RESEARCH LETTER

The importance of *APOB* gene expression as a marker of atherosclerosis severity in coronary vessels

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Introduction Despite enormous progress in the prophylaxis and treatment of atherosclerosis and its complications, the disease still represents a major clinical challenge and is associated with high mortality due to cardiovascular events in many countries. Methods applied in the diagnosis of atherosclerotic lesions in coronary vessels differ in their invasiveness, which depends on the localization of the lesions. For many years, coronary artery revascularization has been the foundation of invasive treatment of acute coronary syndromes (ACS). The aim of revascularization with percutaneous coronary intervention (PCI) or coronary artery bypass grafting (CABG) is to effectively treat ischemia and its unfavorable clinical manifestations in patients with significant lesions in coronary arteries. PCI/CABG is performed in chronic coronary syndromes to lower the risk of cardiovascular (CV) events, including myocardial infarction (MI) and death.

An important clinical issue is to determine an optimal diagnostic pathway for coronary vessel assessment in patients with chronic coronary syndromes. Coronary angiography (CA) is recommended as an alternative examination in the diagnosis of coronary artery disease (CAD) in patients with high clinical CAD probability and significant symptoms that are drug-resistant, or in patients with typical angina induced by little effort, in whom clinical assessment indicates a high risk of CV events.[1,2] Due to the invasiveness of CA, it is very important to accurately identify patients with high CV risk and high probability of essential stenoses in coronary arteries that would require angioplasty. For years there has been a heated debate on clinically significant biomarkers and their configuration in the risk assessment and prognosis of acute events caused by coronary atherosclerosis.

The main modifiable risk factors for coronary artery atherosclerosis are apolipoprotein B (ApoB)-containing lipoproteins, with low-density lipoprotein cholesterol (LDL-C) being the most plentiful among them.[3] ApoB is thought to be a useful additional risk marker of atherosclerotic cardiovascular disease (CVD) because it is present on atherogenic lipoproteins and has been associated with the occurrence of atherosclerotic lesions in coronary arteries.[4,5] Previous analyses indicated that benefits associated with lowering the level of cholesterol in atherogenic lipoproteins depend on lowering the actual count of ApoB particles.[6] According to the guidelines of the European Society of Cardiology (ESC) and the European Atherosclerosis Society,[7] it is recommended to measure the ApoB concentration to improve risk stratification, which is also considered a secondary aim in patients with a high level of triglycerides (TGs). In the currently available literature, there is evidence that the secretion of ApoB is changing simultaneously with the rate of lipogenesis and nutrient supply, but interestingly, the *APOB* mRNA level in the liver does not alter despite an increase in the secretion of lipoproteins.

The aim of this study was to evaluate the ApoB concentration and *APOB* expression (mRNA) depending on the severity of atherosclerosis in coronary vessels.

Patients and methods This retrospective study involved a total of 110 individuals. All participants underwent CA according to the ESC guidelines.[7-11] The inclusion criteria comprised eligibility for CA and the age of at least 18 years. Dissection of a coronary vessel was the only exclusion criterion.

The patients were enrolled in the years 2016 to 2020, and they were divided into the following groups: group A, patients after PCI with stent implantation or patients referred for CABG, with stenoses of a minimum 70%; group B, patients after CA with stenoses of 40% to 50% or with

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stenoses greater than 50% and less than 2-mm wide vessels who were not eligible for PCI/CABG; and group C, patients after CA and without essential lesions in the coronary vessels.

In group A there were 18 women and 36 men. Groups B and C included a total of 29 women and 27 men. The median age of the patients after PCI or referred for CABG (68 years; range, 53–74 years) was similar to the median age in groups B and C (63 years; range, 55–70.5 years).

To quantitate the expression of the *APOB* gene both at the protein level and at the mRNA level, blood samples were collected from all study participants and serum was separated. The biologic materials (serum and whole blood) were stored at –20 °C until preparatory procedures and analyses. The serum concentration of human ApoB was determined using a commercially available enzyme-linked immunosorbent assay kit (SEC003Hu, Cloud-Clone Corp., Katy, Texas, United States). All evaluations were performed according to the manufacturer's instructions, with analysts blinded to the clinical data.

The extraction of mRNA from the blood cells was made using Hybrid-R Blood RNA (GeneAll, Seoul, South Korea), following the manufacturer's protocol. The quantity and purity of RNA samples were assessed with a NanoDrop Spectrophotometer (Thermo Scientific, Waltham, Massachusetts, United States). Complementary DNA (cDNA) was obtained from mRNA templates with the use of the TRANSCRIPTME RNA Kit (Blirt S.A., Gdańsk, Poland) according to the manufacturer's protocol.

Relative quantification of the *APOB* expression was performed using the Rotor-Gene Q thermocycler (QIAGEN, Hilden, Germany) and the commercially available kits of reagents: RT^2 qPCR Primer Assay together with RT^2 qPCR FAST SYBR Green/ROX Mastermix (QIAGEN). The human *APOB* was the gene of interest, and *ACTB*, which encodes human β-actin, was a housekeeping gene. Real-time quantitative polymerase chain reactions were conducted, according to the manufacturer's instructions.

Statistical analysis Reactions for both *APOB* and *ACTB* were carried out in duplicate for every patient. Each batch was run with both negative (no template controls) and positive controls (the pooled cDNA). As part of the method validation, efficiency of the *APOB* and *ACTB* reactions was determined, and the respective values were as follows: 101% (slope = –3294) and 99% (slope = –3352). In order to determine the relative fold change in the *APOB* expression, the double-delta C_t method was used, together with the Pfaffl equation. The normality of distribution of a continuous variable in a given group was assessed with the Shapiro–Wilk test. The Brown–Forsythe test was used to verify the homogeneity of variances between the compared groups. If a variable did not follow the normal distribution or there was no homogeneity of variances, the nonparametric Mann–Whitney test was used. Otherwise, the *t* test for independent samples was applied. The calculations were performed with the STATISTICA 13.3 data analysis software system (StatSoft, Inc., Kraków, Poland). The differences were considered significant at a *P* value lower than 0.013. Because of multiple testing, the Bonferroni correction for $\alpha = 0.05$ was chosen.

Ethics All patients provided their written informed consent to participate in the study. The research was conducted in compliance with the provisions included in the Declaration of Helsinki and its plan had been approved by the Bioethics Committee, Łódź, Poland (43/2016 and 63/2019).

Results The concentration of ApoB and expression of *APOB* at the mRNA level were assessed in the patients requiring PCI/CABG (group A). The results were compared with those obtained in the patients who were not eligible for coronary angioplasty due to atheromatous plaques and those in whom no stenoses were found (groups B and C) (TABLE 1).

In the whole study group (women and men referred for PCI/CABG; group A), the median ApoB concentration was similar to that observed in the patients with no PCI/CABG history (*P* = 0.16).

The *APOB* expression at the mRNA level was 1.28-fold higher in group A than in groups B and C; however, the difference was not significant (*P* = 0.53).

We also performed an analysis of the *APOB* expression at the protein and mRNA levels in relation to the sex of the patients. The median ApoB concentration in the women after PCI or those referred for CABG was almost twice as high as in those with no history of PCI/CABG (*P* = 0.03).

The expression of *APOB* in the women in group A was 0.63-fold higher than in the women included in groups B and C, but not significantly (*P* = 0.43).

The median ApoB concentrations in men (group A vs groups B and C) were similar (*P* = 0.69). The expression of *APOB* at the mRNA level was 2.36-fold higher in the men after PCI or those referred for CABG than in the men with no PCI/CABG history. However, the difference was not significant (*P* = 0.12).

The *APOB* expression in group A was also compared with that observed in group C (no stenoses in the coronary arteries) (TABLE 1). It was found that the median ApoB concentration was higher in the patients included in group A than in those with no relevant lesions in the coronary arteries (*P* = 0.03). The *APOB* expression at the mRNA level was 1.53-fold higher in group A than in group C, but the difference did not reach the level of significance (*P* = 0.31).

The analysis of the *APOB* expression in relation to the sex of the patients showed that the median ApoB concentration was approximately 2-fold higher in the women included in

TABLE 1 Expression of the *APOB* gene at the protein and mRNA level in the study participants

Parameter	Patients after PCI or referred for CABG: group A (n = 54)	Patients with no history of PCI/CABG: groups B and C (n = 56)	*P* value (A vs B+C)	Patients after PCI or referred for CABG: group A (n = 54)	Patients with no significant lesions in the coronary arteries: group C (n = 43)	*P* value (A vs C)
ApoB concentration, μg/ml, median (IQR)	10.4 (6.18–13.19)	7.93 (4.91–11.96)	0.16	10.4 (6.18–13.19)	7.09 (4.23–10.73)	0.03
Increase in the *APOB* expression at the mRNA level	1.28-fold	–	0.53	1.53-fold	–	0.31
Parameter	**Women after PCI or referred for CABG (n = 18)**	**Women with no history of PCI/CABG (n = 29)**	***P* value**	**Women after PCI or referred for CABG (n = 18)**	**Women with no significant lesions in the coronary arteries (n = 23)**	***P* value**
ApoB concentration, μg/ml, median (IQR)	11.73 (6.85–17.26)	6.07 (4.11–10.64)	0.03	11.73 (6.85–17.26)	5.6 (3.53–9.15)	0.01[a]
Increase in the *APOB* expression at the mRNA level	0.63-fold	–	0.43	0.57-fold	–	0.38
Parameter	**Men after PCI or referred for CABG (n = 36)**	**Men with no history of PCI/CABG (n = 27)**	***P* value**	**Men after PCI or referred for CABG (n = 36)**	**Men with no significant lesions in the coronary arteries (n = 20)**	***P* value**
ApoB concentration, μg/ml, median (IQR)	9.31 (5.69–12.57)	8.93 (6.78–16.54)	0.69	9.59 (1.95–19.41)	9.6 (1.21–20.75)	0.99
Increase in the *APOB* expression at the mRNA level	2.36-fold	–	0.12	4.02-fold	–	0.02

P values were derived from the Mann–Whitney test (nonparametric) or the *t* test, as appropriate; *P* values <0.013 were considered significant.

a Significant difference after the Bonferroni correction for multiple testing

Abbreviations: CABG, coronary artery bypass grafting; IQR, interquartile range; PCI, percutaneous coronary intervention

group A than in those without significant lesions in the coronary arteries (group C). The difference was significant ($P = 0.01$) after the Bonferroni correction.

No significant differences in the *APOB* expression at the mRNA level were found between the compared groups of women ($P = 0.38$).

Mean ApoB concentrations in the group of men requiring PCI/CABG and in those without stenoses in the coronary arteries were similar ($P = 0.99$). Interestingly, we found that the *APOB* expression at the mRNA level in the men included in group A was 4-fold higher than in the men in group C. This difference was near the threshold of the corrected significance level ($P = 0.02$).

Discussion ApoB includes apoB48 and apoB100 forms, and it is present in TGs, non–high-density lipoprotein cholesterol (non–HDL-C), and LDL-C. The concentration of ApoB includes a total of the apoB48, apoB100, and lipoprotein(a) amounts, and is considered a direct marker of atherogenic lipoprotein level.[7] In the literature, there are discrepancies concerning the significance of ApoB alone as a marker of CV risk. On the one hand, there are reports that all ApoB-containing lipoproteins have the same effect on CVD risk. On the other hand, according to some studies, ApoB is a more accurate indicator of the CV risk than the levels of TGs, LDL-C, and non–HDL-C, especially in statin-treated patients. In addition, ApoB is considered a more accurate predictor of all-cause mortality and MI than LDL-C.[6,12] A meta-analysis of 8 studies, carried out by Boekholdt et al,[13] showed that non–HDL-C was a slightly more accurate marker of the residual risk than ApoB or LDL-C. It is considered that ApoB particles are the main factor associated with damage to the arterial wall. The more ApoB particles in the arterial lumen, the stronger their accumulation in the wall, and thereby the damage. The uptake of ApoB particles by the arterial wall constitutes the basic step that initiates and promotes atherogenesis from the beginning until ACS, that is, from the development of fatty streaks to erosion of the endothelium and rupture of the atheroma, which precede clinical events.[14]

In the present study, we found that the ApoB concentration in the women included in group A was significantly higher than in the women in group C (11.73 μg/ml vs 5.6 μg/ml; $P = 0.01$).The serum ApoB concentration was approximately 2-fold higher in the women referred for PCI/CABG (group A) than in the women with atherosclerosis who did not require PCI/CABG or those who did not present any lesions in the coronary arteries (groups B and C) (11.73 μg/ml vs 6.07 μg/ml). However, after taking into account the Bonferroni correction for multiple testing, this correlation was not significant ($P = 0.03$) (TABLE 1).

We also showed that the *APOB* expression at the mRNA level in the men requiring PCI/CABG was 4-fold increased in comparison with the men without plaques in the coronary vessels. This relationship was near the threshold of the corrected significance level ($P = 0.02$).

There are several limitations of the present research, including a relatively small number of participants (n = 110), inclusion of data from a single center in a country with a high CVD risk (Poland), the lack of information on the smoking status of the patients, and the use of whole blood as the biological material. Some authors emphasize that there are differences in gene expression among various (sub)populations of cells in blood samples.[15,16] Thus, in order to better characterize the clinical significance of assessing the *APOB* expression at the mRNA level as a parameter of atherosclerosis severity, further studies should be performed, particularly using a single blood cell type defined through standardization.

Increased plasma concentrations of ApoB-containing lipoproteins are essential risk factors for atherosclerosis; therefore, understanding the mechanisms involved in the regulation of ApoB production is important. The expression of ApoB is tissue-specific and primarily limited to the liver and small intestine. *APOB* expression at the mRNA level in the liver remains stable and depends neither on the rate of lipogenesis nor on the diet. The process is regulated in several steps, such as transcription, mRNA editing, and proteosomal degradation.[17,18] In vitro studies showed that mRNA quantities of *APOB* remain constant in various metabolic conditions, which indicates that the gene transcription is closely controlled. Similarly, in in vitro studies, unchanged mRNA quantities were observed even though the amount of the secreted ApoB in a cell culture was modified. Therefore, it can be concluded that transcription of the *APOB* gene, influenced by nutritional, hormonal, and post-transcriptional stimuli, does not change.[19]

The existence of a link between a number of transgene copies and plasma concentration of LDL-C indicates that an increase in the *APOB* expression may lead to enhanced secretion of ApoB-containing lipoproteins. In the course of further research, it has been found that the quantity of the synthetized ApoB surpasses the amount being secreted, which may be explained by post-translational modifications during trafficking of ApoB through the endoplasmic reticulum.[20]

The results of the present study indicate that the ApoB concentration may constitute a major additional marker of atherosclerosis severity in women without ACS during their qualification for noninvasive and invasive examinations of the coronary vessels. The *APOB* expression at the mRNA level did not seem to be a good indicator of atherosclerosis severity; however, it may be an additional marker that could enable clinicians to eliminate the presence of relevant plaques in the coronary arteries in men.

ARTICLE INFORMATION

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