



Clinical research

# Chronic inflammation and increased arterial stiffness in patients with cardiac syndrome X

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## KEYWORDS

Cardiac syndrome X;  
Inflammation;  
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Intima-media thickness;  
Arterial distensibility

**Aims** Endothelial dysfunction and subangiographic atheroma have been reported in patients with cardiac syndrome X (CSX) but little is known regarding chronic inflammation and reduced arterial distensibility as pathogenic mechanisms. We assessed whether markers of inflammation and arterial distensibility differ in CSX patients compared to control subjects.

**Methods and results** We studied 30 consecutive CSX patients (mean age  $57 \pm 6$  years, 25 women) and 30 healthy controls (mean age  $54 \pm 8$  years, 25 women). High sensitivity C-reactive protein (hs-CRP) levels were significantly higher in patients with CSX compared to controls ( $2.6 [1.7-4.5]$  vs  $1.5 [0.7-2.7]$  mg/l,  $P=0.02$ ). Hs-CRP levels correlated with carotid intima-media thickness (IMT) (Spearman's  $\rho=0.51$ ;  $P=0.013$ ). CSX patients also had significantly increased mean IMT values than controls ( $P<0.0001$ ). Arterial stiffness and elastic modulus were also significantly increased in CSX patients compared to control subjects ( $P=0.04$  and  $P=0.04$ , respectively). Distensibility tended to be lower in CSX patients than controls although this difference did not reach statistical significance.

**Conclusions** This study showed for the first time that compared to control subjects, patients with CSX have higher hs-CRP serum levels, increased mean common carotid artery IMT and increased arterial stiffness. The role of these abnormalities in the pathogenesis of CSX deserves investigation.

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## Introduction

Patients with cardiac syndrome X (CSX) have typical exertional chest pain associated with ST-segment depression despite normal coronary arteriograms.<sup>1,2</sup> Coronary endothelial dysfunction leading to microvascular angina<sup>3,4</sup> and impaired coronary flow reserve<sup>5,6</sup> have been proposed as pathogenetic mechanisms in CSX. The mechanisms responsible for endothelial dysfunction in CSX patients are not well understood but risk factors of

coronary artery disease (CAD) such as obesity, hypertension, hypercholesterolaemia and smoking, frequently present in these patients, may play a role. Moreover, plasma levels of circulating intercellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule-1 (VCAM-1) are increased in patients with syndrome X,<sup>7</sup> suggesting endothelial cell activation. Also, subangiographic atheroma has been reported in CSX patients and found to be associated with hypercholesterolaemia and hypertension.<sup>8</sup> C-reactive protein (CRP) is a marker of chronic inflammation and a predictor of vascular disease,<sup>9</sup> which has been found to correlate with disease activity in CSX.<sup>10</sup> Little is known, however, regarding the relationship between chronic inflammation and elasticity parameters in patients with

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CSX. We therefore sought to investigate whether CRP levels and carotid artery stiffness differ in CSX patients compared to apparently normal subjects (controls).<sup>11–15</sup>

## Methods

### Patients

Patients with CSX and apparently healthy controls were studied. The case group consisted of 30 consecutive patients (mean age  $57 \pm 6$  years, 25 women) referred for assessment to St. George's Hospital Chest Pain and Normal Coronary Arteries Clinic. Entry criteria were typical anginal chest pain, normal 12-lead ECGs at rest, a positive exercise ECG stress test response ( $>0.1$  mV ST-segment depression at 80 ms after the J point in two or more contiguous leads) and a completely normal coronary angiogram. Left ventricular dysfunction, valvular heart disease and myocardial hypertrophy were ruled out with M- and B-mode echocardiography. Coronary artery spasm was assessed by hyperventilation and ergonovine testing. Non-cardiac causes of chest pain, such as gastrointestinal and musculoskeletal disorders were also investigated and ruled out as appropriate. Patients with diabetes mellitus were not included. The control group consisted of 30 apparently healthy subjects; 25 women and five men aged between 40 and 70 years (mean  $54 \pm 8$  years) recruited among the medical and nursing staff in our institution. None of these subjects had a previous history of chest pain or acute or chronic diseases. Clinical examination and a resting ECG were normal and none of the control subjects were taking cardiac or non-cardiac medications. A standardized questionnaire was administered to obtain systematic information regarding conventional cardiovascular risk factors, including hyperlipidaemia, hypertension, smoking and family history. The study was approved by the local research ethics committee and all subjects gave written informed consent.

### Biochemical analysis and high sensitivity C-reactive protein measurements

Venous blood samples were obtained in patients and controls at study entry from a large antecubital vein. Blood was centrifuged immediately and serum aliquoted and stored at  $-80^\circ\text{C}$  until analysis. Lipid serum levels were measured immediately by routine methods. CRP measurements were performed on the COBAS Integra (Roche Diagnostics Limited, Lewes, East Sussex, U.K.) using the CRP-Latex assay in both the high sensitivity application (analytical range 0.2–12 mg/l) and the normal application (analytical range 2–160 mg/l). Analytical precision of the high sensitivity CRP-Latex assay was 7.6% at a level of 1.02 mg/l, 3.3% at 1.79 mg/l and 1.3% at a level of 4.36 mg/l. Samples outside the analytical range of the high sensitivity CRP-Latex assay were analysed by the CRP-Latex assay in the normal application. The analytical precision of the normal CRP-Latex assay was 2.4% at a level of 29.5 mg/l and 1.3% at a level of 113 mg/l.

### Ultrasound imaging

Patients and subjects were examined in the supine position (head turned  $45^\circ$ ) with a high resolution ATL (Advance Technology Laboratories) echocardiograph, HDI (high-definition imaging) 3000CV scanner, equipped with a 12.5 MHz linear array transducer. The scanner was connected to a vessel wall tracking system<sup>16,17</sup> for elasticity measurements. All scans were performed by the same trained physician who was blinded to the patients clinical condition. Blood pressure was recorded at

baseline prior to each study using an automated blood pressure recorder.

### Common carotid artery intima-media thickness (CCA-IMT)

The right and left common carotid arteries (CCA) were assessed in the antero-oblique direction. Intima-media thickness was defined as the distance between the leading edge of the luminal echo to the leading edge of the media/adventitia echo.<sup>18</sup> Common carotid artery intima-media thickness measurements were carried out in the artery far wall. Reference points for measurement of IMT were 0.5, 1, 1.5 and 2 cm from the carotid bifurcation, for both right and left CCA. Common carotid artery intima-media thickness values reported in this manuscript represent an average of these measurements. Video images were captured in telediastole of the cardiac cycle by electrocardiogram triggering. All ultrasonic examinations were stored on a super-VHS video system (Panasonic AG-MD830) for subsequent offline processing. The frozen video images were digitally captured on a personal computer using super-VHS frame grabber. Mean CCA-IMT measurements were performed off-line in a blinded fashion using a semi-automated imaging processing software. With this software the blood-intimal and the medial-adventitial borderline were automatically detected using a grey value-based edge detection algorithm combined with higher degree polynome fitting along these borderlines. Differences between these two borderlines were measured along a line orthogonal to the arterial wall. Single IMT values were obtained from pixel-to-pixel measurements on neighbouring lines perpendicular to the vertical line and then averaged and expressed as the mean CCA-IMT. To assess reproducibility of measurements, intra-observer variability of mean CCA-IMT measurements was assessed 1 month prior to study onset in eight volunteers, and a value (mean $\pm$ SD) of  $0.033 \pm 0.023$  mm was obtained.

The presence of atherosclerotic plaques was assessed by evaluating the ultrasonographic images of common, internal and external carotid arteries. The CCA was classified as being affected by plaque if there was a localized thickening  $>1.2$  mm, as reported in previous studies.<sup>19</sup> The severity of internal carotid atherosclerosis was estimated using the maximum percentage diameter stenosis recorded by B-mode ultrasonography, in the case of mild stenoses ( $<30\%$ ),<sup>20,21</sup> and using parameters of flow velocity pattern, measured by Doppler ultrasonography, in severe stenoses ( $>70\%$ ).<sup>22,23</sup> External carotid artery stenoses were reported as percentage diameter reduction as recorded by B-mode ultrasonography.

### Common carotid artery stiffness and distensibility

Artery distensibility, elastic modulus and  $\beta$ -stiffness index were calculated using the following formulae:<sup>24–26</sup>

*Distensibility:*  $(dA/A_d)/dP$ .

*$\beta$ -stiffness index:*  $\text{Ln}(P_s/P_d)/[(D_s-D_d)/D_d]$ .

*Elastic Modulus:*  $dP/[(D_s-D_d)/D_d] \times 100$ ,

where  $dA$ =systolic-diastolic artery cross-sectional area change;  $A_d$ =diastolic artery cross-sectional area;  $dP$ =systolic-diastolic pressure change;  $\text{Ln}$ =logarithm base  $n$ ;  $P_s$ =systolic blood pressure;  $P_d$ =diastolic blood pressure;  $D_s$ =artery diameter at systole and  $D_d$ =artery diameter at diastole.

The measurements were carried out in the distal CCA, 2 cm from the origin of the carotid bulb. The scanner was configured for linear map 1, 35 dB gain and persistence off. Vessel diameter was measured continuously on-line at 25 images per

**Table 1** Demographic and clinical data in cardiac syndrome X patients and control subjects

	Syndrome X (n=30)	Controls (n=30)	P
Age (years)	57±6	54±8	0.10
Women/men	25/5	25/5	1.00
BMI (Kg/m <sup>2</sup> )	28±6	27±4	0.52
Systolic BP (mmHg)	138±23	134±20	0.48
Diastolic BP (mmHg)	78±10	79±11	0.73
Smoking, n (%)	10 (33%)	12 (40%)	0.59
Family history, n (%)	6 (20%)	5 (17%)	0.73
Treatment			
Nitrates, n (%)	8 (27%)	—	
β-blockers, n (%)	10 (33%)	—	
Calcium antagonists, n (%)	22 (73%)	—	
Aspirin, n (%)	14 (47%)	—	
Lipid-lowering drugs, n (%)	12 (40%)	—	
Biochemistry			
Glucose (mmol/l)	5.17±1.01	5.02±0.77	0.53
Cholesterol (mmol/l)	5.08±0.96	5.66±1.03	0.03
Triglycerides (mmol/l)	1.2 [0.9–1.9]	1.0 [0.8–1.5]	0.30

BMI=body mass index, BP=blood pressure.

second over a 1 cm vessel length. Approximately 50 diameter measurements were taken over the vessel length in each image from which the mean diameter was calculated for each image. This represents a large number of measurements points for each study i.e. approximately 90 compared to other methods.<sup>27</sup> The intraobserver variability, regarding measurements of CCA β-stiffness index and CCA distensibility, was assessed in eight volunteers investigated 1 month prior to study onset, and values (mean±SD) of 0.96±0.91 and 0.54±0.90 mmHg<sup>-1</sup>×10<sup>-3</sup> were obtained, respectively.

## Statistical analysis

Results are presented as mean±1 standard deviation (SD) for continuous normally distributed variables, as median [interquartile range] for continuous non-normally distributed data, and as percentages for categorical data. Analysis of normality was performed with the Kolmogorov–Smirnov test. Non-normally distributed data were logarithmically (Log<sub>10</sub>) transformed before being used in comparative analysis. For continuous variables, comparisons between the two groups were performed by use of unpaired, two-tailed t-test. Categorical data and proportions were analysed using Chi-square test. Correlation between non-normally distributed data (hs-CRP) and carotid IMT was carried out with two-tailed Spearman's rho. An adjusted analysis was carried out to account for potential imbalances between both groups. We used a binary logistic regression analysis where we included the clinical status (CSX vs. Controls) as the dependent variable and age, gender, body mass index (BMI) and cholesterol levels as independent variables. The variables assessed in the study, namely hs-CRP levels, CCA-IMT measurements, β-arterial stiffness, elastic modulus, artery distensibility and presence of atherosclerotic plaques, were included as independent variables in the model defined above, independently. A *P* value <0.05 was considered statistically significant. The SPSS 11.0 (SPSS Inc., Chicago, Illinois) statistical software package was used for all calculations.

## Results

The demographic and clinical characteristics of CSX patients and control subjects are presented in Table 1.

There were no significant differences between groups regarding age, BMI, baseline blood pressure and coronary risk factors, such as smoking, hyperlipidaemia and family history. Cholesterol levels were slightly lower in patients with CSX than in controls. Hs-CRP levels were significantly higher in patients with syndrome X than controls (2.6 [1.7–4.5] mg/l vs 1.5 [0.7–2.7] mg/l, *P*=0.02) (Fig. 1). Measurements of carotid artery IMT and other functional variables were successfully performed in all CSX patients and control subjects. Patients with CSX had significantly higher IMT values than controls (0.75±0.14 mm vs 0.63±0.09 mm, *P*<0.0001) (Fig. 1). Arterial stiffness and elastic modulus were also significantly increased in patients with CSX compared to the control group (13.5±5.8 vs 10.9±3.9, *P*=0.04 and 14.2±6.9 vs 11.1±4.2, *P*=0.04, respectively) (Fig. 1). Furthermore, distensibility was lower in syndrome X patients than control subjects although this difference did not reach statistical significance (1.8±0.8 vs 2.6±0.8 mmHg<sup>-1</sup>×10<sup>-3</sup>, *P*=0.22). Of interest, 11 (37%) patients with CSX had atherosclerotic stenoses in both the right and the left internal carotid arteries (6, <30%, 3, 30–49% and 2, 50–69% diameter reduction) compared to 3 (10%) control subjects who had atherosclerotic lesions (two had <30% stenosis in both the right and the left internal carotid artery and one had <30% stenosis in the left internal carotid artery) (*P*=0.01). The differences in hs-CRP levels, CCA-IMT, β-arterial stiffness, elastic modulus and presence of carotid atherosclerotic plaques between CSX patients and control subjects remained significant following binary logistic regression analysis where we included age, gender, BMI and cholesterol levels (Table 2). The differences in IMT, β-arterial stiffness and elastic modulus between syndrome X patients and control subjects remained significant even when patients with carotid plaques (intima-media thickening >1.2 mm) were excluded from analysis (*P*=0.004 for IMT, *P*=0.04 for β-arterial stiffness and *P*=0.03 for elastic modulus).

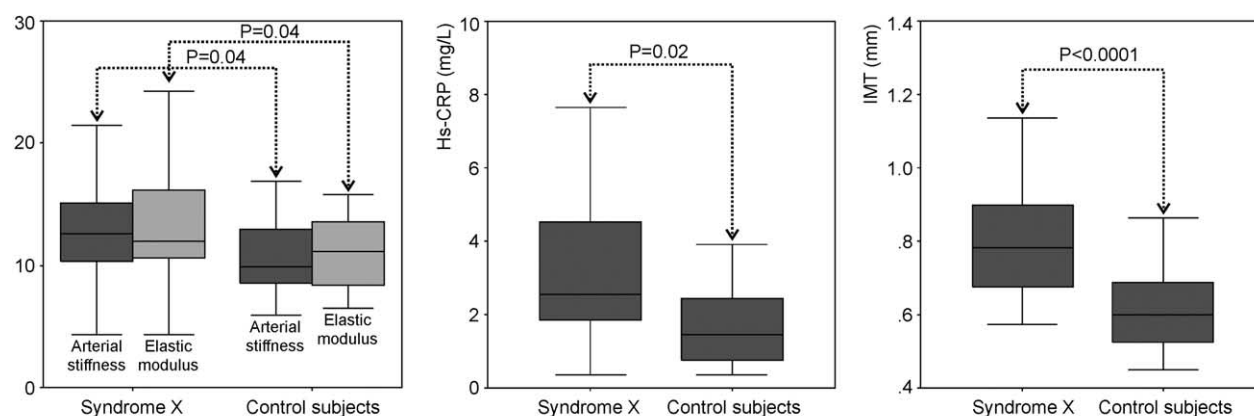


Fig. 1 Boxplots showing differences in elasticity parameters, hs-CRP levels and IMT measurements between patients with cardiac syndrome X and control subjects.

**Table 2** Carotid artery ultrasound results and high sensitivity C-reactive protein levels in cardiac syndrome X patients and control subjects

	Syndrome X (n=30)	Controls (n=30)	P <sup>a</sup>
Hs-CRP (mg/L)	2.6 [1.7–4.5]	1.5 [0.7–2.7]	0.01
Mean CCA-IMT (mm)	0.75±0.14	0.63±0.09	0.002
Atherosclerotic plaques, n (%)	11 (37%)	3 (10%)	0.01
Stiffness	13.5±5.8	10.9±3.9	0.04
Elastic modulus	14.2±6.9	11.1±4.2	0.03
Distensibility (mmHg <sup>-1</sup> ×10 <sup>-3</sup> )	1.8±0.8	2.6±0.8	0.21

CCA-IMT: common carotid artery intima-media thickness. Hs-CRP: high sensitivity C-reactive protein.

<sup>a</sup>P values following logistic regression analysis. All comparisons adjusted by age, gender, body mass index and cholesterol levels.

A significant correlation was found between hs-CRP levels and carotid IMT measurements in patients with CSX (Spearman's  $\rho=0.51$ ;  $P=0.013$ ). Hs-CRP did not correlate with elasticity parameters in CSX patients, although a non-significant trend towards a correlation was found with arterial distensibility ( $r=-0.32$ ;  $P=0.1$ ) and elastic modulus ( $r=0.26$ ;  $P=0.2$ ). When CSX patients and control subjects were considered as a whole ( $n=60$ ), the significance of the correlations improved without changes in the  $r$  value ( $r=-0.29$ ;  $P=0.045$  for distensibility and  $r=0.26$ ;  $P=0.08$  for elastic modulus).

## Discussion

This study showed that patients with CSX have higher serum hs-CRP levels, increased CCA-IMT and increased carotid artery  $\beta$ -stiffness than controls, suggesting the presence of an inflammatory background and an impaired arterial wall structure possibly due to atherosclerotic burden.

Increased hs-CRP levels in CSX patients in the present study support previous observations suggesting a role of inflammation in the pathogenesis of endothelial dysfunction.<sup>8,28</sup> CRP plasma levels are known to be associated with endothelial cell activation<sup>29,30</sup> and coronary endothelial dysfunction.<sup>28</sup> Reduced nitric oxide bioavailability due to endothelial dysfunction<sup>31</sup> and enhanced endothelin-1 (ET-1) expression, promoted by raised CRP

levels<sup>30</sup> may be implicated in the impairment of systemic endothelial vasoreactivity leading to microvascular angina<sup>3,4</sup> and impaired coronary flow reserve<sup>5,6</sup> in CSX. In fact, recent studies from our unit have shown that plasma CRP levels correlate with disease activity in CSX patients.<sup>10</sup> Endothelial dysfunction may be also implicated in phenotypic modulation and proliferation of vascular smooth muscle cells (VSMCs)<sup>32,33</sup> which, in turn, may promote intima-media thickening, considered to represent an early stage of atherosclerosis.

The observation in our study that hs-CRP levels correlated significantly with CCA-IMT is in agreement with previous observations in other patient populations, suggesting a role of chronic inflammation in atherosclerosis.<sup>34</sup> Increased IMT is a marker of early atheroma formation<sup>35</sup> and it is of interest that carotid disease was found in CSX patients despite angiographically normal coronary arteries. This finding confirms previous reports of our group<sup>8</sup> and others<sup>36</sup> regarding the presence of subangiographic atheroma in CSX patients and emphasises the limitations of coronary angiography to detect early signs of atherosclerosis such as intima-media thickening. CAD risk factors, especially hypercholesterolaemia and hypertension, are associated with the development of both carotid IMT<sup>37</sup> and subangiographic atheroma in patients with CSX.<sup>8</sup> In the present study, however, CAD risk factors were similar in CSX patients and controls. Thus, the significant correlation found



between carotid IMT and hs-CRP in patients with CSX suggests that inflammation may modulate the effect of CAD risk factors and promote endothelial dysfunction and intima-media thickening.<sup>34</sup>

It has been suggested that arterial stiffness is a precursor, or at least a predictor of atherosclerosis.<sup>38</sup> Cardiovascular risk factors have been shown to be positively associated with arterial stiffness<sup>39</sup> and men with angiographically documented CAD have been shown to have stiffer arteries than control subjects.<sup>40</sup> In the present study, increased carotid artery stiffness and a reduced carotid artery distensibility were found in CSX patients compared to controls, confirming the presence of an impaired arterial wall structure (and/or function) in these patients, possibly due to subclinical atherosclerotic burden.<sup>13</sup> Patients with CSX were not asked to discontinue cardiac medications and this may have affected results regarding arterial distensibility. It has been reported that pharmacologic and dietary interventions can improve carotid artery distensibility.<sup>41,42</sup> This effect, if present, would have attenuated the abnormalities seen in CSX thus making it possible that the differences observed between CSX and controls would be actually larger than those actually observed in the study.

A limitation of this study is the small sample size of the population investigated. However, well characterized CSX patients fulfilling stringent entry criteria are not extremely common. Lack of statistical power may thus be the reason for the non-significant trend found among hs-CRP, elastic modulus and distensibility in patients with CSX. The increased IMT and abnormalities of elasticity parameters reflect an impaired arterial wall structure in our patients with CSX and suggest a certain degree of atherosclerotic burden. However, this finding makes it difficult to explain the fact that angiographic disease progression to overt atherosclerotic disease is rare in patients with CSX even when these patients have unstable symptoms, as we<sup>8</sup> and others<sup>43</sup> have previously reported. Minimal intraluminal disease, left bundle branch block (LBBB) and the presence of multiple CAD risk factors have been identified as predictors of CAD progression in CSX patients.<sup>8,43</sup> Mononuclear cell activity, lipid and metabolic profiles<sup>44</sup> and platelet aggregability<sup>45</sup> have been reported to differ in CAD versus CSX patients, but these mechanisms alone are unlikely to explain the lack of CAD progression in CSX. It may be speculated that protective mechanisms operate in CSX patients that deserve further investigation. Whether carotid IMT thickening in CSX patients truly represents subclinical atheroma or is rather the expression of reactive vascular hypertrophy should be further elucidated. In fact, increased IMT results in our CSX patients should be interpreted with caution as they may reflect reactive hypertensive arterial wall changes and not necessarily atherosclerosis. A proportion of our CSX could have had borderline hypertension or white-coat hypertension, despite the absence of a history of hypertension, as suggested by the slightly elevated mean systolic blood pressure in the CSX patient group. Both increased IMT and high CRP concentrations are known to increase the risk of cardiovascular events.<sup>9,46</sup> However,

the prognostic implications of these markers in patients with CSX, considered to have an excellent prognosis regarding survival,<sup>47</sup> is not known. Of interest, Zebrack et al.<sup>48</sup> showed that CRP was useful for the evaluation of risk of all-cause mortality or myocardial infarction in patients with stable and unstable angina who underwent diagnostic angiography and were found to have normal coronary angiograms. We have recently reported that CRP levels correlate with clinical disease activity in CSX patients<sup>10</sup> and may thus be a useful marker to identify CSX patients at a high risk of recurrent and severe anginal symptoms who may benefit from aggressive intervention on endothelial dysfunction. The possible diagnostic and therapeutic implications of our findings deserve further investigation. We have previously shown that treatment with angiotensin converting enzyme inhibitors (ACEIs) is effective in CSX.<sup>49</sup> It has been also shown that chronic administration of ACEIs abolished VSMCs proliferation and restored endothelial nitric oxide synthase (eNOS) activity in an animal model of metabolic syndrome X<sup>50</sup> and clinical studies in patients with chest pain and normal coronary arteriograms have shown that ACEIs improve endothelial dysfunction.<sup>51,52</sup> They have also been shown to have a beneficial effect on IMT progression.<sup>53</sup> Normalization of CRP levels over time has been associated with a significant improvement of endothelium-dependent flow responses.<sup>28</sup> Whether a normalization of CRP levels is associated with an improvement in clinical activity and IMT progression in cardiac syndrome X patients requires further assessment in long-term prospective follow up studies.

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