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Association of Low Ficolin–Lectin Pathway Parameters with Cardiac Syndrome X

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Abstract

In patients with typical angina pectoris, inducible myocardial ischaemia and macroscopically normal coronaries (cardiac syndrome X (CSX)), a significantly elevated plasma level of terminal complement complex (TCC), the common end product of complement activation, has been observed without accompanying activation of the classical or the alternative pathways. Therefore, our aim was to clarify the role of the ficolin–lectin pathway in CSX. Eighteen patients with CSX, 37 stable angina patients with significant coronary stenosis (CHD) and 54 healthy volunteers (HC) were enrolled. Serum levels of ficolin-2 and ficolin-3, ficolin-3/MASP-2 complex and ficolin-3-mediated TCC deposition (FCN3-TCC) were determined. Plasma level of TCC was significantly higher in the CSX than in the HC or CHD group (5.45 versus 1.30 versus 2.04 AU/ml, $P < 0.001$). Serum levels of ficolin-2 and ficolin-3 were significantly lower in the CSX compared to the HC or CHD group (3.60 versus 5.80 or 5.20 μg/ml, $P < 0.05$; 17.80 versus 24.10 or 26.80 μg/ml, $P < 0.05$). The ficolin-3/MASP-2 complex was significantly lower in the CSX group compared to the HC group (92.90 versus 144.90 AU/ml, $P = 0.006$). FCN3-TCC deposition was significantly lower in the CSX group compared to the HC and CHD groups (67.8% versus 143.3% or 159.7%, $P < 0.05$). In the CSX group, a significant correlation was found between TCC and FCN3-TCC level ($r = 0.507$, $P = 0.032$) and between ficolin-3/MASP-2 complex level and FCN3-TCC deposition ($r = 0.651$, $P = 0.003$). In conclusion, in patients with typical angina and myocardial ischaemia despite macroscopically normal coronary arteries, low levels of several lectin pathway parameters were observed, indicating complement activation and consumption. Complement activation through the ficolin–lectin pathway might play a role in the complex pathomechanism of CSX.

Introduction

Our understanding of coronary syndromes has evolved in the last two decades from the paradigm of obstructive atherosclerosis of epicardial coronary arteries into the complex concept of anatomo-functional abnormalities of coronary microcirculation. Under normal physiological conditions, the coronary microcirculation regulates myocardial perfusion in response to increased demand by endothelial-dependent and endothelial-independent mechanisms [1].

Patients with typical angina pectoris and inducible myocardial ischaemia, but with macroscopically healthy coronaries has became commonly known as 'cardiac syndrome X' (CSX, with incidence of 19% in men and up to 48% in women) [2, 3]. As an underlying pathomechanism, microvascular angina characterized by reduced coronary microvascular dilatory responses and increased coronary resistance has been suggested and consistently found in patients with CSX [4]. In stable angina patients with proven myocardial ischaemia and normal coronary arteries, significantly increased risks of future major adverse cardiac events and all-cause mortality were found compared to a normal population without ischaemic heart disease, even after adjusting for traditional cardiac risk factors [3, 4]. Although obvious explanation – namely endothelial dysfunction and other microvascular abnormalities in both coronary and peripheral arteries – has been suggested a decade ago, the complex pathomechanism has remained undetermined [5–8]. Taken together, stable angina patients with normal coronary arteries should be recognized as a unique clinical entity [9].

SCANDINAVIAN JOURNAL OF Immunology AN INTERNATIONAL MONTHLY JOURNAL

Data about the role of complement system in angina patients with macroscopically normal coronary arteries are lacking. As previously published [10], the level of terminal soluble C5b-9 complex (TCC) was increased in plasma of this patient group compared to those with angiographically proven coronary atherosclerosis. In these patients, despite the lacking morphologically evident atherosclerotic lesions, complement system activation seems to be present.

The complement system can be activated *via* three different routes, namely by the classical, the alternative or the lectin pathway [11, 12]. The lectin pathway is triggered by the binding of mannose-binding lectin (MBL) or ficolins to special carbohydrate structures on the surface of micro-organisms, apoptotic cells or altered-self structures [13]. In humans, five initiation molecules of the lectin pathway have been described: MBL, the recently recognized ficolin-1 (M-ficolin), ficolin-2 (L-ficolin), ficolin-3 (H-ficolin or Hakata antigen) and lately also collectin-11 [14–16]. While ficolin-1 can be predominantly found intracellularly in leucocytes, ficolin-2 and ficolin-3 can be found mainly in serum [17]. MBL and ficolins in serum are complexed with MBL/ficolin-associated serine proteases (MASPs – i.e. MASP-1, MASP-2 or MASP-3) and their truncated proteins (sMAP and MAP-1) [16, 18, 19]. The initiation of the lectin pathway finally results in the formation of terminal pathway activation complex (TCC or C5b-9), similar to the previously mentioned classical or alternative pathways. Importantly, MASPs can activate C4 and C2 leading to the generation of C3 convertase (C4b2b) of the complement classical and lectin pathways [13, 19].

As we published previously [10], the high C1rC1sC1-INH level (observed in patients with significant coronary atherosclerosis) was an independent biomarker of ischaemic heart disease. In this study, the level of TCC was increased in patients with macroscopically healthy coronary arteries compared to those with angiographically proven coronary atherosclerosis. As no parallel activation of the classical or the alternative complement pathway was observed in this study, our findings indicated the potential involvement of the lectin pathway in the CSX patient population. Based on these results, our aim was to clarify the role of lectin pathway activation and ficolins in the CSX patient cohort.

Materials and methods

Patient population. In this case–control study, we enrolled 55 patients scheduled for elective coronary angiography at our institution with diagnosis of stable angina pectoris. In each patient, non-invasive tests (exercise stress test or myocardial perfusion scan) were performed before coronary angiography and were positive for inducible myocardial ischaemia. The coronary angiography showed macroscopically normal coronary arteries, despite the history of typical angina and positive ischaemia provocation non-invasive tests in 18 patients (CSX group). In 37 patients, angiography indicated significant coronary artery stenosis (coronary heart disease: CHD group). These patients were referred to percutaneous coronary intervention or to coronary artery bypass graft (CABG) surgery or were advised for conservative therapy. The cohort of patients with stable angina was examined in a previous study, determining the classical and alternative pathways of complement activation [10]. Fifty-four healthy volunteers served as controls (HC group).

Blood samples. In each patient, 8 ml of venous blood was drawn from the cubital vein into serum-separating tubes before coronary angiography. Peripheral blood samples were drawn from healthy subjects similarly. The serum was separated by centrifugation at 3000 rpm for 10 min at room temperature. The samples were immediately frozen at −80 °C in aliquots and were thawed only before the measurement of lectin pathway parameters. Patient exclusion criteria were as follows: acute coronary syndrome, cardiogenic shock, history of severe renal or hepatic disease, haematological disorders, acute or chronic inflammatory disease and malignancy. The study protocol was approved by both the institutional review board of Semmelweis University of Budapest and the Hungarian Defence Forces Medical Centre. Informed consent was obtained in accordance with the Declaration of Helsinki.

Measurement of the lectin pathway parameters. The concentrations of ficolin-2 [20], ficolin-3 [21], the ficolin-3/MASP-2 complex [22], MAP-1 [23] and sC5b-9 (TCC) [10] were determined by previously described standard sandwich ELISA techniques, using monoclonal antibodies specific for each molecule. Biotinylated antibodies were added to the second layer, and streptavidin/HRP complexes were used for detection. All samples were tested in duplicate against a standard serum pool with known content of each analyte. Ficolin-3-mediated terminal complement complex deposition (FCN3-TCC) was measured as described previously by Hein *et al.* [24]. In brief, acetylated bovine serum albumin (acBSA) was immobilized in Maxisorp ELISA plates and used as a ficolin-3 ligand. To block any interference from the classical pathway or the alternative pathway [25], full serum samples were pre-incubated with sodium polyanethol sulphonate (SPS). Serum samples were diluted 1:25 in barbital buffer containing 0.05% Tween-20 (VBS-T) and incubated on the plate for 45 min at 37 °C. Thereafter, mouse anti-human TCC was applied for 2 h at room temperature, and then, rabbit anti-mouse HRP was added to the wells as secondary antibody, for 1 h at 37 °C. Finally, the plates were developed using OPD substrate, and the optical density was determined at 490/630 nm by ELISA reader (BioTek).

Statistical analysis. The statistical calculations were performed with PRISM for Windows v5.02 (GraphPad Software Inc., San Diego, CA, USA; www.graphpad.com)

and SPSS v13.0 (SPSS Inc., Chicago, IL, USA). As most of the variables were non-Gaussian, nonparametric tests were applied. Mann–Whitney *U*-test was used to compare two independent groups. Spearman rank correlation analysis was performed to analyse correlation between continuous variables. All the statistical analyses were two-tailed, and $P < 0.05$ was considered to represent a significant difference.

Results

Demographic data

Demographic data of the CSX and CHD populations are presented in Table 1. The 54 healthy volunteers (21 men and 33 women, median age: 33 years, 25–75th percentiles: 21–58 years) did not have any known disease. Within the healthy control group, age and BMI ($P < 0.0001$ for both comparisons) were significantly lower, compared to the CSX and CHD groups, respectively.

Both patient groups had increased presence of conventional cardiovascular risk factors, such as hypertension, type 2 diabetes mellitus, hyperlipidaemia, obesity, previous cardiovascular event and tobacco use. The majority of the patients received antiplatelet therapy, ACE inhibitor, beta-blocker, statin or nitrate. No significant difference was found regarding medical therapy upon admission between patient groups (Table 1). Compared to patients with CSX, a significantly higher incidence of previous myocardial infarction ($P = 0.003$, Fisher's exact test) and percutaneous coronary intervention ($P = 0.003$, Fisher's exact test) was observed in the CHD group. Interestingly, there were no significant differences in body mass index; both groups were in the 'overweight' category. The HDL-cholesterol level was significantly higher in patients with CSX, compared to patients with CHD ($P = 0.013$, Mann–Whitney *U*-test).

When comparing the laboratory parameters, we found no significant differences in kidney or liver function parameters between the patient groups.

Serum and plasma levels of lectin pathway components

The plasma level of TCC, the common end product of the complement activation pathways, was significantly higher in the CSX group compared to the CHD and HC groups (5.45 AU/ml versus 2.04 AU/ml, $P = 0.0001$; 5.45 AU/ml versus 1.30, $P < 0.0001$) (Table 2). These data represent a subgroup analysis of our previously published results [10].

The serum levels of ficolin-2 were significantly lower in the CSX group compared to the HC group (3.60 μg/ml versus 5.80 μg/ml, $P = 0.005$) and also lower compared to the CHD group (3.60 μg/ml versus 5.20 μg/ml, $P = 0.052$). Similarly, the serum levels of ficolin-3 were significantly lower in the CSX group compared to the HC group (17.80 μg/ml versus 24.10 μg/ml, $P = 0.035$) and CHD (17.80 μg/ml versus 26.80 μg/ml, $P = 0.016$) group, as well (Table 2).

The ficolin-3/MASP-2 complex was significantly lower in the CSX group compared to the HC group (92.90 AU/ml versus 144.90 AU/ml, $P = 0.006$). Interestingly, the ficolin-3/MASP-2 complex was also significantly lower in the CHD group compared to the HC group (87.0 AU/ml versus 144.90 AU/ml, $P = 0.011$).

The FCN3-TCC deposition was significantly lower in the CSX group compared to the HC group (67.8% versus 143.3%, $P = 0.008$) and the CHD (67.8% versus 159.7%, $P = 0.037$) group, as well. There were no significant differences in serum MAP-1 levels; however, the levels tended to be the lowest in the CSX group (CSX: 178.60 ng/ml, HC: 194.50 ng/ml, CHD: 212.40 ng/ml) (Table 2).

Correlations between serum lectin pathway parameters in the CSX group

We found a significant correlation between TCC and FCN3-TCC in the CSX group ($r = 0.507$, $P = 0.032$) (Fig. 1A). Similarly, the ficolin-3/MASP-2 complex level and FCN3-TCC deposition correlated significantly ($r = 0.651$, $P = 0.003$) (Fig. 1B).

Cardiovascular risk factors and serum lectin pathway components

When analysing the whole patient cohort ($n = 55$), a significantly higher TCC level (5.04 AU/ml versus 2.05 AU/ml, $P = 0.0002$) was observed in non-smoking individuals compared to smoking patients. Presence of hypertension or diabetes mellitus, similar to age, body mass index, kidney and liver function parameters and CRP, was not associated with the measured lectin pathway parameters.

Importantly, among cardiovascular risk factors, patients with hyperlipidaemia had significantly different levels of lectin pathway products. Ficolin-2, ficolin-3, ficolin-3/MASP-2 complex and FCN3-TCC deposition were significantly higher in hyperlipidaemic CHD patients (Table 3). Remarkably, this phenomenon was not observed in the CSX group (Table 3).

Further evaluating hyperlipidaemic patients, we analysed the serum lectin pathway parameters with respect to serum cholesterol and triglyceride levels. When Spearman rank test was performed, serum lectin pathway component levels showed no significant correlation with serum cholesterol values. In contrast, we found a significant correlation with ficolin-2, ficolin-3, ficolin-3/MASP-2 complex, FCN3-TCC deposition and MAP-1 levels and serum triglyceride levels in the CHD group (Table 4). It is

Table 1 Demographic parameters.

Demographic data	CHD (n = 37)	CSX (n = 18)	HC (n = 54)
Age, mean ± SD, years	61.44 ± 8.69	61.08 ± 8.81	35.00 ± 11.25[a]
Males/females, n (%)	32 (86.4)/5 (13.6)	9 (50)/9 (50)	21 (39)/33 (61)[c]
BMI, mean ± SD, kg/m^2	29.06 ± 5.61	28.56 ± 4.73	23.69 ± 4.60[a]
Risk factors			
History of tobacco use, n (%)	28 (75.6)	8 (44.4)	6 (11.1)[a]
Hypertension, n (%)	32 (86.4)	14 (77.7)	5 (9.26)[a]
Diabetes mellitus, n (%)	17 (45.9)	3 (16.6)	0 (0)[a]
Hyperlipidaemia, n (%)	17 (45.9)	7 (38.8)	2 (3.70)[a]
Previous AMI, n (%)	18 (48.6)[b]	0 (0)	0 (0)
Previous PCI, n (%)	19 (51.3)[b]	0 (0)	0 (0)
Previous CABG, n (%)	6 (16.2)[b]	0 (0)	0 (0)
Medication on admission			
Aspirin, n (%)	26 (70.27)	9 (50)	2 (3.70)[a]
Clopidogrel, n (%)	25 (67.6)	5 (27.7)	0 (0)[a]
ACE inhibitors, n (%)	31 (83.7)	11 (29.7)	1 (1.85)[a]
Beta-blockers, n (%)	34 (91.9)	15 (83.3)	1 (1.85)[a]
Lipid-lowering agents, n (%)	32 (86.4)	9 (50)	0 (0)[a]
Nitroglycerine/nitrates, n (%)	9 (24.3)	5 (27.7)	0 (0)[a]
Laboratory parameters			
CK, mean ± SD (U/l)	136.94 ± 58.63	101.40 ± 44.40	n.m.
LDH, mean ± SD (U/l)	335.23 ± 63.93	268.36 ± 139.51	n.m.
Cholesterol, mean ± SD (mmol/l)	4.15 ± 1.03	4.66 ± 1.31	4.84 ± 0.88[c]
HDL-cholesterol, mean ± SD (mmol/l)	0.98 ± 0.20[b]	1.29 ± 0.41	1.55 ± 0.59
LDL-cholesterol, mean ± SD (mmol/l)	2.44 ± 0.90	2.53 ± 0.94	2.57 ± 0.96
Triglycerides, mean ± SD (mmol/l)	1.58 ± 0.71	1.56 ± 0.72	0.95 ± 1.01[c]
GOT, mean ± SD (U/l)	25.75 ± 11.29	31.18 ± 19.45[d]	20.00 ± 5.46
Creatinine, mean ± SD (μmol/l)	74.87 ± 14.70	78.83 ± 21.23	67.00 ± 12.29[c]
CN, mean ± SD (mmol/l)	5.66 ± 1.46	6.66 ± 2.42	4.40 ± 1.02[a]
CRP, mean ± SD (mg/l)	3.16 ± 2.23	2.49 ± 2.83	n.m.
Fructosamine, mean ± SD (μmol/l)	233.82 ± 41.96	236.88 ± 32.57	n.m.

SD, standard deviation; CHD, patients with coronary heart disease; CSX, patients with negative coronary angiography; HC, healthy control individuals; BMI, body mass index; CK, creatine kinase; LDH, lactate dehydrogenase; AST, aspartate aminotransferase; CN, carbamide; n.m., not measured.
The values in parentheses represent percentages. Values are means ± SD or n (%).
Fisher's exact test for the categorical variables and Mann–Whitney test for the continuous variables. Individuals in the HC group received ASA and statin for primary prevention of cardiovascular events.
[a]Significant differences in HC group compared to CSX and CHD groups, $P < 0.01$ respectively.
[b]Significant differences in CHD group compared to CSX and HC groups, $P < 0.05$ respectively.
[c]Significant differences between CHD versus HC group, $P < 0.05$.
[d]Significant differences between CSX versus HC group, $P < 0.05$.

Table 2 Serum lectin pathway parameter levels in the patient groups (CHD and CSX) and healthy controls (HC).

	CHD	CSX	HC	P value
TCC, AU/ml	2.04 (1.54–2.46)	5.45 (3.71–7.62)	1.30 (0.93–2.29)	0.0001[a] <0.0001[b]
Ficolin-2, μg/ml	5.20 (3.50–7.70)	3.60 (3.20–4.70)	5.80 (4.20–8.0)	0.052[a] 0.005[b]
Ficolin-3, μg/ml	26.80 (18.30–37.10)	17.80 (12.90–25.30)	24.10 (18.20–30.10)	0.016[a] 0.035[b]
MAP-1, ng/ml	212.40 (169.80–237.60)	178.60 (123.40–253.60)	194.50 (149.60–279.50)	NS
Ficolin-3/MASP-2 complex, AU/ml	87.0 (54.90–188.60)	92.90 (39.40–121.70)	144.90 (89.80–336.20)	0.506[a] 0.006[b]
Ficolin-3-mediated TCC deposition, %	159.70 (72.70–203.0)	67.80 (27.80–129.0)	143.30 (91.90–195.30)	0.037[a] 0.008[b]

CHD, patients with coronary heart disease; CSX, patients with cardiac syndrome X.
The values in parentheses represent 25–75th percentile. Values are given as medians.
[a]CHD versus CSX.
[b]CSX versus HC.

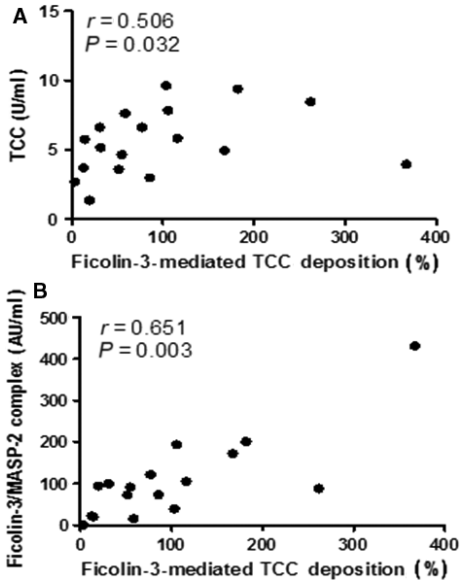

Figure 1 Correlations between serum lectin pathway components in the CSX group. (A) Correlation between TCC and FCN3-TCC in the CSX group. (B) Correlation between ficolin-3/MASP-2 complex level and FCN3-TCC deposition.

Table 3 Serum lectin pathway component levels according to the presence/absence of hyperlipidaemia.

	CHD HL +/HL −	CSX HL +/HL −
C5b-9, AU/ml	2.05/2.03	5.80/5.10
Ficolin-3-mediated TCC deposition, %	170.50/81.60 (**0.003**)	77.30/51.60
Ficolin-3/MASP-2 complex, AU/ml	132.60/72.02 (**0.013**)	94.40/88.0
Ficolin-2 μg/ml	6.90/4.60 (**0.047**)	3.50/3.60
Ficolin-3 μg/ml	34.30/18.40 (**0.0001**)	19.0/16.70
MAP-1 ng/ml	227.90/207.70	185.0/171.0

HL, hyperlipidaemia; CHD, patients with coronary heart disease; CSX, patients with cardiac syndrome X.
The values in parentheses represent *P* value. Values are given as medians.

important to note that such correlations were absent in the CSX group.

Medical therapy and serum lectin pathway parameters

Intake of acetylsalicylic acid, ACE inhibitors, beta-blockers and lipid-lowering agents was not associated with the measured lectin pathway parameters (data not shown).

Patients on chronic clopidogrel therapy had significantly higher ficolin-3/MASP-2 complex (121.67 AU/ml versus 73.50 AU/ml, $P = 0.015$) and ficolin-3 (30.40 μg/ml versus 16.60 μg/ml, $P = 0.006$) levels in the CSX subgroup, when compared to patients without such therapy. Similar phenomenon was not observed in the CHD group.

Table 4 Correlation between serum lectin pathway parameter levels and serum triglyceride levels in different patient subgroups.

Correlation between serum triglyceride levels and:	CHD	CSX
sC5b-9	$R = 0.086/P = 0.691$	$R = -0.414/P = 0.098$
Ficolin-3-mediated TCC deposition	$\mathbf{R = 0.424/P = 0.043}$	$R = -0.098/P = 0.737$
Ficolin-3/MASP-2 complex	$\mathbf{R = 0.426/P = 0.042}$	$R = -0.143/P = 0.626$
Ficolin-2	$\mathbf{R = 0.528/P = 0.009}$	$R = 0.055/P = 0.851$
Ficolin-3	$\mathbf{R = 0.512/P = 0.012}$	$R = -0.200/P = 0.493$
MAP-1	$\mathbf{R = 0.449/P = 0.031}$	$R = 0.402/P = 0.154$

CHD, patients with coronary heart disease; CSX, patients with cardiac syndrome X.
Values represent the results of Spearman rank correlation analysis.

Discussion

Cardiac syndrome X remains a major diagnostic and therapeutic challenge causing significant deterioration in patient's functioning and quality of life. Although substantial data report microvascular and endothelial dysfunction within this patient group, the complex pathomechanism is still unclear.

In the present study, we have demonstrated consumption of multiple parameters along the ficolin–lectin pathway. We observed significantly lower serum levels of ficolin-2, ficolin-3, ficolin-3/MASP-2 complex and FCN3-TCC deposition accompanied by significantly higher TCC level in patients with CSX compared to healthy controls and to patients with angiographically proven coronary heart disease. Furthermore, we found significant correlations between TCC and FCN3-TCC deposition and between ficolin-3/MASP-2 complex level and FCN3-TCC deposition.

According to these results, in the group of patients with CSX, consumption and activation of the ficolin–lectin pathway are present, best marked by low levels of ficolin-2 and ficolin-3. We consider lower ficolin-3/MASP-2 complex levels as a consequence of the decreased level of the ficolin-3 component. Measurement of the FCN3-TCC, where the serum is activated with a ficolin-3 specific activator agent and the induced TCC formation is analysed, is indicative of the remaining activity of the pathway. Decreased ficolin-3-mediated TCC deposition is therefore a sign of prior *in vivo* activation and consumption of the ficolin–lectin pathway.

Lectin pathway activation appears to have a controversial role in the cardiovascular system. While several studies pointed to a beneficial effect of the lectin pathway activation resulting in antiatherosclerotic effect [26, 27], others showed adverse cardiovascular effects of high MBL plasma concentration [28]. MASPs were associated with cardiovascular risk factors including

dyslipidaemia, obesity and hypertension in patients with stable coronary artery disease [29]. Furthermore, MASP-2 plasma level was lower in myocardial infarction and stroke patients compared to patients with stable coronary artery disease [29]. In a magnetic resonance study, ficolin-2, MBL and MAP-1 were associated with left ventricle dilatation after myocardial infarction, indicating a potential role for lectin pathway products in myocardial remodelling [30].

Although our results suggest changes in lectin pathway complement cascade in patients with CSX, our data cannot verify the causality between the measured parameters and pathogenesis of CSX. There is a general agreement that the main pathological feature in the majority of patients with CSX is microvascular/endothelial dysfunction [31]. *Dollard et al.* [32] demonstrated that C-reactive protein (CRP) remains significantly higher in patients with CSX who remain symptomatic than in either healthy controls. Furthermore, coronary endothelial dysfunction may be associated with an increased release of constricting factors and the production of pro-inflammatory cytokines, cell adhesion molecules and growth factors (i.e. intracellular cell adhesion molecule-1 and vascular cell adhesion molecule-1). Inflammatory and proliferative changes in the vessel wall might cause arteriole hyperplasia and perivascular fibrosis leading to microvascular dysfunction [33–35].

Several members of the complement system were found to be deposited in the microvascular system of patients with diabetes (glomeruli and glomerular capillaries [36], retinal vessels [37] and choriocapillaris [38]). Based on our findings, namely the significantly lower serum levels of ficolin-2 and ficolin-3 and the ficolin-3/MASP-2 complex, we hypothesize that lectin pathway product deposition might also occur in the subendothelial matrix and contribute to microvascular dysfunction. Besides, these activated molecules might bind to pathological structures on the surface of endothelial cells. It was described that ficolins recognize acetyl-group-containing substances including N-acetylglucosamine [39, 40] and glucan [41]. Furthermore, in addition to sugars, they also could bind to substances such as elastin and DNA [18]. An intact endothelium is a fully biocompatible surface that is not recognized by the complement system. However, blood contact with a damaged endothelium will lead to a certain degree of activation of the complement system [42].

The end product of the complement activation, composed of components C5b, C6, C7, C8 and C9, called terminal complement complex C5b-9 (TCC), or 'membrane attack complex' (MAC). TCC facilitates the killing of bacteria and other pathogens by altering the permeability of their membranes. TCC was initially considered to be involved mainly in cytolytic processes of either pathogenesis or host cells [11]. Endothelial cells represent a potential target of TCC, which exerts a number of non-cytolytic effects [43]. Hoffmeister *et al.* [44] described elevated C5b-9 levels in patients with stable angina compared to healthy individuals. Furthermore, in patients with acute coronary syndromes the C5b-9 levels were even higher [44]. Our data are in agreement with these observations; TCC levels were lowest in healthy controls and significantly elevated in the CHD group. Interestingly, TCC levels were the highest in patients with CSX. Excess amount of this membrane attack complex might have a direct cytolytic effect on endothelial cells, contributing to the well-documented above-described endothelial dysfunction in these patients.

There are several potential triggers of microvascular dysfunction, including many of the conventional cardiac risk factors such as hypertension, dyslipidaemia and smoking [45]. Triglyceride levels correlated significantly with all the lectin pathway components in patients with CHD but not in patients with CSX. One can speculate that the well-known relationship between high serum lipid levels, lipid accumulation and atherosclerotic plaques is absent in these patients.

Emerging evidence suggests that platelets may have an ability to interact with both the classical and alternative pathways of complement activation [46]. Our results indicate that medical therapy with the platelet ADP receptor inhibitor clopidogrel was associated with the lack of ficolin–lectin pathway consumption in patients with CSX. Clopidogrel has a pleiotropic, anti-inflammatory and immune response/modulating effect and might influence the lectin pathway of the complement system as well. However, low number of individuals on chronic clopidogrel therapy in the CSX cohort was inadequate to further analyse the complex effect.

MBL, similar to ficolins, is a pattern recognition molecule and is an upstream component of the lectin pathway complement cascade [11, 12]. High level of MBL was found in ST-elevation myocardial infarction patients with reduced left ventricular systolic function [47]. Elevated MBL level was shown to be a risk factor for future development of coronary heart disease in healthy men [28]. In contrast, in a large prospective study high MBL level was associated with decreased risk of myocardial infarction independently of other cardiovascular factors [48]. Furthermore, MBL deficiency-associated genotypes were linked to increased early incidence of myocardial infarction [49]. Taken together, the role of MBL in the development of cardiovascular disease and/or prognosis is controversial, and data regarding angina patients with macroscopically normal coronaries are completely missing. In the future, determination of the MBL level might be relevant in patients with cardiac syndrome X.

In summary, we demonstrate significantly lower serum levels of lectin pathway parameters, namely ficolin-2,

ficolin-3, ficolin-3/MASP-2 complex and FCN3-TCC deposition, and significantly higher TCC levels in patients with CSX compared to healthy controls or to patients with angiographically proven coronary heart disease. Low levels of several lectin pathway products might reflect upstream consumption and consequent, downstream terminal complement complex activation. As we hypothesized, complement activation might contribute to the increased cardiovascular risk of patients with CSX by promoting endothelial and microvascular dysfunction. The topic of complement system activation via the lectin pathway requires further evaluation in a larger patient cohort with cardiac syndrome X.

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Conflict of interest

Authors disclose any and all relationships that could be perceived as real or apparent conflict of interest.

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