuous variables between groups. Categorical variables were compared using the chi-square test and one-way ANOVA. Correlation between serum anti β2GPI antibody levels and SYNTAX score was demonstrated with Pearson's correlation analysis and Mann-Whitney U test. In addition, univariate and multivariate binary logistic regression analysis was performed to detect independent factors affecting severity of CAD. The baseline variables for which evident significance ($p < 0.10$)

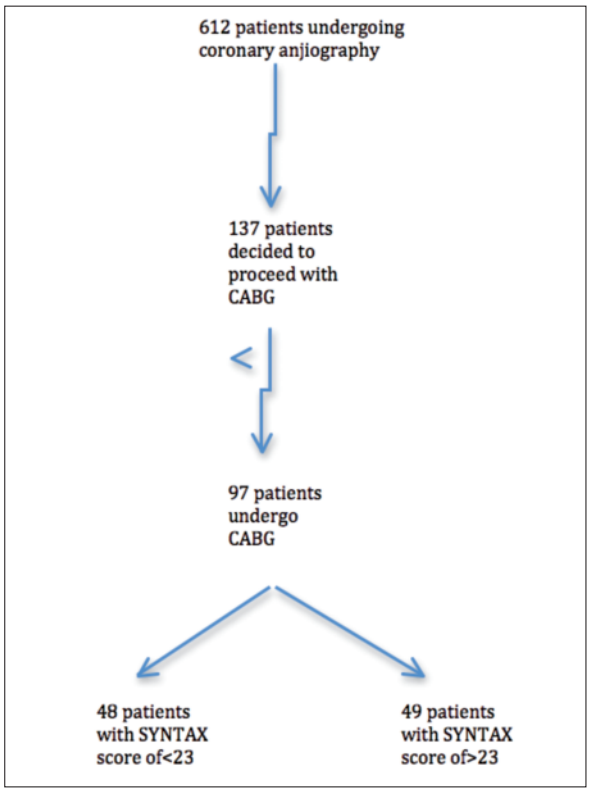

Figure 1. Flow chart of study.

Table I. Baseline characteristics of the patients.

	Group 1 (SYNTAX score < 23) n=48	Group 2 (SYNTAX score > 23) n=49	*p* value
Age	56±8	59±8,3	NS
Sex (M/F)	38/10	40/9	NS
BMI	27±3,0	28±3,4	NS
Cigarette smoking	27%	35%	0.010*
Alcohol	18	20	NS
Systolic BP	137±11	139±10	NS
Hypertension	38	37%	NS
Hyperlipidemia	33%	41%	0.02*
Diabetes mellitus	27%	38%	<0.001*
Serum creatinine	0.9±0.13	0.9±0.11	NS
LDL-Cholesterol	139±43	152±45	<0.001*
HDL- Cholesterol	44±14	42±13	NS
Triglyceride	178±46	189±33	0.02*
CRP	4.0±1.9	5.2±2.1	0.0017*
HbA1c	5.8±2.7	6.7±2.9	<0.001*
Uric acid	8.3±1.41	8.2±1.37	NS
Leukocyte count	8720±3200	8850±2700	NS
Anti-β2GPI IgA	7.6±0.19	7.3±.020	NS
Anti-β2GPI IgM	11.9±2.9	12.1±2.7	NS
Anti-β2GPI IgG	13.9±4.6	20.8±3.5	<0.001*

by univariate analysis were included in multivariate logistic regression analysis. The results of the model were reported as 95% Confidence Interval and *p* values. All *p* values were two-sided in the tests and *p* values less than 0.05 were considered to be statistically significant.

Results

A total of 97 patients were included in this study. Group 1 included 48 patients with SYNTAX score of equal or less than 23 and Group 2 included 49 patients with SYNTAX score higher than 23. Comparison of baseline characteristics of patients is shown in Table I. Both groups were similar in terms of age, sex and BMI. Blood pressure, alcohol intake and serum creatinine levels were also similar between the two groups. However, presence of diabetes and hyperlipidemia, CRP levels, $HbA1_C$ levels and smoking history were significantly higher in patients with higher SYNTAX scores. There was significant correlation between elevated anti β2GPI IgG levels and higher SYNTAX score which indicate advanced and complex CAD (Table II). Anti β2GPI IgA and IgM levels were not statistically different between 2 groups. Univariate and multivariate regression analysis also showed significant correlation between anti β2GPI IgG levels and advanced CAD (Table III).

Discussion

In this study, the risk of significant lesion complexity increased progressively with increasing anti-β2GPI-IgG type of antibody levels. According to our results, anti-β2GPI-IgG is a strong predictor of higher SYNTAX score. But we did not show any association between anti-β2GPI-IgA and IgM types with SYNTAX Score.

In this study, we used SYNTAX score to determine extent and complexity of atherosclerotic

Table II. Univariate logistic regression analysis for the determinants of advanced coronary artery disease which defined as SYNTAX score ≥ 23.

Variable	95%CI	*p*
Age (years)	0.993; 1.029	0.27
Sex (male/female)	0.346; 0.378	0.37
Smoking	0.878; 0.983	0.06*
Creatinine	0.919; 1.282	0.29
Hiperlipidemia	0.977; 1.109	0.02*
Hypertension	0.851; 1.802	0.26
Diabetes mellitus	1.019; 1.348	0.02*
HbA1c	0.917; 1.107	0.01*
CRP	0.326; 0.764	0.001*
Leukocyte count	0.851; 0.937	0.17
LDL-Cholesterol	1.642; 2.208	0.001*
Anti β2GPI IgA	0.837; 1.231	0.27
Anti β2GPI IgM	0.927; 0.982	0.32
Anti β2GPI IgG	0.738; 0.806	0.001*

$p < 0.10$ is indicated as significant in univariate analysis.

Table III. Multivariate logistic regression analysis for the determinants of advanced coronary artery disease which defined as SYNTAX score ≥ 40.

Variable	95%CI	p
Smoking	0.094; 0.385	0.001*
Diabetes mellitus	1.003; 1.023	0.01*
HbA1c	0.929; 1.623	0.01*
LDL-cholesterol	0.923; 1.572	0.001*
CRP	0.432; 0.644	0.45
Hyperlipidemia	0.838; 0.934	0.13
Anti β2GPI IgG	0.879; 1.526	0.001*

$p < 0.05$ is indicated as significant in multivariate analysis.

CAD. The SYNTAX score is a new angiographic tool used to grade the complexity of coronary artery lesions that shows greater discrimination ability both in patients with multivessel disease and in patients with left main disease[15,16]. This scoring system was developed to assist patient's selection and risk stratification for the SYNTAX trial (Synergy Between Percutaneous Coronary Intervention with TAXUS and Cardiac Surgery) and provides the first evidence-based approach in using optimal revascularization strategies for patients with multivessel and/or left main CAD. Our patient population was entirely composed of patients undergoing CABG surgery. By taking these patients we aimed to include solely patients with critical atherosclerotic coronary narrowings.

We only enrolled patients undergoing elective coronary angiography and we exclude patients with acute coronary syndromes. By doing these we tried to eliminate positive antibody response induced by tissue necrosis.

Beta-2 glycoprotein is the major antigen bound by antibodies demonstrable in the antiphospholipid syndrome (APS)[17]. Beta 2 GPI was first described in the early 1960s as a component of the beta-globulin fraction of human serum. This molecule was classified as an apolipoprotein, and it was initially termed apolipoprotein H.

Beta 2 GPI is produced in the liver and the placenta. The mean serum level of β2GPI is about 200 mg/ml, which makes β2GPI one of the most abundant proteins in human serum, second only to fibrinogen, among the plasma proteins involved in clotting.

Antiphospholipid antibodies particularly antibodies against β2-glycoprotein I (anti-β2GPI) are casually associated with both arterial and venous thromboses in patients with autoimmune diseases[3-6,18,19]. However, their exact prevalence and role in the pathogenesis of arterial and venous thromboses in the absence of autoimmune disease is still inconclusive.

A number of clinical studies have established that anti-CL/β2-GPI antibodies are associated with thromboembolic events such as cerebral vascular disorder (CVD), ischemic heart disease (IHD), deep vein thrombosis, and pulmonary embolism[4,20-22].

Atherosclerosis is a chronic inflammatory response to the deposition of lipoproteins (cholesterol and triglycerides) in the walls of arteries. Atherosclerosis usually occurs due to well-known risk factors such as hypercholesterolemia, hypertension, diabetes mellitus, adiposity, and smoking[23,26]. In inflammation, many components of the immune system, including monocytes and macrophages, T cells, autoantibodies and their respective autoantigens, and cytokines secreted by cells within atherosclerotic plaques, are thought to be involved in the pathologic processes that underlie the development of atherosclerosis[27,28].

β2GPI antibodies have been shown to evoke procoagulant activity, either in the presence or the absence of the antiphospholipid syndrome. George et al[6] have provided data that β2GPI may be proatherogenic by inducing an immune response in mice which accelerated atherosclerosis. They also demonstrated the presence of β2GPI in atheroma and when β2GPI-reactive lymphoid tissue was administered to an atherosclerotic mouse model this promoted fatty streak formation[29]. The same group also reported that inducing immunological tolerance of β2GPI by prior oral feeding with the antigen resulted in a significant reduction in the extent of atherosclerotic lesions[30]. They concluded that, β2GPI is implicated in the progression of the atherosclerotic plaque, and may be utilized as an immunomodulator of plaque progression and that cellular immunity to β2GPI exists in patients with the antiphospholipid syndrome.

It has been reported that β2GPI specifically binds to Cu^2+-oxidized low-density lipoprotein (oxLDL) and that the β2GPI-oxLDL complex is then targeted by β2GPI antibodies. Ligands for ß2GPI purified from oxLDL are omega-carboxylated 7-ketocholesteryl esters, such as 7 ketocholesteryl-9-carboxynonanoate (oxLig-1) and 7-ketocholesteryl-12-carboxy (keto) dodecanoate (oxLig-2). These ligands form oxLDL- β2GPI complexes, which are taken up by macrophages via anti-ß2GPI autoantibody-mediated phagocytosis[10,31-32]. The presence of ß2GPI-oxLDL complexes and IgG antibodies recognizing these

complexes were strongly associated with arterial thrombosis. These antibodies correlated with IgG immune complexes containing ß2GPI or LDL suggesting that the β2GPI-oxLDL complexes acting as autoantigens are associated with autoimmune-mediated atherogenesis[33]. The oxidative-modification of low-density lipoproteins (oxLDL) and oxLDL/β2GPI complex formation have been reported in patients with autoimmune disorders and the interaction of oxLDL with β2GPI in circulation suggests that oxLDL/β2GPI complexes may also play a role in the development of atherosclerosis and/or cardiovascular complications in diabetes mellitus[34].

Conclusions

In this study we aimed to evaluate the relationship between anti-β2GPI of IgA, IgM and IgG types and the extent and complexity of CAD in a group of stable coronary artery patients undergoing CABG surgery. Beside traditional risk factors like diabetes, smoking and LDL-cholesterol we found that the higher SYNTAX score is related to the higher levels of anti-β2GPI of IgG type. In addition to the traditional risk factors for atherosclerosis, the proinflammatory and procoagulant activities of aPLs appear to be important risk factors for the development and progression of atherosclerotic occlusive disease.

Larger experimental and clinical studies are needed to evaluate mechanistic role of these antibodies in CAD.

Competing of interest

Authors have declared that no competing interest exists.

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