Short Communication

Presence of *Chlamydia pneumoniae* DNA in the Artery Wall – Biomarker of Coronary Artery Disease

(*Chlamydia pneumoniae* / biomarker / coronary artery disease)

J. KIRBIŠ[1], D. KEŠE[2], D. PETROVIČ[3]

[1]Department of Cardiovascular Surgery, University Medical Centre Ljubljana
[2]Institute of Microbiology and Immunology, Medical Faculty, University of Ljubljana
[3]Institute of Histology and Embryology, Medical Faculty, University of Ljubljana

Abstract. Many authors have shown an association between *Chlamydia pneumoniae* (CPn) infection and coronary artery disease (CAD). However, whether CPn infection demonstrated by CPn DNA presence in the artery wall plays an important role in pathogenesis of CAD and acute coronary events (i.e. unstable angina) remains to be elucidated. One hundred and fifteen consecutive patients with CAD (51 with unstable angina and 64 with stable angina) were compared with 52 control subjects with aortic valve disease without angiographic evidence of CAD. The presence of CPn DNA in the aortic wall was assessed with nested polymerase chain reaction (PCR), and the IgM, IgG and IgA anti-CPn titres were assessed with microimmunofluorescence test. CPn DNA presence in the artery (i.e. aortic) wall was associated with 3.7-fold increased risk of CAD (95% CI 1.2-11.3, P < 0.01); however, no statistically significant difference in CPn DNA presence was demonstrated between unstable and stable angina (17.6% vs. 25%). In the CPn DNA positive group more often than in the CPn DNA negative group, serological signs of chronic infection (55.2% vs. 27%, P = 0.004) were demonstrated, whereas no statistically significant differences were demonstrated in prevalence of either acute infection (9.3% vs. 0%) or reinfection (0% vs. 0%). In conclusion, CPn DNA presence in the artery (i.e. aortic) wall was associated with CAD, therefore may be used as a biomarker for CAD. Moreover, no statistically significant differences in CPn DNA presence in the artery wall and in serology were present between unstable and stable angina; therefore, CPn infection does not seem implicated in triggering an acute coronary event.

Since the first report in 1988 by Saikku and co-workers (Saikku et al., 1988), *C. pneumoniae* (CPn) has been implicated in the pathogenesis of coronary artery disease (CAD) by seroepidemiological studies (Blasi et al., 1997; Mazzoli et al., 1998). Due to a high prevalence of anti-CPn antibodies in subjects older than 20 years, however, more specific markers for CPn infection are needed (Kalayoglu et al., 2002).

The aim of this study was to find whether a CPn DNA infection demonstrated by the presence of CPn DNA in the artery (i.e. aortic) wall was associated with CAD, and could therefore be used as a biomarker of CAD. Moreover, we assessed the prevalence of CPn DNA in the vessel wall in patients with acute coronary event (i.e. unstable angina) in comparison with stable angina.

Material and Methods

The research protocol was approved by the national medical ethics committee. Subjects were recruited consecutively from individuals referred for open heart surgery due to CAD (CAD group, 115 cases: 51 cases with acute coronary syndrome, i.e. unstable angina, and 64 cases with stable angina) or because of aortic valve disease (control group, 52 subjects without angiographic evidence of CAD on whom aortic valve replacement was to be performed). The ascending aorta samples approximately 1 cm above the right coronary ostium of CAD group patients and of control group subjects were put in the transport media and transported to the laboratory on ice. The aortotomy line for aortic valve replacement was performed transversally 1 cm above the right coronary ostium. DNA was isolated, purifed and treated with a kit according to the manufacturer's protocol (QIAGEN, Santa Clara, CA). The presence of CPn DNA was detected by a nested PCR method using the specific primers HL-1 and HL-2, (gtt-gtt-cat-gaa-ggc-cta-act, tgc-ata-acc-tac-ggt-gtg-tgt-t). The PCR reaction yielded a 437-bp product. Consensus recommendations on standardized testing for CPn by the microimmunofluorescence (MIF) method was used to confirm acute infection, reinfection, and chronic infec-

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Corresponding author: Daniel Petrovič, Institute of Histology and Embryology, Medical Faculty of Ljubljana, Korytkova 2, 1105 Ljubljana, Slovenia. Tel: (+386) 1 543 7367; Fax: (+386) 1 543 7361; e-mail: daniel.petrovic@mf.uni-lj.si

Abbreviations: CAD – coronary artery disease, CPn – *Chlamydia pneumoniae*.

tion (Saikku et al., 1988; Dowell et al., 2001). Differences in mean values were analysed using analysis of variance. Chi-square test was used to compare discrete variables. Statistical analysis was performed using the SPSS program for Windows 2000 version 12 (SPSS Inc., Chicago, IL). Statistical significance was set at $P < 0.05$.

Results and Discussion

The presence of CPn DNA in the artery (i.e. aortic) wall was associated with a 3.7-fold increased risk of CAD (95% CI 1.2-11.3, $P < 0.01$). Our finding is in accordance with the report of Wong and co-workers, who reported an association between the circulating CPn DNA and CAD in men (Wong et al., 1999). Moreover, in the CPn DNA positive group more often than in the CPn DNA negative group, serological signs of chronic infection (55.2% vs. 27%, $P = 0.004$) were demonstrated, whereas no statistically significant difference was demonstrated in the prevalence of either acute infection or reinfection (Table 1).

Acute infection (MIF IgM titre ≥ 1 : 16 or greater, or IgG titre ≥ 1 : 512) was observed in 15.5%, 8.2%, and 0% in the acute coronary syndrome, stable angina, and the control group, respectively ($P = 0.015$), and reinfection was not observed in any case. Chronic infection (MIF $32 < IgG < 512$ or $32 \geq IgA < 256$) was observed in 40%, 68.9%, and 61.5% in the acute coronary syndrome, stable angina, and the control group, respectively (P = ns).

The comparison of patients with acute coronary syndrome (i.e. unstable angina) and stable angina failed to demonstrate a statistically significant difference in the presence of CPn DNA in the artery wall (17.6% vs. 25%), frequency of acute infection (15.5% vs. 8.2%) and chronic infection (40% vs. 68.9%); therefore, CPn infection does not seem implicated in triggering an acute coronary event.

The presence of CPn DNA was, interestingly, demonstrated in the ascending aorta near the right coronary ostium in a few subjects without serological signs of either acute or chronic infection (3 cases in the CAD group: 1 with unstable angina and 2 with stable angina, and 2 cases in the control group); this is an interesting finding that is in accordance with our previous report (Zorc et al., 2005). We speculate that these subjects are antibody non-responders.

In conclusion, CPn infection is associated with CAD, and may therefore be used as a biomarker of CAD. However, no statistically significant differences in CPn DNA presence in the artery (aortic) wall and in serology were present between unstable and stable angina; therefore, CPn infection does not seem implicated in triggering an acute coronary event.

References

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Table 1. Clinical and laboratory characteristics of subjects with the presence of CPn DNA in the vessel wall (aortic wall).

	CPn positive n (%)	CPn negative n (%)	P	Odds ratio (95% CI)
Number	29	129		
Male sex	26 (89.7)	104 (80.6)	0.3	2.1 (0.6–7.4)
Arterial hypertension	16 (55.2)	76 (58.9)	0.7	0.9 (0.4–1.9)
Diabetes	7 (24.1)	30 (23.3)	0.9	1.0 (0.4–2.7)
Age	49 ± 11	52 ± 8	0.03	
CRP level	23 ± 11	26 ± 33	0.7	
Coronary artery disease	25 (86.2)	81 (62.8)	0.01	3.7 (1.2–11.3)
Acute infection[2]	12 (9.3)	0 (0)	0.09	1.2 (1.1–1.4)
Chronic infection[3]	16 (55.2)	33 (27)	0.004	3.3 (1.4–7.6)
Reinfection[4]	0	0	n.s.	

[1]the number of CPn positive and CPn negative subjects was 158 and not 167, since the PCR reaction for the detection of CPn in the vessel (aortic) wall was not successful in 9 subjects
[2]Acute infection = IgM titre ≥1 : 16 or greater or IgG titre ≥ 1 : 512
[3]Chronic infection = $32 < IgG < 512$ or $32 \leq IgA < 256$
[4]Reinfection = IgG ≥ 512 and IgA ≥ 256
CRP – C-reactive protein

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