# The role of inflammation and *Chlamydia pneumoniae* infection in patients with chronic occlusion of the terminal aorta

## Rolul inflamației și al infecției cu *Chlamydia pneumoniae* la pacienții cu ocluzie cronică a aortei terminale

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## Abstract

*Background: Chronic Chlamydia pneumoniae (CP) and cytomegalic virus (CMV) infections are considered important risk factors for atherosclerosis, but without a certain benefit from the antimicrobial therapy. Infection has yet an unknown role in the appearance and progression of peripheral arterial disease (PAD) that is why conventional therapy for PAD does not include antimicrobial treatment.*

*Aim: to investigate the association of inflammation and Chlamydia pneumoniae infection with the presence of chronic aortic occlusion (Leriche syndrome).*

*Methods: A number of 122 subjects, divided into two groups, were assessed using clinical criteria, laboratory findings and invasive imaging (classic angiography +/- digital subtraction angiography). The study group included 62 patients with Leriche syndrome. The diagnostic inclusion criteria were the classic ones and the angiographic presence of Leriche syndrome. The control group included 60 patients without any cardiovascular pathology, recruited from general population. C reactive protein (CRP), fibrinogen and specific antibodies for Chlamydia pneumoniae, IgM and IgG presence were determined for both groups.*

*Results: A higher incidence of serological evidence of CP infection was found in the patients from the study group (43/62) than in controls (6/60). There is no statistically association between serological evidence of*

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*CP infection and age, provenience, or smoking. In the study group 28 patients had fibrinogen values over 400 mg% compared to just 2 patients in the control group and also 28 patients had high levels of CRP compared to just 2 in control group. CRP (p=0.003), IgG (p=0.011) and fibrinogen were significantly correlated with severe stages of chronic aortic occlusion.*

*Conclusions: This study supports the hypothesis that inflammation (high levels of CRP, fibrinogen) and chronic CP infection (IgG seropositivity) have an important role in lower limb atherosclerosis and correlate with the severity of the disease.*

*Key words: chronic aortic occlusion, inflammation, Chlamydia pneumonia, atherosclerosis*

## Rezumat

*Infecțiile cronice cu Chlamydia pneumoniae (CP) și citomegalovirus (CMV) sunt considerate factori de risc importanți pentru ateroscleroză, dar terapia antimicrobiană nu aduce un beneficiu cert. Infecția are un rol încă nedeterminat în apariția și progresia bolii arteriale periferice și, de aceea, terapia convențională pentru aceasta din urmă nu include tratament antimicrobian. Scop: investigarea asocierii inflamației șia infecției cu Chlamydia pneumoniae cu prezența ocluziei aortice cronice (sindromul Leriche). Metode. Un număr de 122 de subiecți divizați în două grupuri au fost investigați folosind criterii clinice, date de laborator și investigații imagistice invazive (angiografie clasică +/- angiografie cu substracție digitală). Grupul de studiu a inclus 62 pacienți cu sindrom Leriche. Criteriile diagnostice de includere au fost cele clasice și prezența angiografică a sindromului Leriche. Grupul de control a inclus 60 pacienți fără nici o patologie cardiovasculară, recrutați din populația generală. Proteina C reactivă, fibrinogenul și anticorpii specifici de tip IgM și IgG pentru Chlamydia pneumoniae, au fost determinate la ambele grupuri. Rezultate. La pacienții din grupul de studiu dovada serologică de infecție cu Chlamydia pneumoniae a fost mai frecventă (43/62) decît în grupul de control (6/60). Nu a fost găsită nici o asociere statistică între dovada serologică a infecției cu Chlamydia pneumoniae și vîrstă, mediul de proveniență și fumat. În grupul de studiu, 28 pacienți au avut valori ale fibrinogenului peste 400 mg%, comparativ cu numai 2 pacienți din grupul de control și, de asemenea, 28 pacienți au avut nivele crescute ale proteinei C reactive, comparativ cu doar 2 pacienți din grupul de control. Proteina C reactivă (p=0,003), IgG (p=0,011) și fibrinogenul au fost asociate semnificativ cu severitatea stadiului de ocluzie aortică cronică. Concluzii. Acest studiu susține ipoteza că inflamația (nivele crescute de proteină C reactivă, fibrinogen) și infecție cronică cu Chlamydia pneumoniae (seropozitivitate IgG) au un rol important în ateroscleroza membrului inferior și sunt corelate cu severitatea acestei boli.*

*Cuvinte-cheie: ocluzie aortică cronică, inflamație, Chlamydia pneumoniae, ateroscleroză*

## Introduction

The association of atherosclerosis and different risk factors (increased levels of plasmatic cholesterol and mainly LDL-cholesterol, decreased levels of HDL-cholesterol, LDL modifications like oxidation and glycosilation, increased homocystein levels, smoking, hypertension, diabetes, viral (CMV)[2] or other infections like *Chlamydia pneumoniae* (CP)[2, 5] have generated a hypothesis that explains the progression of lesions: the hypothesis of reaction to aggression, the most probable hypothesis up-to-date. Chronic CP infection has been suspected as being a triggering factor of atherosclerosis, but there is no certain evidence concerning the benefits of anti-Chlamydia therapy in reducing the number of cardiovascular events[3, 11]. A positive association between the level of infection and peripheral arterial disease was observed, but only in women with high CRP levels[1]. Recent seroepidemiologic studies suggest that CP infection predicts the development of vascular disease, just as smoking, hypertension, and elevated low-density lipoprotein (LDL) cholesterol[4]. Circulating bacterial endotoxins may am-

plify the proatherogenic status and endothelial dysfunction. Infection has a questionable role in the appearance and progression of chronic aorto-iliac occlusion, and therefore antimicrobial therapy is not a standard in the actual treatment of peripheral arterial ischemia. Although the mechanism of association between infection and atherosclerosis is not known, it is probable that infection stimulates inflammation. Several studies point out the benefits of antibiotic therapy (macrolides prevent progression of peripheral arterial occlusive disease), while others found no evidence of these benefits[7, 12].

## Methods

The study included 122 subjects of both gender, with ages between 37-79 years, and a mean age of 60,26 years (SD +/- 7,95 years). The patients were divided into two groups, a study group of 62 patients with Leriche syndrome, and a control group of 60 patients respectively.

The inclusion criteria for the study group were the past medical history, clinical, labor findings and angiographic confirmation of Leriche syndrome. The exclusion criteria were the major amputations of the lower limb, or any other amputations that may limit the patients walking ability, surgical or endovascular interventions on the peripheral arteries over the study period, suspicion or treatment of tuberculosis, immunosuppressive therapy, active infection that requires the systemic or oral administration of antibiotics, autoimmune diseases, history of excessive alcohol abuse, drug abuse, severe psychiatric diseases, chronic antibiotics consumption, uncontrolled arterial hypertension (resting hypertension higher than 160/100 mmHg), uncontrolled congestive heart failure or arrhythmias. The control group consisted of 60 patients with no evidence of cardiovascular pathology, recruited from general population.

To confirm the implication of both inflammatory and infectious factors in the process

**Table I. Age distribution of patients in the study group and presence of anti-*Chlamydia pneumoniae* IgG.**

| IgG | Age groups (years), number of patients | | | Total |
|---|---|---|---|---|
| | <40 | 40-60 | >60 | |
| Negative | 1 | 7 | 11 | 19 |
| Positive | 1 | 23 | 19 | 43 |
| Total | 2 | 30 | 30 | 62 |

of atherosclerosis, we have determined CRP and fibrinogen (as inflammation markers), as well as the presence of specific antiCP antibodies, IgM as a marker of acute infection and IgG as a marker of a prior infection, for both groups, using the ELISA test, and Labsystems Anti CP IgG and IgM EIA kits[10]. Anti CP antibodies have been detected in the patient's serum, harvesting 6 ml of integral blood from each patient. Because all samples were processed simultaneously, patient's serum were frozen to -70C° and maintained at this temperature over the study period till at the time of determination. No sodium azide was used and samples were not refrozen. Antibody titers were expressed qualitatively (positive/negative), as well as semi-quantitatively. AntiCP IgM and IgG kits do not have crossed reactivity with *Chlamydia trachomatis* antibodies.

All collected data were stored in an electronic database using Microsoft Excel 2007 for Windows Vista Ultimate software and statistical calculations were performed. The significance of data was analyzed using Student's t-test (paired, so that each member of one sample has a unique relationship with a particular member of the other sample. If the calculated p-value is below the threshold chosen for statistical significance - the 0.05 level, then the null hypothesis which usually states that the two groups do not differ is rejected in favor of an alternative hypothesis, which typically states that the groups do differ) and chi-square test (statistical hypothesis test in which the test statistic

**Table II. Correlations between high levels of IgG and fibrinogen values in the study group.**

| IgG | Fibrinogen level (number of patients) | | Total |
|---|---|---|---|
| | <400mg% | >400mg% | |
| Negative | 11 | 8 | 19 |
| Positive | 23 | 20 | 43 |
| Total | 34 | 28 | 62 |

**Table III. Correlations between high levels of IgG and fibrinogen values in the control group.**

| IgG | Fibrinogen level (number of patients) | | Total |
|---|---|---|---|
| | <400mg% | >400mg% | |
| Negative | 53 | 1 | 54 |
| Positive | 5 | 1 | 6 |
| Total | 58 | 2 | 60 |

has a chi-square distribution when the null hypothesis is true, or any in which the probability distribution of the test statistic, assuming the null hypothesis is true, can be made to approximate a chi-square distribution as closely as desired by making the sample size large enough). To evaluate the correlation between CRP positivity and other risk factors, a multivariate analysis was performed (SPSS package).

Our study complies with the Declaration of Helsinki concerning medical ethics. The ethics committee has approved the research protocol and the informed consent has been obtained from the subjects recruited in this study.

## Results

Patients in the study group presented a higher incidence of CP infection, with 43 infected patients out of 62 (69.35%) (*Table 1*), and compared with the control group, where 6 of the 60 patients (10%) were infected with CP. There is no statistically significant connection between age and the serological confirmation of CP infection (p=0,445), environment of origin (p=0,669) or smoking habits (p=0,544) of patients in the study group.

None of the 62 patients of the study group showed suggestive immunological modifications of an acute infection, proving that in the case of patients with aorto-iliac occlusion, the *Chlamydia pneumoniae* infection is a chronic one (IgM antibodies were never detected in our patients).

Chronic CP infection was present in 42 of the 60 smoking patients and 1 of the 2 non-smoking patients of the study group. In the control group, chronic CP infection was present in 4 of the 13 smoking patients and 2 of the 47 non-smoking patients. There were no correlations between the number of smoking patients and chronic CP infection in the study group (p=0,546), or in the control group (p=0,524). There is no statistically significant correlation between IgG and fibrinogen levels in patients with Leriche syndrome (p=0,747), or in the control group (p=0,05513) (*Tables II, III*).

In the study group, there is no statistically significant connection between IgG and CRP values, 28 patients presenting positive CRP, compared with 2 patients of the control group (*Tables IV, V*).

From all the IgG positive patients, 25 had a Fontaine stage IV, 13 stage III and 5 stage II occlusive arterial disease. Most of the IgG negative patients had a Fontaine stage III (11 patients) and stage II (8 patients) disease (*Table VI*). CRP (p = 0,003), IgG (p = 0,011) and fibrinogen levels were significantly correlated with advanced stages of terminal aorta occlusion. These results correlate with previous data about the frequency of infection in patients with peripheral atherosclerotic arterial disease. We could not establish a strong cause-effect relationship between bacterial infection and peripheral arterial disease, but the high level of seropositivity may be considered as a target in the treatment of peripheral atherosclerotic arterial disease.

**Table IV. Correlations between high levels of IgG and CRP values in the study group.**

| IgG | CRP (number of patients) | | Total |
|---|---|---|---|
| | Negative | High levels | |
| Negative | 11 | 8 | 19 |
| Positive | 23 | 20 | 43 |
| Total | 34 | 28 | 62 |

## Discussions

The present paper approaches a subject of actuality, regarding the involvement of a chronic infectious factor in the etiology of atherosclerosis – *Chlamydia pneumoniae* infection[1, 2, 11]. The infectious agent was evidenced by determining IgM and IgG antibodies specific for CP. The analysis of these values shows that CP infection is chronic in patients with Leriche syndrome, but there are no statistically significant correlations with age and environment of origin. Other risk factors involved in the appearance of atherosclerosis like smoking or inflammation are independent of the infectious factor. Clinical stages of peripheral arterial disease on the other hand have a statistically significant correlation with chronic CP infection. This infection plays a role in the etiology and progress of aortoiliac atherosclerosis, but it is not a decisive factor in its pathogenesis. The association of two pro-atherosclerotic factors

**Table VI. Correlations between Fontaine stages of Leriche syndrome patients and IgG levels. Chi test: p= 0,0000586175**

| Fontaine stages | IgG (number of patients) | | Total |
|---|---|---|---|
| | Negative | Positive | |
| II | 8 | 5 | 13 |
| III | 11 | 13 | 24 |
| IV | 0 | 25 | 25 |
| Total | 19 | 43 | 62 |

**Table VIII. Correlations between high levels of IgG and CRP values in the control group.**

| IgG | CRP (number of patients) | | Total |
|---|---|---|---|
| | Negative | Positive | |
| Negative | 53 | 1 | 54 |
| Positive | 5 | 1 | 6 |
| Total | 58 | 2 | 60 |

(chronic infection evidenced by the presence of CP specific IgG and inflammation evidenced by the presence of CRP and fibrinogen) presents a significant correlation with the clinical stages of chronic arterial ischemia, specific for Leriche syndrome[6, 9, 11].

## Conclusions

The results of this study supports the previously reported association between *Chlamydia pneumoniae* infection and peripheral arterial disease. These data are consistent with previous studies indicating a chronic *Chlamydia pneumoniae* antibody pattern as a possible risk factor marker for atherosclerosis.

Our findings suggest a link between *Chlamydia pneumoniae* chronic infection and the presence of chronic aortic occlusion. Due to the small size of our study, the present data do not allow any definitive conclusion about a cause-effect relationship between infection and lesion. This study provides data on a possible involvement of *Chlamydia pneumoniae* in the pathogenesis of chronic occlusion of the terminal aorta and additional evidence for an association between this agent, other markers of atherosclerosis (fibrinogen, CRP) and atherosclerosis.

The presence of anti-chlamydia IgG antibody is indicative of chlamydial infection at an undetermined time. High levels of IgG antibodies are of diagnostic value in chronic chlamydial infection.

The patients with Leriche syndrome and with serological infection with *Chlamydia pneumoniae* are not a homogenous group.

Interlaboratory standardization of direct and indirect detection methods of *Chlamydia pneumoniae* infection is required to elucidate the role of these microorganisms in chronic aortic occlusion development.

For the future more studies are needed to investigate the association between *Chlamydia pneumoniae* infection and the development of atherosclerotic disease. Additional laboratory studies are also needed to suggest mechanisms by which such an infection could contribute to atherogenesis.

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