Low-grade endotoxemia and NOX2 in patients with coronary microvascular angina

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ABSTRACT

Background: Endothelial dysfunction and oxidative stress were hypothesized to be involved in the pathogenesis of coronary microvascular angina (MVA). NADPH oxidase-2 (NOX2) activation could provoke increased oxidative stress and endothelial dysfunction, but data on MVA have not been provided yet.

Aims: This study aimed to evaluate the interaction among NOX2 activation, serum lipopolysaccharide (LPS) levels, as well as oxidative stress production as potential causes of endothelial dysfunction in MVA patients.

Methods: In this study, we wanted to compare serum levels of soluble NOX2-dp (sNOX2-dp), H_2O_2 production, hydrogen peroxide breakdown activity (HBA), nitric oxide (NO) bioavailability, endothelin 1 (ET-1), serum zonulin (as intestinal permeability assay), and LPS in 80 consecutive subjects, including 40 MVA patients and 40 controls (CT), matched for age and sex.

Results: Compared with CT, MVA patients had significantly higher values of sNOX2-dp, H_2O_2, ET-1, LPS, and zonulin. Conversely HBA and NO bioavailability were significantly lower in MVA patients. Simple linear regression analysis showed that sNOX2 was associated with serum LPS, serum zonulin, H_2O_2, and ET-1. Furthermore, an inverse correlation between sNOX2, HBA, and nitric oxide bioavailability was observed. Multiple linear regression analysis showed that LPS and zonulin emerged as the only independent predictive variables associated with sNOX2.

Conclusions: This study provides the first report attesting that patients with MVA have high LPS levels, NOX2 activation, and an imbalance between pro-oxidant and antioxidant systems, in favor of the oxidizing molecules that could be potentially implicated in the endothelial dysfunction and vasoconstriction of this disease.

Key words: coronary microvascular angina, LPS, NADPH oxidase, NOX2, oxidative stress

WHAT'S NEW?

The role of oxidative stress and low-grade endotoxemia is unclear in patients with coronary microvascular angina (MVA). This study aimed to evaluate NADPH oxidase-2 activation, serum lipopolysaccharide levels, as well as oxidative stress production, and endothelial dysfunction in MVA patients and controls. The results of this study show that MVA patients have high circulating levels of serum lipopolysaccharide, oxidative stress, and endothelin 1. NADPH oxidase-2 activation could contribute to provoking an imbalance between pro-oxidant and antioxidant systems, which could determine endothelial dysfunction and vasoconstriction in MVA.

INTRODUCTION

Microvascular angina (MVA) is defined as microvascular dysfunction of the coronary arteries. It is characterized by typical chest pain, evidence of myocardial ischemia on an electrocardiogram, and the absence of obstructive coronary artery disease (CAD) [1]. MVA represents an increasingly growing problem when we consider that up to 50% of patients with angina who undergo coronary angiography have not any significant artery obstruction [2–4]. Despite the absence of significant coronary lesions, its prognosis is not benign, because MVA is an independent risk factor for major adverse cardiovascular events [5, 6].

Microvascular dysfunction is studied invasively with coronary angiography and use of pharmacological tests to evaluate the endothelial reactivity [7]. It has been demonstrated that it mainly embraces two aspects: firstly, inadequate vasodilatation to a stimulus, and secondly, exaggerated vasoconstriction (coronary microvascular spasm) [7]. Experimental studies suggested a major role of oxidative stress in the initiation and progression of microvascular dysfunction in MVA [8]. NADPH oxidase-2 (NOX2) is considered one of the main sources of superoxide anion in humans, a modulator of the arterial tone [9–12], and an enzyme directly implicated in microvascular dysfunction [13]. The role of NOX2 in MVA has not been yet elucidated.

Lipopolysaccharide (LPS) is an endotoxin derived from the membrane of gram-negative bacteria that, by binding to Toll-like receptor 4 (TLR4), activates intracellular transcription of several inflammatory mediators in the vessels [14]. An impairment of tight junction causes translocation of LPS from the gut to the systemic circulation where it initiates pro-inflammatory and pro-oxidant effects in the vessels [15]. Moreover, a recent experimental study showed that LPS from *Escherichia coli* was localized in human plaque and may contribute to atherosclerotic damage *via* TLR4-mediated oxidative stress and NOX2 activation [16, 17]. A relationship between LPS and NOX2 activation has been previously described in other clinical settings such as non-alcoholic fatty liver disease (NAFLD), pneumonia [18], and neurodegenerative disease [19], but no data have showed its role in MVA yet. Thus, in this study, we evaluated the potential role of low-grade endotoxemia by LPS in eliciting systemic Nox2 activation and the balance of pro-oxidant molecules, such as H_2O_2, and the antioxidant system, such as hydrogen peroxide breakdown activity (HBA), in MVA.

METHODS

The study was supported by the Sapienza University in cooperation with Nicolae Testemitanu State University of Medicine and Pharmacy, Chisinau, Republic of Moldova, to strengthen institutional competencies and the management of partner universities, finalized to cardiovascular prevention [20].

Forty consecutive Moldovan patients with coronary MVA and 40 controls (CT) matched for age and sex agreed to participate in the study, which was performed between July 2018 and December 2020. These patients were recruited at the Moldavian Research Institute of Cardiology, Chisinau, Republic of Moldova.

Coronary MVA was diagnosed according to the criteria suggested by the Coronary Vasomotion Disorders International Study Group [21]; these criteria include (1) presence of symptoms suggestive of myocardial ischemia; (2) objective documentation of myocardial ischemia, as assessed by currently available techniques; (3) absence of obstructive CAD (<50% coronary diameter reduction and/or fractional flow reserve >0.80) documented by CT coronary scan or coronary angiography, as in our case; (4) confirmation of a reduced coronary blood flow reserve and/or inducible microvascular spasm [21]. Microvascular disorder was confirmed by using the angiographic flow assessment criteria based on the thrombolysis in myocardial infarction (TIMI) flow grade or TIMI frame count (TFC/cTFC). TIMI-2 flow grade, which means partial perfusion (i.e. requiring three or more beats to opacify the distal vessel) or a corrected TIMI frame count >27 frames, in at least one major coronary vessel, have been frequently used and were, therefore, preliminarily assumed. The latter is based upon images acquired at 30 frames/second and a correction factor of 1.7 for the left anterior descending artery (LAD). The speed with which the dye reaches the distal bifurcation of LAD from the ostium is measured in frame counts. In right coronary artery, the flow was measured from the ostium to the origin of the first posterolateral branch. In the left circumflex, the flow was measured from the ostium to the most distal branch of the last OM. A correction factor of 1.7 was used for LAD (for corrected TFC). The normal count was considered 21 ± 3 frames.

Considering the COVADIS (Coronary Vasomotion Disorders International Study Group) criteria, our cohort of patients had a definitive diagnosis of MVA.

From all patients included in the study, blood samples for analysis of oxidative stress, LPS, and zonulin levels were collected after a fasting period of 8 hours.

Subjects were excluded from the study if they had liver insufficiency, advanced chronic kidney disease, acute cerebrovascular disease, acute myocardial infarction, recent abdominal surgery or were taking antioxidants. We also excluded patients with active cancer, uncontrolled blood pressure or diabetes, inflammatory bowel disease, and those taking or with a recent intake of antibiotics.

Informed written consent was obtained from all subjects: the study conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Ethical Committee of the Nicolae Testemitanu State University of Medicine and Pharmacy, Chisinau, Republic of Moldova (no. 3-9-19/41/49).

Serum sNOX2-dp detection

NOX2 activation was measured as soluble NOX2-derived peptide (sNOX2-dp) with an ELISA method, as previously described [22]. Briefly, the peptide is recognized by binding to a specific monoclonal antibody against the amino acid sequence (224–268), the extra membrane portion of NOX2. Values were expressed as pg/ml; intra-assay and inter-assay coefficients of variation were 8.95% and 9.01%, respectively.

H_2O_2 production

The H_2O_2 was evaluated by a Colorimetric Detection Kit (ArborAssay, Ann Arbor, MI, US) and expressed as µM. Intra-assay and inter-assay coefficients of variation were 2.1% and 3.7%, respectively.

Determination of % HBA in serum

Serum hydrogen peroxide (H_2O_2) HBA was measured with an HBA assay kit (Aurogene, Rome, Italy; code HPSA-50). The percentage of HBA was calculated according to the following formula: % of HBA = [(Ac–As) / Ac] × 100, where Ac is the absorbance of H_2O_2 1.4 mg/ml, and As is the absorbance in the presence of the serum sample.

NO bioavailability

A colorimetric assay kit (Abcam, Cambridge, UK) was used to determine NO bioavailability as previously described [23]. Intra-assay and inter-assay coefficients of variation were 2.9% and 1.7%, respectively.

Serum zonulin

Serum zonulin was used as an intestinal permeability assay. Serum zonulin levels were measured using a commercial ELISA kit (Elabscience, Houston, TX, US). Antibody specific for zonulin has been pre-coated onto a microplate and 100 µl of standards, and patient sera samples were added and incubated for 90 min at 37°C. Then, a biotinylated detection antibody specific for zonulin and Avidin-Horseradish Peroxidase (HRP) conjugate was added to each microplate. Values were expressed as ng/ml; both intra-assay and inter-assay coefficients of variation were within 10%.

Serum LPS assay

LPS levels in serum were measured using a commercial ELISA kit (Cusabio, Houston, TX, US). The standards and samples were plated for 2 hours at room temperature into a micro-plate pre-coated with the antibody specific for LPS. After incubation, samples were read at 450 nm. Values were expressed as pg/ml; intra-assay and inter-assay coefficients of variation were <10%.

Serum endothelin 1 assay

Serum endothelin 1 (ET-1) levels were measured using a commercial ELISA kit (Thermo Fisher Scientific, Waltham, MA, US).

Statistical analysis

Statistical analyses were undertaken using SPSS 25.0 software for Windows (IBM, Armonk, NY, US). The Kolmogorov–Smirnov test was used to determine whether variables were normally distributed. Normally distributed data are described as means and standard deviations (SD). Between-group differences were analyzed by Student t-test. Differences between percentages were assessed by the χ^2 test. Bivariate analysis was performed by Spearman correlation; the variables with evidence of an association P <0.10 were included in a multivariable linear regression using an automated procedure. The results of the multivariable linear regression analysis were expressed as standardized coefficient beta (β) with standard error (SE). Moreover, the coefficient of determination was provided (R2). A P-value of <0.05 was considered statistically significant.

Sample size determination

We computed the minimum sample size with respect to a two-tailed, one-sample Student t-test considering, on the basis of data from a previous pilot study (data not shown): a difference of 7.3 pg/ml for sNOX2-dp levels between patients and controls (mean patients, 32; mean controls, 24.2 pg/ml), 10 as SD, 0.05 (α) as type-I error probability and 0.95 as power 1–β. The sample size was 40 patients/group.

RESULTS

Clinical characteristics of MVA patients and controls are reported in Table 1. There was no significant difference in age, sex, and classic cardiovascular risk factors between the two groups (Table 1).

LPS and zonulin

MVA patients had increased levels of LPS (mean [SD], 37.5 [12.9] pg/ml vs. 20.7 [10.1] pg/ml; P <0.001) and zonulin (3.0 [0.9] ng/ml vs. 1.57 [0.5] ng/ml; P <0.001) compared to the control group (Figure 1A and B).

Table 1. Clinical characteristics of the patients with and without microvascular angina

	Patients with microvascular angina (MVA) n = 40	Patients without microvascular angina (control group) n = 40	*P*-value
Age, years	62.7 (8.1)	61.7 (5.7)	0.56
Male sex	14 (35)	20 (50)	0.17
Diabetes	10 (25)	4 (10)	0.11
Dyslipidemia	36 (90)	38 (95)	0.39
Hypertension	37 (92)	36 (90)	0.69
Current smokers	6 (15)	8 (20)	0.46
Obesity	15 (37)	18 (45)	0.49
Anticoagulants	3 (7.5)	2 (5)	0.74
Beta-blockers	34 (85)	19 (47)	<0.01
Calcium antagonists	28 (70)	10 (35)	<0.01
ACE inhibitors	23 (57)	15 (37)	0.14
Renin-angiotensin-aldosterone system inhibitors	12 (30)	2 (5)	0.01
Statins	33 (82)	25 (62)	0.11
Diuretics	17 (42)	3 (7.5)	<0.01
Anti-platelet drugs	35 (87)	21 (52)	<0.01

Continuous variables are reported as mean (SD); categorical variables are expressed as n and percentage

Abbreviations: ACE, angiotensin-converting enzyme

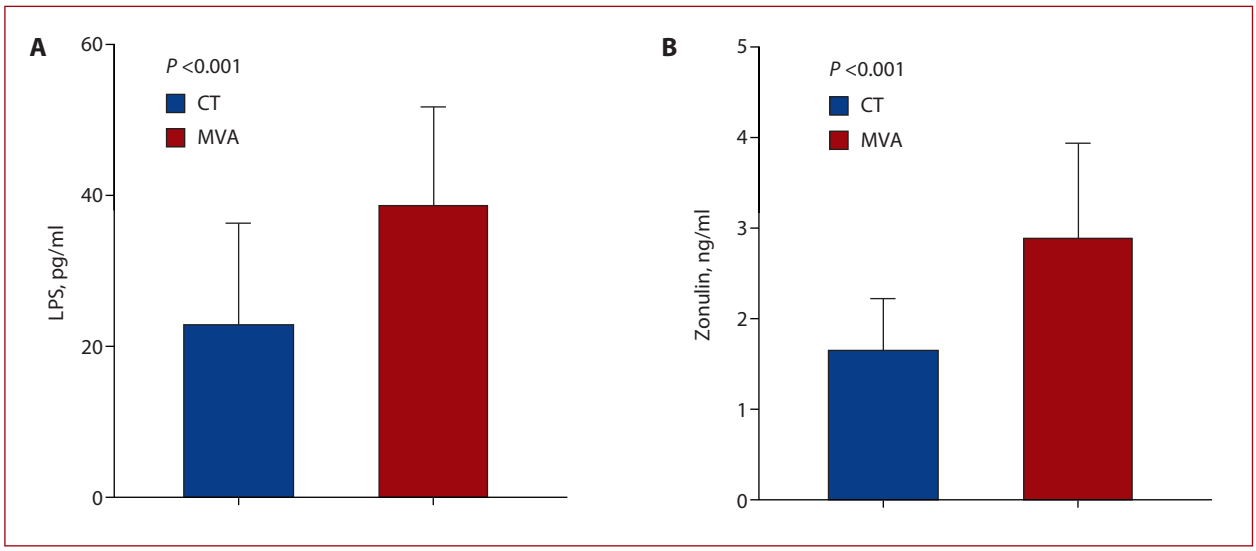

Figure 1. Gut-permeability analysis: serum lipopolysaccharide concentration (**A**) and serum levels of zonulin (**B**) in microvascular angina (MVA) patients (n = 40) and controls (CT) (n = 40)

Oxidative stress

Compared with CT, MVA patients had significant higher mean values of sNOX2-dp (57.4 [13.1] pg/ml vs. 27.4 [11.5] pg/ml; *P* <0.001) and H_2O_2 production (47.7 [21.4] µM vs. 22.1 [10.3] µM; *P* <0.001) (Figure 2A and B). Conversely HBA were significantly lower in patients with MVA compared to controls (27.8 [10.8] vs. 56.1 [21.2] %; *P* <0.001) (Figure 2C).

Endothelial dysfunction and NO bioavailability

MVA patients had significant lower NO bioavailability (28.1 [10.5] µM vs. 35.6 [14.4] µM; *P* = 0.01) and higher levels of ET-1 (15.6 [6.2] pg/ml vs. 10.2 [5.6] pg/ml; *P* <0.001) (Figure 3A and B).

Associations among the studied variables

Univariate analysis by Spearman correlation test showed that sNOX2 was associated with serum LPS (Rs = 0.629; *P* <0.001), serum zonulin (Rs = 0.641; *P* <0.001), H_2O_2 (Rs = 0.590; *P* <0.01), and ET-1 (Rs = 0.410; *P* <0.001). Furthermore, an inverse correlation between sNOX2 and HBA (Rs = –0.646; *P* <0.001) and nitric oxide bioavailability (Rs = –0.312; *P* <0.01) was observed. LPS was also associated with serum ET-1 (Rs = 0.243; *P* = 0.03), zonulin (Rs = 0.474; *P* <0.001), and oxidative stress, as shown by its correlation with H_2O_2 (Rs = 0.490; *P* <0.001) and HBA (Rs = –0.560; *P* <0.001).

Multiple linear regression analysis, adjusted for statins, beta-blockers, calcium antagonists, diabetes and hyperten-

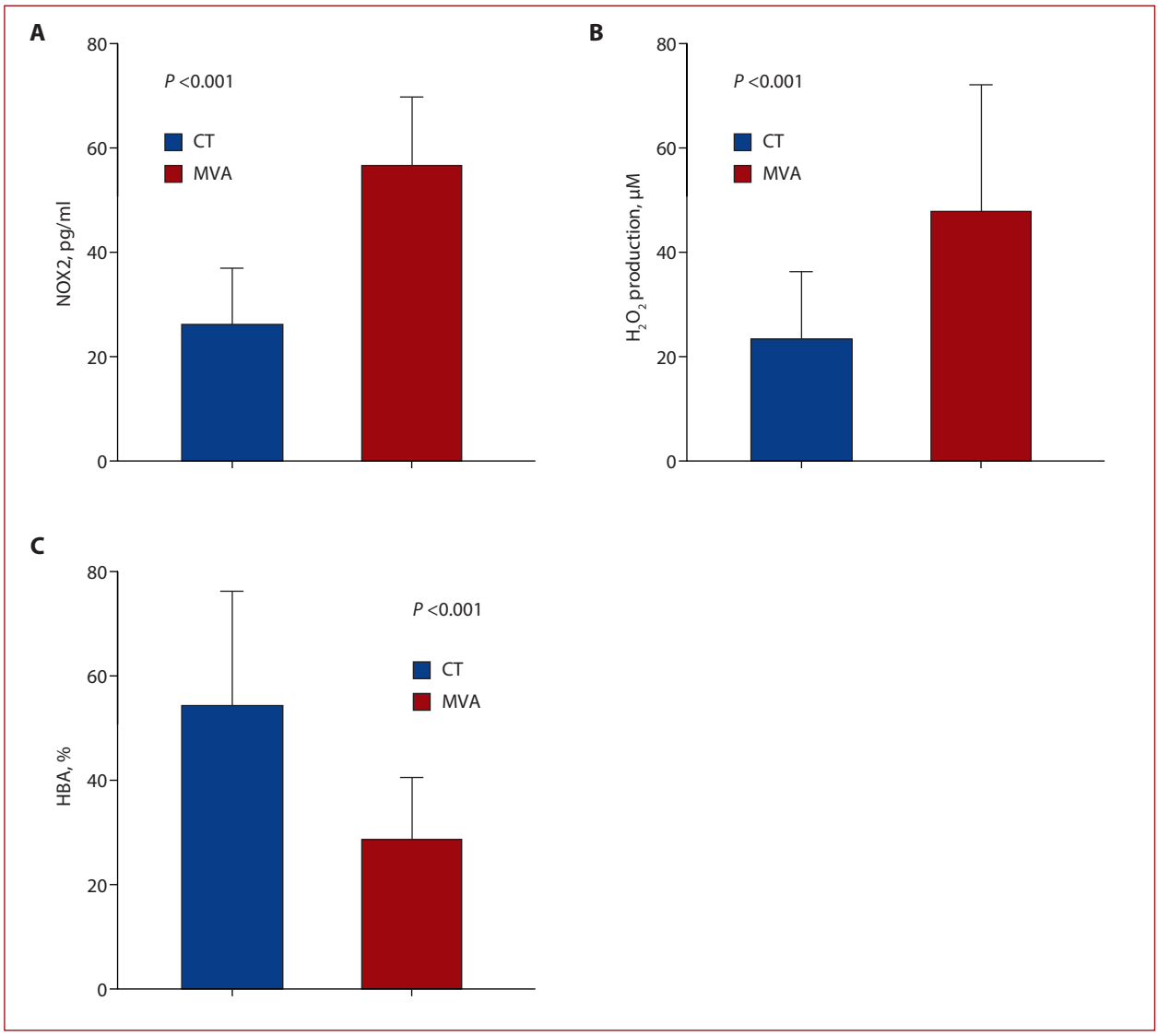

Figure 2. Oxidative stress production: serum sNOX2-dp levels (**A**), serum H_2O_2 production (**B**), and serum hydrogen peroxide breakdown activity (**C**) in microvascular angina (MVA) patients (n = 40) and controls (CT) (n = 40)

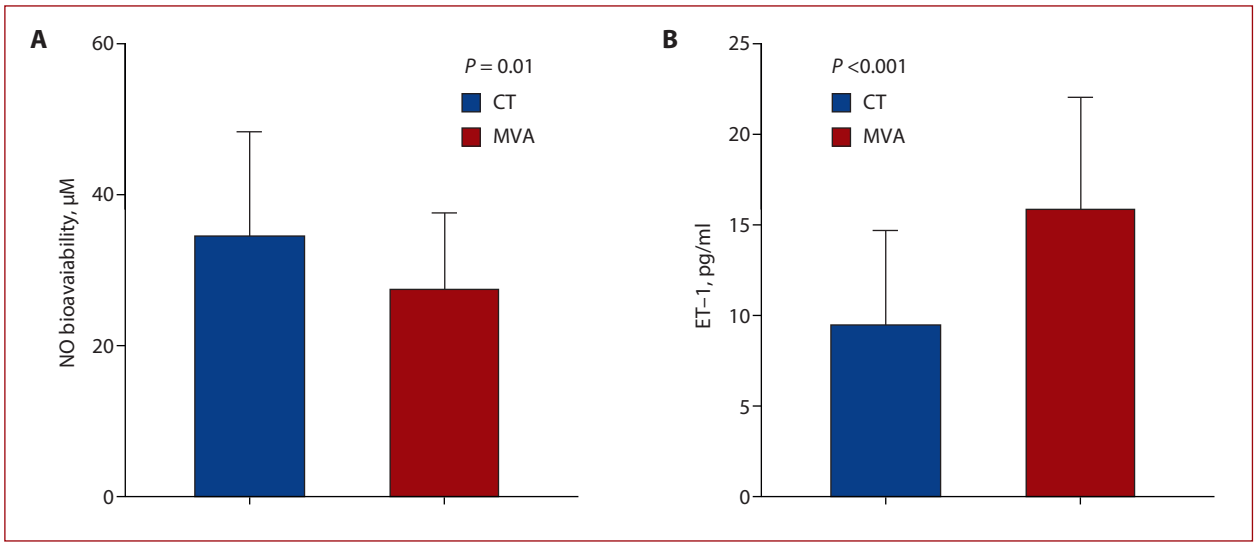

Figure 3. Endothelial dysfunction evaluation: serum NO bioavailability (**A**) and serum levels of endhotelin 1 (ET-1) (**B**) in microvascular angina (MVA) patients (n = 40) and controls (CT) (n = 40)

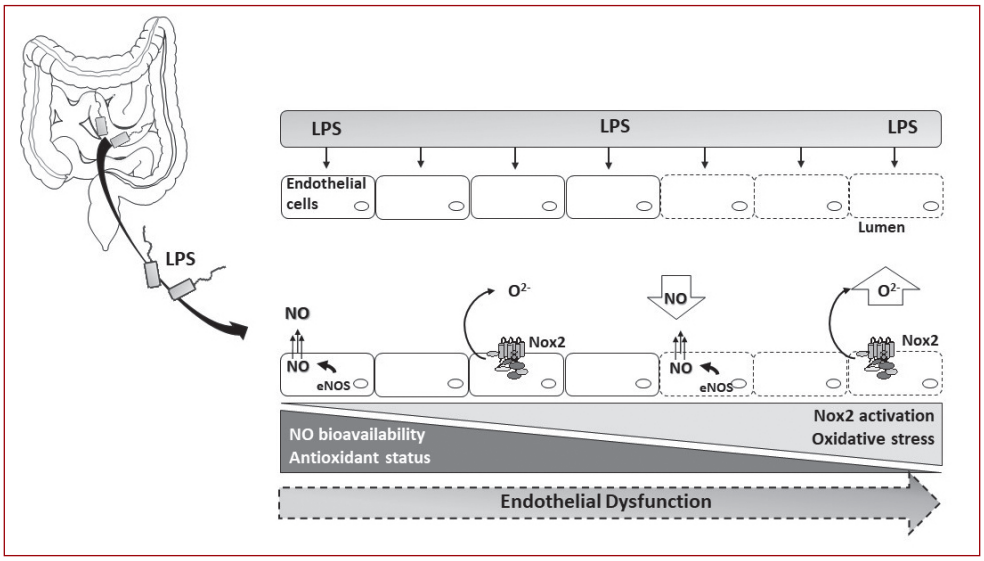

Figure 4. Low-grade endotoxemia could trigger activating NOX2, which increases oxidative stress and decreases antioxidant status and nitric oxide bioavailability, causing endothelial dysfunction in patients with microvascular angina (MVA)

sion, showed that LPS (SE, 0.129; standardized coefficient β, 0.335; P =0.001) and zonulin (standardized coefficient β, 0.279; SE, 1.828; P =0.006) emerged as the only independent predictive variables associated with sNOX2 (R^2 = 61%). Furthermore, sNOX2 (standardized coefficient β, 0.469; SE, 0.099; P = 0.001) and HBA (standardized coefficient β, –0.258; SE, 0.082; P = 0.04) were the independent predictive variables associated with LPS (R^2 = 44%).

DISCUSSION

The study shows for the first time that MVA patients have increased LPS serum levels. To find if gut permeability may account for LPS increase in patients with MVA, we measured the circulating levels of zonulin, which modulates gut permeability by disassembling the intercellular tight junctions [24]. Experimental and clinical studies demonstrated that zonulin up-regulation increases gut permeability [25]. The increased serum levels of zonulin in patients with MVA and its correlation with serum LPS could provide evidence that gut permeability is enhanced in this cohort and may be responsible for the high circulating levels of LPS.

LPS is a pro-inflammatory molecule that may favor endothelial dysfunction with an oxidative stress-mediated mechanism. In particular, LPS binds to TLR4 complex and activates a series of proteins and kinases in endothelial cells (as nuclear factor-kappa B [NF-κB]), interleukin 1 receptor-associated kinase (IRAK), tumor necrosis factor receptor-associated factor 6, NF-kB-inducing kinase, and inhibitor kappa B, which increase the production of proinflammatory molecules (as cytokines/chemokines) [26].

In accordance with this, LPS directly correlated with NOX2, suggesting a role for LPS as a trigger for oxidative stress [8]. The high levels of H_2O_2 and the close relation with sNOX2 observed in this study suggest that systemic oxidative stress in MVA could derive from LPS-induced NADPH oxidase activation as hypothesized by previous papers [8, 27].

NOX2 is considered an important modulator of artery vasodilation in endothelial cells; previous studies showed that NOX2 activation increases oxidative stress and impaired NO biosynthesis determining vasoconstriction [9, 11, 28].

No description of an association between LPS, NOX2, and endothelial dysfunction in MVA has been reported. Previously, in other settings, such as neurodegenerative diseases and neuropsychiatric disorders, associations among increased LPS, NOX2, oxidative stress, and endothelial dysfunction were reported [19, 29]. Experimental studies in animals showed that LPS could induce cardiac microvascular dysfunction [30]. Mice treated with LPS showed cardiac endothelial dysfunction and higher mortality that could be related to the loss of pericytes [30]. Other studies showed that LPS impaired endothelial dysfunction by reducing vasodilatory response to acetylcholine and eNOS phosphorylation [31, 32].

Our report supports and extends these previous findings in patients with MVA by demonstrating that NOX2 activation could increase oxidative stress and could determine endothelial dysfunction by reduction of NO bioavailability [33] and by increased ET-1 production. Reactive oxygen species generated by NOX2 activation could stimulate ET-1 secretion and provoke microvascular dysfunction [34]. In line with several previous studies, we found increased serum ET-1 levels in MVA patients [8, 35]; however, we cannot exclude that genetic dysregulation of ET-1 could be implicated [36].

The study has some limitations and implications. We did not perform all the invasive tests to evaluate the coronary function, including measurements of CFR, microvascular resistance, acetylcholine, or adenosine provocation

test. We did not evaluate other NADPH isoforms, such as NOX1 and NOX4, and other antioxidant systems such as catalase, SOD, and glutathione peroxidase that could also contribute to increased oxidative stress in MVA. The mechanism accounting for LPS translocation from the gut microbiota to systemic circulation was not addressed by the present study. However, changes in gut permeability might be a plausible mechanism as increased serum zonulin, which reflects enhanced gut permeability, is significantly correlated with blood LPS. Nevertheless, zonulin is an indirect marker of gut permeability, and we cannot exclude that serum LPS could also be derived from other sources, thereby a further study is necessary to elucidate this issue. Furthermore, some sources of chronic inflammation such as periodontitis [37] that may have affected LPS levels were not analyzed in this study.

Future studies are necessary to assess if improvement of gut permeability and eventually lowering of low-grade endotoxemia improve artery dysfunction in MVA.

In conclusion, this study provides the first report attesting that patients with MVA have high LPS levels, NOX2 activation, and an imbalance between pro-oxidant and antioxidant systems, in favor of the oxidizing molecules that could be potentially implicated in the endothelial dysfunction and vasoconstriction of this disease. These results could open new therapeutic strategies to modulate gut microbiota and oxidative stress to reduce the cardiovascular risk in MVA patients.

Article information

Conflict of interest: None declared.

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REFERENCES

1. Villano A, Lanza GA, Crea F, et al. Clinical spectrum and outcome of patients with non-st-segment elevation acute coronary syndrome and no obstructive coronary atherosclerosis. J Cardiovasc Med (Hagerstown). 2018; 19(Suppl. 1): e36–e39, doi: 10.2459/JCM.0000000000000638, indexed in Pubmed: 29538142.
2. Rakowski T, De Luca G, Siudak Z, et al. Characteristics of patients presenting with myocardial infarction with non-obstructive coronary arteries (MINOCA) in Poland: data from the ORPKI national registry. J Thromb Thrombolysis. 2019; 47(3): 462–466, doi: 10.1007/s11239-018-1794-z, indexed in Pubmed: 30565147.
3. Schindler TH, Dilsizian V. Coronary microvascular dysfunction: clinical considerations and noninvasive diagnosis. JACC Cardiovasc Imaging. 2020; 13(1 Pt 1): 140–155, doi: 10.1016/j.jcmg.2018.11.036, indexed in Pubmed: 30982670.
4. Chen C, Wei J, AlBadri A, et al. Coronary microvascular dysfunction — epidemiology, pathogenesis, prognosis, diagnosis, risk factors and therapy. Circ J. 2016; 81(1): 3–11, doi: 10.1253/circj.CJ-16-1002, indexed in Pubmed: 27904032.
5. Vancheri F, Longo G, Vancheri S, et al. Coronary microvascular dysfunction. J Clin Med. 2020; 9(9), doi: 10.3390/jcm9092880, indexed in Pubmed: 32899944.
6. Taqueti VR, Di Carli MF. Coronary microvascular disease pathogenic mechanisms and therapeutic options: JACC state-of-the-art review. J Am Coll Cardiol. 2018; 72(21): 2625–2641, doi: 10.1016/j.jacc.2018.09.042, indexed in Pubmed: 30466521.
7. Jansen TPJ, Konst RE, Elias-Smale SE, et al. Assessing microvascular dysfunction in angina with unobstructed coronary arteries: JACC review topic of the week. J Am Coll Cardiol. 2021; 78(14): 1471–1479, doi: 10.1016/j.jacc.2021.08.028, indexed in Pubmed: 34593129.
8. Sucato V, Corrado E, Manno G, et al. Biomarkers of coronary microvascular dysfunction in patients with microvascular angina: a narrative review. Angiology. 2022; 73(5): 395–406, doi: 10.1177/00033197211034267, indexed in Pubmed: 34338554.
9. Violi F, Sanguigni V, Carnevale R, et al. Hereditary deficiency of gp91(phox) is associated with enhanced arterial dilatation: results of a multicenter study. Circulation. 2009; 120(16): 1616–1622, doi: 10.1161/CIRCULATIONAHA.109.877191, indexed in Pubmed: 19805647.
10. Violi F, Carnevale R, Loffredo L, et al. NADPH oxidase-2 and atherothrombosis: insight from chronic granulomatous disease. Arterioscler Thromb Vasc Biol. 2017; 37(2): 218–225, doi: 10.1161/ATVBAHA.116.308351, indexed in Pubmed: 27932349.
11. Loffredo L, Carnevale R, Sanguigni V, et al. Does NADPH oxidase deficiency cause artery dilatation in humans? Antioxid Redox Signal. 2013; 18(12): 1491–1496, doi: 10.1089/ars.2012.4987, indexed in Pubmed: 23216310.
12. Violi F, Sanguigni V, Loffredo L, et al. Nox2 is determinant for ischemia-induced oxidative stress and arterial vasodilatation: a pilot study in patients with hereditary Nox2 deficiency. Arterioscler Thromb Vasc Biol. 2006; 26(8): e131–e132, doi: 10.1161/01.ATV.0000229710.13054.2d, indexed in Pubmed: 16857955.
13. Li Y, Pagano PJ. Microvascular NADPH oxidase in health and disease. Free Radic Biol Med. 2017; 109: 33–47, doi: 10.1016/j.freeradbiomed.2017.02.049, indexed in Pubmed: 28274817.
14. Grylls A, Seidler K, Neil J. Link between microbiota and hypertension: Focus on LPS/TLR4 pathway in endothelial dysfunction and vascular inflammation, and therapeutic implication of probiotics. Biomed Pharmacother. 2021; 137: 111334, doi: 10.1016/j.biopha.2021.111334, indexed in Pubmed: 33556874.
15. Stoll LL, Denning GM, Weintraub NL. Potential role of endotoxin as a proinflammatory mediator of atherosclerosis. Arterioscler Thromb Vasc Biol. 2004; 24(12): 2227–2236, doi: 10.1161/01.ATV.0000147534.69062.dc, indexed in Pubmed: 15472123.
16. Carnevale R, Nocella C, Petrozza V, et al. Localization of lipopolysaccharide from Escherichia Coli into human atherosclerotic plaque. Sci Rep. 2018; 8(1): 3598, doi: 10.1038/s41598-018-22076-4, indexed in Pubmed: 29483584.
17. Nocella C, Carnevale R, Bartimoccia S, et al. Lipopolysaccharide as trigger of platelet aggregation via eicosanoid over-production. Thromb Haemost. 2017; 117(8): 1558–1570, doi: 10.1160/TH16-11-0857, indexed in Pubmed: 28492699.
18. Cangemi R, Pignatelli P, Carnevale R, et al. Low-grade endotoxemia, gut permeability and platelet activation in community-acquired pneumonia. J Infect. 2016; 73(2): 107–114, doi: 10.1016/j.jinf.2016.05.013, indexed in Pubmed: 27288596.
19. Loffredo L, Ettorre E, Zicari AM, et al. Oxidative Stress and Gut-Derived Lipopolysaccharides in Neurodegenerative Disease: Role of NOX2. Oxid Med Cell Longev. 2020; 2020: 8630275, doi: 10.1155/2020/8630275, indexed in Pubmed: 32089785.
20. Loffredo L, Ivanov V, Ciobanu N, et al. Is there an association between atherosclerotic burden, oxidative stress, and gut-derived lipopolysaccharides? Antioxid Redox Signal. 2020 [Epub ahead of print], doi: 10.1089/ars.2020.8109, indexed in Pubmed: 32336107.
21. Ong P, Camici PG, Beltrame JF, et al. International standardization of diagnostic criteria for microvascular angina. Int J Cardiol. 2018; 250: 16–20, doi: 10.1016/j.ijcard.2017.08.068, indexed in Pubmed: 29031990.

22. Carnevale R, Silvestri R, Loffredo L, et al. Oleuropein, a component of extra virgin olive oil, lowers postprandial glycaemia in healthy subjects. Br J Clin Pharmacol. 2018; 84(7): 1566–1574, doi: 10.1111/bcp.13589, indexed in Pubmed: 29577365.
23. Loffredo L, Carnevale R, Cangemi R, et al. NOX2 up-regulation is associated with artery dysfunction in patients with peripheral artery disease. Int J Cardiol. 2013; 165(1): 184–192, doi: 10.1016/j.ijcard.2012.01.069, indexed in Pubmed: 22336250.
24. Fasano A, Not T, Wang W, et al. Zonulin, a newly discovered modulator of intestinal permeability, and its expression in coeliac disease. Lancet. 2000; 355(9214): 1518–1519, doi: 10.1016/S0140-6736(00)02169-3, indexed in Pubmed: 10801176.
25. Sapone A, de Magistris L, Pietzak M, et al. Zonulin upregulation is associated with increased gut permeability in subjects with type 1 diabetes and their relatives. Diabetes. 2006; 55(5): 1443–1449, doi: 10.2337/db05-1593, indexed in Pubmed: 16644703.
26. Bowman JD, Surani S, Horseman MA. Endotoxin, Toll-like Receptor-4, and Atherosclerotic Heart Disease. Curr Cardiol Rev. 2017; 13(2): 86–93, doi: 10.2174/1573403X12666160901145313, indexed in Pubmed: 27586023.
27. Konst RE, Guzik TJ, Kaski JC, et al. The pathogenic role of coronary microvascular dysfunction in the setting of other cardiac or systemic conditions. Cardiovasc Res. 2020; 116(4): 817–828, doi: 10.1093/cvr/cvaa009, indexed in Pubmed: 31977015.
28. Violi F, Pignatelli P, Pignata C, et al. Reduced atherosclerotic burden in subjects with genetically determined low oxidative stress. Arterioscler Thromb Vasc Biol. 2013; 33(2): 406–412, doi: 10.1161/ATVBAHA.112.300438, indexed in Pubmed: 23288160.
29. Loffredo L, Spalice A, Salvatori F, et al. Oxidative stress and gut-derived lipopolysaccharides in children affected by paediatric autoimmune neuropsychiatric disorders associated with streptococcal infections. BMC Pediatr. 2020; 20(1): 127, doi: 10.1186/s12887-020-02026-8, indexed in Pubmed: 32188439.
30. Zeng H, He X, Tuo QH, et al. LPS causes pericyte loss and microvascular dysfunction via disruption of Sirt3/angiopoietins/Tie-2 and HIF-2α/Notch3 pathways. Sci Rep. 2016; 6: 20931, doi: 10.1038/srep20931, indexed in Pubmed: 26868537.
31. La Mura V, Pasarín M, Rodriguez-Vilarrupla A, et al. Liver sinusoidal endothelial dysfunction after LPS administration: a role for inducible-nitric oxide synthase. J Hepatol. 2014; 61(6): 1321–1327, doi: 10.1016/j.jhep.2014.07.014, indexed in Pubmed: 25038487.
32. La Mura V, Pasarín M, Meireles CZ, et al. Effects of simvastatin administration on rodents with lipopolysaccharide-induced liver microvascular dysfunction. Hepatology. 2013; 57(3): 1172–1181, doi: 10.1002/hep.26127, indexed in Pubmed: 23184571.
33. Paolocci N, Biondi R, Bettini M, et al. Oxygen radical-mediated reduction in basal and agonist-evoked NO release in isolated rat heart. J Mol Cell Cardiol. 2001; 33(4): 671–679, doi: 10.1006/jmcc.2000.1334, indexed in Pubmed: 11341236.
34. Dammanahalli KJ, Sun Z. Endothelins and NADPH oxidases in the cardiovascular system. Clin Exp Pharmacol Physiol. 2008; 35(1): 2–6, doi: 10.1111/j.1440-1681.2007.04830.x, indexed in Pubmed: 18047620.
35. Monti LD, Piatti PM. Role of endothelial dysfunction and insulin resistance in angina pectoris and normal coronary angiogram. Herz. 2005; 30(1): 48–54, doi: 10.1007/s00059-005-2638-0, indexed in Pubmed: 15754155.
36. Ford TJ, Corcoran D, Padmanabhan S, et al. Genetic dysregulation of endothelin-1 is implicated in coronary microvascular dysfunction. Eur Heart J. 2020; 41(34): 3239–3252, doi: 10.1093/eurheartj/ehz915, indexed in Pubmed: 31972008.
37. Szczepaniak P, Mikołajczyk TP, Cześnikiewicz-Guzik M, et al. Periodontitis as an inflammatory trigger in hypertension: From basic immunology to clinical implications. Kardiol Pol. 2021; 79(11): 1206–1214, doi: 10.33963/KP.a2021.0161, indexed in Pubmed: 34847238.