RESEARCH LETTER

Increased endocan expression as a biomarker of endothelial dysfunction in patients with metabolic syndrome

Sylwia Iwańczyk, Anna Smukowska-Gorynia, Patrycja Woźniak, Marek Grygier, Maciej Lesiak, Aleksander Araszkiewicz

First Department of Cardiology, Poznan University of Medical Sciences, Poznań, Poland

Introduction Metabolic syndrome (MS) is an increasing global health problem, especially in developing countries, associated with rapid socioeconomic and demographic transitions.[1] In the Polish population, the prevalence of MS and its components increased significantly over the last decade by 3.3 percentage points in women (26.6% vs 29.9%; P <0.001) and 8.8 percentage points in men (30.7% vs 39.4%; P <0.001).[2]

MS is characterized by central obesity, insulin resistance, dyslipidemia, and elevated blood pressure.[1] All of its components, if left untreated, can lead to the early development of atherosclerosis and, consequently, adverse cardiovascular events.

Excessive visceral adipose tissue in obese individuals increases the risk of endothelial dysfunction, which affects the vascular homeostasis by impaired vasorelaxation, increased leukocyte adhesion, activation of platelets, oxidative stress, and inflammation.[3] Thus, it is believed that endothelial dysfunction is a precursor of both the development of atherosclerotic lesions and thrombotic complications.[4]

Endocan, known as endothelial cell–specific molecule-1, is an endothelium-derived circulating proteoglycan that is considered an early immune-inflammatory marker of endothelial dysfunction.[5] An increased concentration of endocan has been found in patients with pediatric metabolic syndrome,[6] type 2 diabetes mellitus (T2DM),[7] hypertension,[8] and coronary artery disease (CAD).[9] However, little is known about the level of this molecule in the adult population with MS.[10] Thus, this study aimed to evaluate the endocan concentration in patients with and without MS.

Patients and methods The presented data result from a subanalysis of the study previously published by the authors.[11] Currently, we analyzed all 90 patients undergoing coronary angiography for typical chest pain or discomfort. All the patients were prospectively enrolled between October 2018 and March 2020. Those who met the criteria for the diagnosis of MS were included in the MS group (n = 34). The remaining patients were assigned to the non-MS group (n = 56). MS was diagnosed if at least 3 of the following criteria were met: (1) waist circumference equal to or above 94 cm for men and equal to or above 80 cm for women; (2) blood pressure equal to or greater than 130/85 mm Hg or pharmacologic antihypertensive treatment; (3) triglyceride level equal to or greater than 150 mg/dl; (4) fasting blood glucose level equal to or above 100 mg/dl or pharmacologic treatment for hyperglycemia; (5) high-density lipoprotein cholesterol level (HDL-C) below 40 mg/dl for men and below 50 mg/dl for women.

We excluded patients with acute coronary syndrome, history of severe hepatic and renal dysfunction, leukemia, leukopenia, thrombocytopenia, or ongoing inflammatory and malignant diseases, connective tissue diseases; interferon treatment, and those who did not provide informed consent to participate in the study.

Ethylenediamine tetraacetic acid–coagulated blood samples (10 ml) were collected from all the patients on the first day after the cardiac catheterization and were processed within 30 minutes of the collection. The samples were centrifuged at 1300 g for 15 minutes at room temperature. The supernatant was stored at –80 °C. Determination of the endocan concentration was performed using the human endothelial cell–specific molecule 1 (ECSM1 / ENDOCAN) enzyme-linked immunosorbent assay (ELISA) kit (DRG International, Springfield, New Jersey, United States). This ELISA kit is based on the principle

Correspondence to:
Sylwia Iwańczyk, MD,
First Department of Cardiology,
Poznan University of Medical Sciences, ul. Długa 1/2,
61-848 Poznań, Poland,
phone: +48 61 854 92 22,
email: syl.iwanczyk@gmail.com
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TABLE 1 Baseline clinical characteristics and laboratory test results

Parameter	Metabolic syndrome group (n = 34)	Non–metabolic syndrome group (n = 56)	*P* value[a]
Male sex	24 (70.1)	42 (75.0)	0.64
Age, y	64.7 (6.6)	63.7 (6.9)	0.53
BMI, kg/m^2	31.3 (4.3)	27.5 (5.0)	<0.001
Hypertension	30 (88.2)	43 (76.8)	0.12
T2DM	32 (94.1)	4 (7.1)	<0.001
Hyperlipidemia	28 (82.3)	27 (48.2)	0.002
CAD	25 (73.5)	22 (39.2)	0.002
Previous MI	15 (44.1)	18 (32.1)	0.25
CAE	11 (32.3)	17 (30.3)	0.77
WBC, 10^9/l	8.3 (2.5)	7.0 (2.0)	0.01
Neutrophils, 10^9/l	5.4 (2.4)	4.3 (1.6)	0.02
Lymphocytes, 10^9/l	1.9 (0.6)	1.9 (0.6)	0.68
Monocytes, 10^9/l	0.5 (0.4–0.6)	0.4 (0.3–0.5)	0.02
Triglycerides, mmol/l	1.4 (1.1–2.4)	1.2 (0.9–1.6)	0.03
TC, mmol/l	4.3 (1.0)	4.6 (1.3)	0.26
HDL-C, mmol/l	1.4 (0.5)	1.6 (0.4)	0.04
LDL-C, mmol/l	2.2 (0.9)	2.4 (1.1)	0.32
Glucose, mmol/l	6.5 (6.1–7.1)	5.5 (5.1–6.1)	<0.001

Continuous variables are presented as mean (SD) or median (interquartile range), and categorical variables as number (percentage).

a *P* <0.05 significant for all comparisons (analysis of variance)

Abbreviations: BMI, body mass index; CAD, coronary artery disease; CAE, coronary artery ectasia; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MI, myocardial infarction; T2DM, type 2 diabetes mellitus; TC, total cholesterol; WBC, white blood cells

of double-antibody sandwich technique to detect human ECSM1/ENDOCAN. The sensitivity of this assay was determined by sub-tracing 2 standard deviations to the mean optical density value of 20.0 standard replicates and calculating the corresponding concentration. The assay range was between 8 and 2000 ng/l.

Statistical analysis All continuous variables were presented as means (SD) for normal distribution or medians (interquartile range [IQR]) for nonnormal distribution. The normality of the distribution of variables was assessed using the Kolmogorov–Smirnov test. Categorical variables were presented as numbers and percentages or frequencies. The significance of differences between the mean values of the normally distributed continuous data was evaluated using the *t* test. The Mann–Whitney test was used to compare the continuous data that did not follow normal distribution. Categorical variables were compared using the χ^2 test. *P* values below 0.05 were considered significant. The prognostic relevance of individual variables regarding the prediction of end points was estimated using univariable logistic regression analysis. The multivariable logistic regression model included the variables with *P* values lower than 0.1 in the univariable model. We used the PQStat Software (PQStat v.1.8.0.476, Poznań, Poland) for all statistical analyses.

Results Clinical characteristics of the study population are summarized in TABLE 1. Due to the profile of the analyzed groups, obesity, T2DM, and lipid disorders were diagnosed significantly more often in patients with MS. Moreover, serum levels of glucose and triglycerides were significantly higher, and the level of HDL-C was significantly lower in the MS group than in the patients without MS. CAD was significantly more common in the patients with MS. Interestingly, the total white blood cell (WBC) count and the fraction of neutrophils and monocytes were significantly higher in the patients with MS. Other demographic data and comorbidities did not differ significantly between the 2 groups.

In addition, multivariable logistic regression analysis showed that the endocan concentration and CAD were the independent risk factors for the occurrence of MS. Detailed data are presented in Supplementary material, *Table S1*.

The results of the analysis showed that the serum concentration of endocan significantly differed between the 2 subsets of patients (Supplementary material, *Figure S1*), and was higher in the MS group than in the non-MS group (median [IQR], 746.7 [610.8–1533.6] ng/l vs 571.4 [519.3–689.0] ng/l; *P* = 0.009).

Discussion The present study evaluated the degree of endothelial dysfunction measured by serum endocan concentration in patients with MS.

Endocan, secreted by the endothelium, stimulates endothelial cells to release inflammatory cytokines and increase vascular permeability. Moreover, it is independently associated with the levels of soluble intercellular adhesion molecule-1 and soluble vascular cell adhesion molecule-1, thereby increasing the adhesion of leukocytes to endothelial cells and enhancing leukocyte migration.[12,13] This upregulated inflammatory response plays a crucial role in atherosclerotic plaque formation and cardiovascular diseases.[14]

The current study showed a higher serum concentration of endocan in the MS group than in the non-MS subset, confirming that endothelial dysfunction is one of the initial steps in the development of MS and, eventually, cardiovascular diseases. Apart from endocan, WBC count, as well as neutrophil and monocyte levels were higher in the MS patients, which corresponds with well-known low-grade inflammation observed in MS. The association between MS and endothelial dysfunction has been assessed previously. Suzuki et al[10] showed that patients with MS and coexistent endothelial dysfunction, characterized by disturbances in brachial artery flow–mediated dilation, were at the highest risk for cardiovascular events.[12] Each component of MS, including visceral adiposity, dyslipidemia, hypertension, hyperglycemia, and insulin resistance, can individually alter the vascular function and,

in consequence, disturb the endothelial homeostasis.[4] Indeed, in our study, a higher endocan concentration was an independent risk factor for MS. Thus, endocan is a potential biomarker of MS and confirms the key role of endothelial dysfunction in MS development. In a study by Osmenda et al,[15] endothelial function in T2DM was significantly affected by polymorphisms of the cytochrome b-245 alpha chain (*CYBA*) gene encoding p22phox, which is a crucial subunit of nicotinamide adenine dinucleotide phosphate oxidase. The above data prove that the pathomechanism of endothelial dysfunction in MS and its components is complex and not fully understood.[15] Furthermore, in several studies, MS was shown to be associated with an increased risk of incident cardiovascular morbidity and mortality.[10] Our findings are in line with these results: in the MS group, CAD prevalence was significantly higher than in the patients without MS.

Conclusions Endocan is significantly overexpressed in the serum of patients with MS, which suggests a more severe endothelial dysfunction in these individuals. Moreover, endocan was an independent risk factor for the occurrence of MS in multivariable analysis. The significantly higher level of endocan and WBC count in individuals with MS suggest that low-grade inflammation is characteristic of this group of patients.

SUPPLEMENTARY MATERIAL

Supplementary material is available at www.mp.pl/paim.

ARTICLE INFORMATION

CONFLICT OF INTEREST None declared.

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