Elżbieta Mazur[1], Justyna Niedźwiadek[1], Andrzej Wolski[2], Zofia Siezieniewska-Skowrońska[3], Witold Żywicki[2], Agnieszka Korolczuk[3], Elżbieta Korobowicz[3], Maria Kozioł-Montewka[1]

Chlamydia pneumoniae Infection in Abdominal Aortic Aneurysm (AAA) Patients and Its Clinical Impact

[1]Department of Medical Microbiology, University School of Medicine, Lublin,
[2]Department of Vascular Surgery, University School of Medicine, Lublin,
[3]Department of Clinical Pathomorphology, University School of Medicine, Lublin

The aim of our study was to assess the presence of *Chlamydia pneumoniae* infection in AAA patients and to evaluate its association with clinical symptoms and histological signs of inflammation in the aortal wall. Fifty-two AAA patients participated in the research. Thirty healthy controls took part in serological examination. *C. pneumoniae* was detected by PCR and immunofluorescence *in situ* reaction in aorta samples of 84.6% and 86.54% of the patients, respectively. Serological markers of chronic *C. pneumoniae* infection were detected in 86.5% of AAA patients and in 33.3% of healthy controls. High titers of specific IgG and IgA were found in 37.8% of AAA patients with serologically defined chronic infection. All patients in "high serology" group had symptomatic aneurysm and inflammatory infiltrations in their aortal wall samples. Conclusions: AAA patients infected with *C. pneumoniae* are not a homogenous group. "High serology" group is much more prone to have symptomatic aneurysm than the remaining of AAA patients. Serology can be very useful in predicting the risk of AAA rupture. Inter-laboratory standardization of direct and indirect detection methods of *C. pneumoniae* infection is required to elucidate the role of these bacteria in AAA development.

Introduction

Aneurysms have been recognized for their morbidity and mortality for centuries. Epidemiological investigations have revealed a 4% prevalence of aneurysms in persons over 65 years of age [29]. Although aneurysms can develop in any artery, the most striking morphological alterations occur in abdominal aorta and other large arteries. The pathogenesis of abdominal aortic aneurysm (AAA) involves the complex interaction of variety of factors, which, acting over many years, weaken the aortic wall [5, 21, 26, 45]. Traditionally, AAA was viewed as a complication of atherosclerosis [4]. Today, the crucial pathologic processes underlying degeneration of the aortic media in aneurysms appear to be inflammation and proteolysis – elastin degradation is considered a primary event in AAA development [38, 44].

Chlamydiae are obligate intracellular parasites that are classified as bacteria because of the composition of their cell wall and their growth by binary division. They have a unique biphasic life cycle with a smaller extracellular form, the elementary body (EB), and a larger replicating intracellular form, the reticulate body. The EBs attach to susceptible host cells and are phagocytized. Within the phagosome they transform to reticulate bodies, which replicate by using the host cell energy stores and form characteristic cytoplasmic inclusions. The reticulate bodies revert to the EB form prior to cell lysis. *C. pneumoniae* organisms have a characteristic pear-shaped EB surrounded by a periplasmatic space that is morphologically distinct from the round EBs of *C. trachomatis* and *C. psittaci* [16].

The frequent finding of the organism in atheroma and not in normal artery tissue by different methods and many investigators suggests that *C. pneumoniae* may play a role in clinical manifestations of atherosclerosis, including AAA. Several studies, principally with the use of polymerase chain reaction (PCR), have demonstrated the presence of *C. pneumoniae* in the wall of 50–100% of abdominal aortic aneurysms [2, 10, 11, 27, 31]. The bacte-

ria were also successfully cultured from a half of aorta samples, in which they had been detected by immunocytochemistry [11]. Serological evidence of *Chlamydia pneumoniae* infection has been recently associated with the expansion of abdominal aortic aneurysms [20, 41]. Moreover, in two randomized, double-blind, placebo-controlled pilot studies the expansion of small aneurysms was reduced in patients treated with roxithromycin and doxycyclin [25, 42]. In addition, in an experimental model *C. pneumoniae* membrane antigens appeared to be the cause of aneurysmal dilation and associated macrophage recruitment [39]. Also, it has been shown that the presence of *C. pneumoniae* is associated with increased degradation of elastin in aortic tissue *in vitro* [33].

The aim of our study was to assess the presence of *Chlamydia pneumoniae* infection in AAA patients by means of direct detection methods (immunofluorescence antibody technique, PCR, electron microscopy) and serological studies. Also, we tried to evaluate an association of this infection with clinical symptoms and histological signs of inflammation in the aortal wall.

Material and Methods

Material

Fifty-two AAA patients (9F, 43M), mean age 68.3 years (50–81) participated in the research. All patients were operated for AAA at the Department of Vascular Surgery, University School of Medicine in Lublin. The indications for surgery were: aneurysm size 4.5–8.0cm or the presence of clinical symptoms. All procedures were elective. Aorta samples were obtained during operation and divided into several parts for further investigation. The samples for histopathological examination and electron microscopy were preserved in formalin and glutharaldehyde, respectively; for immunofluorescence antibody technique and PCR they were frozen in -70°C until performing the procedures. Blood samples for serological examination were obtained from patients on the day before operation.

Controls (for serological examination)

Thirty control subjects, matched for age and sex (5F, 25M) without clinical signs and symptoms of cardiovascular and pulmonary disease took part in our study.

PCR

DNA was extracted from frozen aorta samples using a Qiagen DNA mini-kit according to the manufacturer's instructions. DNA was eluted in a final volume of 100µl, aliquoted, and stored at -20°C. For the PCR using CP1-CP2 with nested primer pair CPC-CPD the conditions were as follows: the first round of amplification employed 1.5mM $MgCl_2$, 0.4µM primers, and 0.625U of Taq polymerase, 50Mm KCl, 200µM dNTP, 10mM Tris HCl (pH=8.3) and 2.5µl sample DNA and involved 20 cycles of 1min at 94°C, 1min at 65°C minus 0.5°C per cycle, and 1min at 72°C plus an additional 20 cycles of 1min at 94°C, 1min at 55°C, and 1min at 72°C. The PCR products amplified by the outer primers (CP1-CP2) were diluted 1:10, and a volume of 2.5µl was added to a new 25-µl PCR mixture for a second amplification with nested primer pair CPC-CPD. The second round of amplification employed 3mM $MgCl_2$, 1µM primers, and 0.625U of Taq polymerase, 50Mm KCl, 200µM dNTP, 10mM Tris HCl (pH=8.3) and involved 30 cycles of 1min at 94°C, 1min at 50°C, and 1min at 72°C [22]. All amplification products (333bp and 207bp) were analyzed by agarose gel electrophoresis followed by ethidium bromide staining. DNA isolated from the reference strain *C. pneumoniae* ATCC 1310-VR was used as a positive control.

Immunofluorescence antibody technique (IDFA)

The specimens were initially treated with primary mice antibodies (RR402 clone by DAKO company), next with the secondary goat antibody against mice immunoglobulins marked with fluorescein isothiocyanate. The procedure was conducted according to manufacturer's recommendations.

Histopathological evaluation

The specimens for the evaluation in the light microscope were routinely prepared by HE staining.

Electron microscopy

Aorta samples taken from 7 patients were examined by means of electron microscopy. Samples for EM were prepared according to the standard procedure and examined in the 900 Zeiss Electron Microscope (Philips).

Serological studies

To evaluate the level of specific IgA, IgM and IgG in patients' serum samples microimmunofluorescence method (MIF) was applied. *Chlamydia pneumoniae* Micro-IF test (Labsystems, Finland) was used according to manufacturer's instructions. Sera analyzed for *C. pneumoniae* IgM and IgA were diluted in IgG blocker (Labsystems, Finland) to remove possible interference with IgG.

The following serological criteria were adopted to assess the type of infection:

1. IgG<1:128, IgA≤1:8, IgM=0 – contact with the pathogen in the past;
2. IgG≥1:512, IgM≥1:8 – primary acute infection;
3. IgG≥1:128, IgA≥1:32, IgM=0 – chronic (persistent) infection; IgG≥1:512, IgA≥1:64 among chronic infection group was called active infection.

Statistical analysis

All statistical analyses were performed with SPSS for Windows program version 8.0. For dichotomous variables, χ^2 test or Fisher's exact test were used. All tests were 2-tailed. Differences were considered significant at $p<0.05$.

Results

C. pneumoniae DNA was detected by nested PCR in aorta samples of 44/52 (84.6%) patients. Positive immunofluorescence *in situ* reaction (IDFA) was observed in aorta samples of 45/52 (86.54%) patients (Fig. 1). In 40/52 (76.9%) patients the presence of *C. pneumoniae* in aorta samples was assessed by means of two employed direct detection methods (PCR and IDFA). In aorta samples of 2 patients *C. pneumoniae* was not detected by any of applied direct detection methods.

The histopathological evaluation of all aorta samples yielded the diagnosis of a true aneurysm in each case. Atherosclerotic changes in the aortic wall were observed in all specimens. In 11 samples abundant inflammatory infiltrations were found, which consisted of mononuclear cells in the whole aneurysm wall. In 32 specimens inflammatory infiltrations were much less abundant and located mainly in the muscular coat and the adjacent fatty tissue. Lymphocytes, plasma cells and macrophages prevailed in the inflammatory infiltrations (Fig. 2). In 9 samples no inflammatory reaction in the aneurysm wall was observed.

The aorta samples from 7 patients with directly detected *C. pneumoniae* antigen or DNA and serologically defined active infection were examined in electron microscopy. In all 7 cases atherosclerotic changes have been found (atheromatous ulcers covered by mural thrombi, foam cells, macrophages, collagen and elastic fibers, cholesterol crystals, lymphocytes, granulocytes and plasma cells). The presence of *C. pneumoniae* inclusions depended on the progression of the lesion. In 3 specimens (patients No. 5, 14, 20), in which the presence of macrophages, foam cells, and inflammatory cells mixed with collagen, elastic fibers, and cholesterol crystals was observed, the intracellular inclusions (IB) of *C. pneumoniae* were visible containing elementary (EB) and reticulate bodies (RB), as well as extracellular EB (Fig. 3). IB were mainly seen in the cytoplasm of macrophages. In one sample (patient No. 5) we found cells filled with IB. In next 2 specimens (patients No. 9 and 22) only the components of thrombi, ulceration and cell fragmentation were observed morphologically. In these samples we have not detected the presence of *C. pneumoniae*.

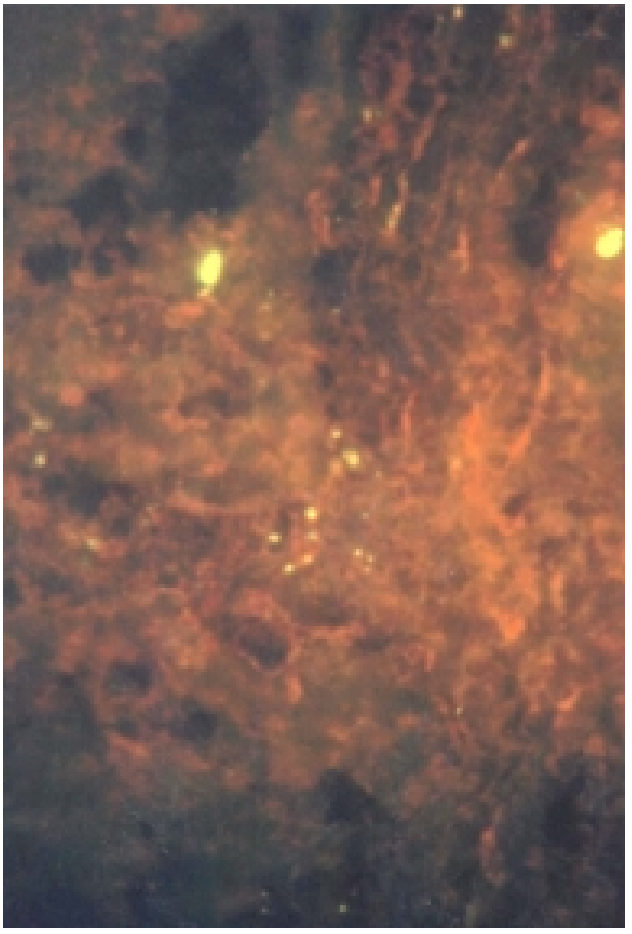

Fig. 1. Positive fluorescence reaction with antibodies against *Chlamydia pneumoniae* RR402 clone. Magn. 450x.

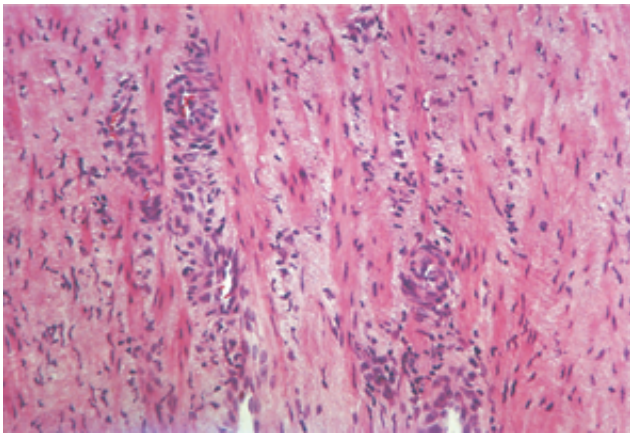

Fig. 2. Inflammatory infiltration of aneurysm wall containing lymphocytes and plasma cells. HE. Magn. 360x.

In still next 2 specimens (patients No. 23 and 24) the result of our observation was controversial. IB and EB were seen in the remnants of fragmented cells and among them. Single macrophages and foam cells have been found, but the inclusions were not seen in any of them.

Serological markers of chronic *C. pneumoniae* infection were detected in 45/52 (86.5%) AAA patients and in 10/30 (33.3%) healthy controls (χ^2=24.4; df=1; p<0.001). Serological markers of previous contact with the pathogen were detected in 7/52 (13.5%) AAA patients and in 20/30 (66.6%) healthy controls. We did not detect serological markers of primary acute *C. pneumoniae* infection in any of our patients or healthy controls (lack of specific IgM) (Figs. 4 and 5). Seventeen out of 45 (37.8%) AAA patients with serological signs of chronic infection had high titers of specific IgG and IgA – they were called "active infection group". Interestingly, all members of this group had symptomatic aneurysm. Statistical analysis revealed that symptomatic aneurysm was more frequent in active infection group in comparison with the remaining patients (exact Fisher's test, p<0.001) (Fig. 6).

In active infection group the presence of *C. pneumoniae* in aorta samples was detected by means of IDFA or nested PCR in15/17 (88.2%) patients. In two of them the bacteria was detected only by IDFA, in others two it was not detected by any of applied methods. All patients in this group had inflammatory infiltration in their samples of aortic wall, but only in two of them the infiltrations were numerous. Statistical analysis showed that in active infection group the presence of inflammatory infiltration in aortic wall samples was more frequent that in the remaining patients (exact Fisher's test, p<0.05) (Table 1).

In 43/52 (82.7%) patients *C. pneumoniae* infection was detected serologically and, simultaneously, using at least one of applied direct detection methods. Two patients who were positive in serology but negative in IDFA or PCR belonged to the active infection group; they had moderate infiltrations in their aorta samples and symptomatic aneurysm.

Seven out of 52 (13.5%) patients were negative in serology (they have serological markers of previous contact with the pathogen but not of infection) but positive in at least one of applied direct detection methods.

The histopathological evaluation detected inflammatory infiltration in aorta samples of 43/52 (82.7%) patients. In aorta samples of 11/43 (25.6%) patients abundant inflammatory infiltrations were found, which consisted of mononuclear cells in the whole aneurysm wall. We called these patients "abundant inflammatory infiltration group". All but one patient in this group had

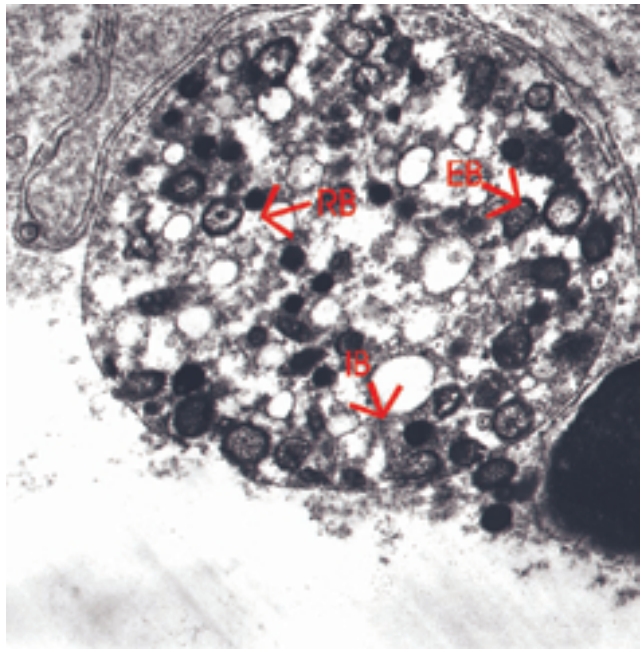

Fig. 3. IB (inclusion bodies), EB (elementary bodies) and RB (reticulate bodies) in aneurysmal wall. Electron microscopy. Magn. 20,000x.

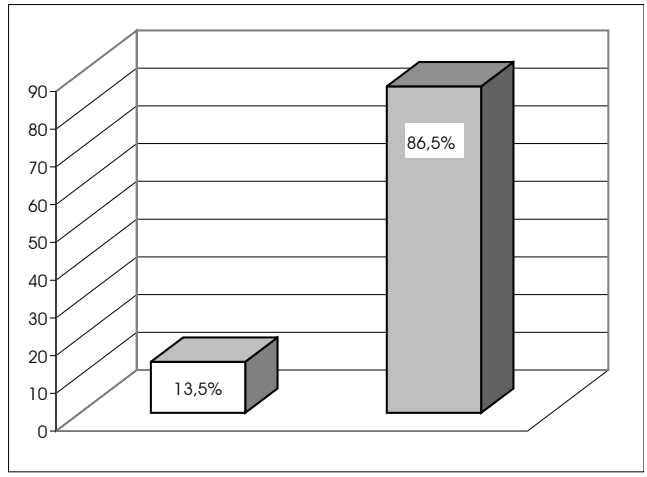

Fig. 4. Previous contact with the pathogen (13.5%) and chronic *C. Pneumoniae* infection (86.5%) in AAA patients.

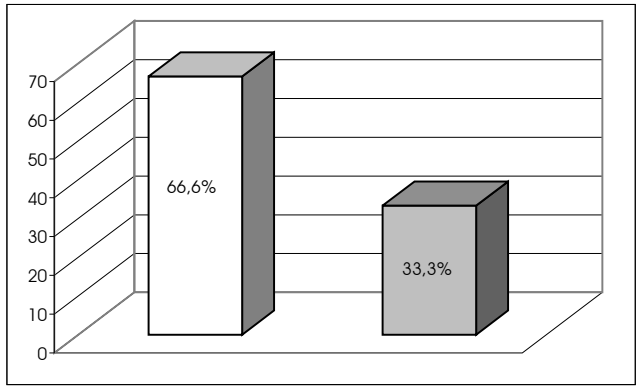

Fig. 5. Previous contact with the pathogen (66.6%) and chronic *C. pneumoniae* infection (33.3%) in healthy controls

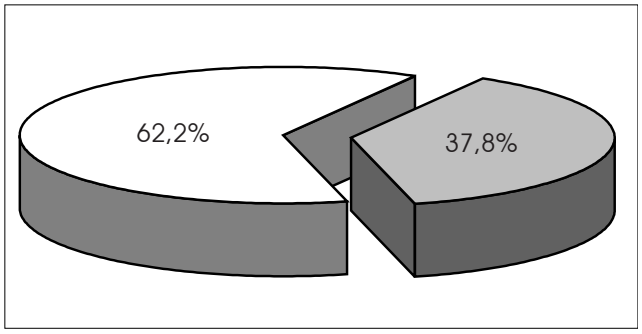

Fig. 6. Chronic active (37.8%) and chronic non-active (62.2%) *C. pneumoniae* infection in AAA patients.

TABLE 1

Results of PCR, IDFA, and histopathological examination in serologically defined chronic active *C. pneumoniae* infection group of AAA patients

No	PCR	IDFA	INFLAMMATION
5.	+	+	1
10.	+	+	1
11.	-	-	1
14.	-	+	1
19.	+	+	2
21.	+	+	1
22.	+	+	1
23.	+	+	1
28.	+	+	1
33.	+	+	1
34.	-	-	1
37.	-	+	1
42.	+	+	2
44.	+	+	1
45.	+	+	1
46.	+	+	1
51.	+	+	1

PCR and IDFA: + – positive result of PCR or IDFA; – – negative result of PCR or IDFA; Inflammation: 1 – moderate inflammatory infiltrations in aortal wall sample; 2 – abundant inflammatory infiltrations in aortal wall sample

symptomatic aneurysm. However, since the group was small in number, Fisher's exact test revealed that there had been no statistically significant association between numerous inflammatory infiltration and symptomatic aneurysm (p=0.154).

In all patients belonging to abundant inflammatory infiltration group the presence of *C. pneumoniae* in aorta samples was detected using direct detection methods – in 7 of them by means of two applied methods and in 4 others – by one method only. Nine out of 11 (81.8%) patients in this group had serological signs of chronic non-active *C. pneumoniae* infection and 2/11 (18.2%) of active infection (Table 2).

TABLE 2

Results of PCR, IDFA, serological examination and the presence of symptoms in abundant inflammatory infiltration group of AAA patients

No	SEROLOGY	PCR	IDFA	SYMPTOMS
2.	1	+	+	+
8.	1	+	-	+
12.	1	+	+	+
13.	1	-	+	-
19.	2	+	+	+
25.	1	+	+	+
31.	1	+	-	+
35.	1	+	+	+
36.	1	-	+	+
42.	2	+	+	+
48.	1	+	+	+

Serology: 1 – chronic non-active *C. pneumoniae* infection; 2 – chronic active *C. pneumoniae* infection; Symptoms: + symptomatic aneurysm; - – lack of aneurysm symptoms

Overall results of the serological examination, direct microorganism identification, histological traits of the inflammation in the AAA wall and the symptoms of aneurysm are summarized in Table 3.

Discussion

The methods of direct *C. pneumoniae* detection in arterial tissues include antigen detection by immunocytochemistry (ICC) or immunofluorescence antibody technique (IDFA), DNA detection by PCR and *in situ* hybridization, EM, and isolation in culture. In our study PCR, immunofluorescence antibody technique (IDFA), and, in selected samples, EM were used. Detection rates of *C. pneumoniae* DNA by PCR in aorta wall samples taken from AAA patients varies between 50% and 100% among studies and investigators [2, 10, 11, 27, 31]. However, novel multicenter comparison trial reported that the rate of detection of *C. pneumoniae* DNA within endarterectomy specimens by PCR varies between 0% and 100% [1]. Possible reasons of the discrepancy in detection rates include the localized nature of chlamydial pathology (the distribution of bacteria in atherosclerotic lesions is localized or patchy), the small amount of tissue examined, differences in laboratory procedures applied (lack of standardization of PCR assays) [15, 22]. In our study *C. pneumoniae* DNA was detected by PCR in 84.62% of AAA patients. Rela-

Table 3
Overall results of the serologic examination, direct microorganism identification (PCR, IDFA, electron microscopy), histological traits of inflammation in the AAA wall and the symptoms of aneurysm in AAA patients

NO	IDFA	PCR	INFLAMMATION	EM	SEROLOGY	SYMPTOMS
1.	–	+	1		0	–
2.	+	+	2		1	+
3.	+	+	1		1	–
4.	+	+	1		1	–
5.	+	+	1	+	2	+
6.	+	+	0		1	+
7.	+	+	0		1	–
8.	–	+	2		1	+
9.	+	+	0		1	+
10.	+	+	1	–	2	+
11.	-	–	1		2	+
12.	+	+	2		1	+
13.	+	–	2		1	–
14.	+	–	1	+	2	+
15.	+	–	0		1	+
16.	+	+	1		1	–
17.	+	+	1		1	–
18.	+	+	1		0	+
19.	+	+	2	+	2	+
20.	+	+	1		0	+
21.	+	+	1	–	2	+
22.	+	+	1	+/–	2	+
23.	+	+	1	+/–	2	+
24.	–	+	1		0	–
25.	+	+	2		1	+
26.	+	+	1		1	–
27.	+	+	1		1	–
28.	+	+	1		2	+
29.	+	+	0		1	+
30.	+	+	0		1	–
31.	-	+	2		1	+
32.	+	+	0		1	+
33.	+	+	1		2	+
34.	–	–	1		2	+
35.	+	+	2		1	+
36.	+	–	2		1	+
37.	+	–	1		2	+
38.	+	–	0		1	+
39.	+	+	1		1	–
40.	+	+	1		1	–
41.	+	+	1		0	+
42.	+	+	2		2	+
43.	+	+	1		0	+
44.	+	+	1		2	+
45.	+	+	1		2	+
46.	+	+	1		2	+
47.	–	+	1		0	–
48.	+	+	2		1	+
49.	+	+	1		1	–
50.	+	+	1		1	+
51	+	+	1		2	+
52	+	+	0		1	–

PCR and IDFA: + – positive result of PCR or IDFA; – – negative result of PCR or IDFA; Inflammation: 0 – lack of inflammatory infiltrations in aortal wall sample; 1 – moderate inflammatory infiltrations in aortal wall sample; 2 – abundant inflammatory infiltrations in aortal wall sample; Serology: 0 – previous contact with the pathogen; 1 – chronic non-active *C. pneumaniae* infection; 2 – chronic active *C. pneumoniae* infection; Symptoms: + – symptomatic aneurysm; – – lack of aneurysm symptoms; Electron microscopy: + – presence of *C. pneumoniae* EB, RB and IB in aortic wall sample; +/– – controversial result of EM examination; – – lack of *C. pneumoniae* EB, RB and IB aortic wall sample.

tively high rate of positive results in our study may depend on the method – nested PCR is, in general, more sensitive than single-step PCR because of the 2-step amplification and the use of 2 sets of primers. However, while increased sensitivity is an advantage of nested PCR, carryover contamination is a major disadvantage of this method. The procedure of nested PCR used by us had higher sensitivity in comparison with conventional PCR (0.06 IFU vs. 4 IFU) [22]. Moreover, in recent recommendations for standardizing *C. pneumoniae* assays, this procedure was considered as satisfying the optimal criteria for a validated assay [6].

A lack of correlation between ICC or IDFA and PCR in the detection of *C. pneumoniae* in arterial tissue has been shown in latest review, in which 30 published papers devoted to this subject were summarized [15]. It appeared that only 25–50% of the specimens were positive by both tests. Moreover, ICC or IDFA had higher detection rates than PCR (37% vs. 24%, or 68% vs. 29%, depending on the studies). This finding is contrary to the sensitivity of the tests, since PCR is more sensitive than ICC or IDFA. Lower detection rate with PCR has been attributed to the difficulty in extracting DNA from atheromatous tissue and to the presence of inhibitors for PCR in atherosclerotic vessels [15]. Moreover, in recent study Vammen at al. stated that detection of *C. pneumoniae* by ICC procedures should be interpreted with caution due to the potential cross-reaction with non-chlamydial proteins [43]. In our study detection rates for IDFA and PCR were 86.54% and 84.62%, respectively. IDFA detection rate was only insignificantly higher than that of PCR. Accordance rate was higher as compared with literature data – 78.8% of aorta samples in our study were positive by both tests.

Serology constitutes an important tool for the diagnosis of *C. pneumoniae* infection. MIF test, which was used in our study, had become the serological "gold standard" for diagnosis of infections with this pathogen, being highly specific and sensitive when compared with culture. It was the use of the MIF that led to the identification of *C. pneumoniae* as a distinct species of *Chlamydia*. It is the only species-specific antibody test available that can measure isotype-specific antibody titers to all *Chlamydia* species simultaneously. The specificity of the MIF test can be attributed to the use of purified elementary bodies of all 3 species of *Chlamydia* rather than reticulate bodies that express predominantly genus-specific epitopes [6]. However, the assay is technically complex, interpretation is subjective, and neither reagents nor diagnostic criteria have been standardized [30, 40]. The criteria used in our work were restricted. IgG and IgA titers applied by us for MIF results interpretation were the highest in comparison with the ones used by other authors [28].

One of the most challenging aspects of *C. pneumoniae* testing is the identification of persons with persistent or chronic infection by means of serological testing. Persistently elevated IgG or the presence of IgA antibodies have been frequently used [3, 35, 36]. The persistence of elevated antibody titers is generally considered to be a sign of chronic infection [12, 37]. There are no means to distinguish between chronic and recurrent infections reliably, although the constant presence of IgA antibodies has been proposed to indicate chronic infection. IgA is a short-living immunoglobulin (half-life: 5–7 days), hence its presence implies that the antigen is still present. A high IgA titer is considered to be a marker for various chronic bacterial infections, including *Pseudomonas aeruginosa* infection in cystic fibrosis. Circulating specific IgA is undoubtedly a marker of the presence of *C. pneumoniae* and it may indicate that the infection is chronic and still active [12]. Constantly elevated levels of more long-living IgG antibodies (their half-life is measured in weeks or months) may reflect recurrent infections in the past. IgM antibodies are considered to indicate primary acute infection [9]. However, there is at present no validated serologic marker of persistent or chronic infection [6].

In our study we observed statistically significant higher frequency of serologically defined chronic *C. pneumoniae* infection in AAA patients, as compared with healthy controls (86.5% vs. 33.3%). Our results are in accordance with those obtained by other investigators [18].

Studies using direct detection methods of *C. pneumoniae* DNA or antigen in the aneurysm wall or atherosclerotic plaques and indirect serological detection have reported poor concordance between the two methods [15, 20, 34]. This inconsistency may be explained by inhibitors of PCR reaction, which are present in human specimens and patchy occurrence of *C. pneumoniae* in lesions but, in fact, it remains an unresolved paradox with no good explanation. There is a suggestion that PCR and serum MIF should not be compared because they probably detect an infection at different stages; PCR may detect cases at an acute stage whereas in chronic cases DNA detection by PCR is probably unsuccessful [8]. Accordingly to other studies direct detection of *C. pneumoniae* antigens and DNA in lesions seems more frequent in people with low rather than with high titers of specific antibodies [2].

In our work *C. pneumoniae* infection was detected serologically and, simultaneously, at least by one of two applied direct detection methods in 82.7% of AAA patients. Direct detection proved to be negative in 2 patients who had high titers of specific IgG and IgA. Seven patients who had low

titers of specific IgG and IgA, indicating previous contact with the pathogen but not infection, appeared positive in direct detection methods.

Inflammation and proteolysis are the twin processes considered to underlie aortic dilation, with matrix metalloprotease-9 being considered as one of the pivotal proteolytic enzymes involved in aneurysm expansion in men and experimental animals [38]. *C. pneumoniae* is able to induce an inflammatory response in infected tissues [23]. The chlamydial membrane antigens and the secreted heat shock protein-60 stimulate the secretion of matrix metalloprotease-9 from human macrophages *in vitro* [13, 14]. By contrast, in novel study, Petersen at al. did not confirm the hypothesis that the presence of *C. pneumoniae* DNA in the AAA wall is associated with increased activity of matrix metalloproteases [32]. This suggests that factors other than metalloproteases may contribute to matrix degradation and rupture of *C. pneumoniae* DNA- positive AAA. Membrane antigens of *C. pneumoniae* were found in all samples of AAA, even in the absence of DNA and heat shock protein [24]. In recent rabbit experimental model chlamydial membrane antigens (rather than living organisms) appeared to cause aneurysmal dilation and associated macrophage recruitment and activation [39].

In our study special attention have been paid to 2 groups of patients with symptomatic aneurysm namely serologically defined active infection group and patients with abundant inflammatory infiltration in their aorta samples (in this group there was one patient without symptomatic aneurysm).

In 88.2% of the patients in active infection group *C. pneumoniae* was detected by at least one of the applied direct detection methods. All but 2 patients in this group had moderate inflammatory infiltrations and all had symptomatic aneurysm. This may suggest that in the patients with high titers of specific anti-*C. pneumoniae* antibodies local inflammatory reaction is not the main process responsible for aneurysm rupture. In our previous study [46] it was found that in this group of patients IL-12 and IFN-gamma serum concentrations were significantly lower than in the remaining AAA patients and healthy controls suggesting down-regulated cell-mediated immune response, which in turn could be the cause of chronic infection reactivation. Living and actively replicating intracellular bacteria may activate the processes of aortic wall degradation in another way than chronic (cryptic) form of bacteria or persistent antigens [7, 20].

In the recent work of Lindholt and al. the progression of AAA in men was positively correlated with the presence of indicators of *C. pneumoniae* infection. A significant positive correlation between both IgA and IgG titers and mean annual expansion of AAA was observed. An IgG titer of 1:128 or higher was present significantly more often in cases with an expansion greater than 1cm annually [19]. In the study of Vammen et al. it was claimed that antibodies against *C. pneumoniae* predict the need for elective intervention on small aneurysms. IgG antibodies were most predictive for cases expanding to operation recommendable sizes [41]. Similarly, our study also confirmed that high titers of anti-*C. pneumoniae* IgG and IgA correlated with symptomatic aneurysm.

In all patients with abundant inflammatory infiltration *C. pneumoniae* was detected serologically and at least by one of two direct detection methods. In 81.8% of the patients in this group serologically defined chronic non-active infection was detected and active infection in 18.2%. Providing that membrane antigens rather than living bacteria enhance inflammatory infiltration in aortic wall [39], the data suggest that in serologically defined chronic non-active infection *C. pneumoniae* antigens or perhaps special, cryptic form (typical for persistent infection) are present in lesions rather than actively replicating bacteria. In our previous study serum IL-12 and IFN-gamma concentrations appeared to be higher in this group of patients in comparison with the active infection group, suggesting that the patients with chronic non-active infection were able to control the infection [46]. So, it can be concluded that *C. pneumoniae* can participate in AAA formation *via* different pathways, depending on the form of infection. Consequently, AAA patients infected with *C. pneumoniae* seem not to be a homogenous group. Active infection group is much more prone to have symptomatic aneurysm than chronic non-active infection group. Serology can be very useful in differentiating these two groups and in predicting AAA expansion and the risk of rupture and therefore standardization of serological techniques and the development of uniform criteria for interpretation of serologic findings is necessary [40].

It has been shown that *C. pneumoniae* can replicate in human macrophages, endothelial cells, and smooth muscle cells [7]. The presence of all forms of *C. pneumoniae*, namely IB, EB and RB in aorta samples examined by us in electron microscope confirms these findings. However, the exact role of *C. pneumoniae* in the pathogenesis of vascular disease, including AAA, is still not known. Inter-laboratory standardization of both, direct and indirect detection methods of *C. pneumoniae* is absolutely required to precisely elucidate the role of these bacteria in AAA development.

Conclusions

1. AAA patients infected with *C. pneumoniae* are not a homogenous group. "High serology" group is much more prone to have symptomatic aneurysm than the remaining AAA patients.
2. Serology can be very useful in predicting the risk of AAA rupture.
3. Inter-laboratory standardization of direct and indirect detection methods of *C. pneumoniae* infection is required to elucidate the role of these bacteria in AAA development.

References

1. *Apfalter P, Blasi F, Boman J et al:* Multicenter comparison trial of DNA extraction methods and PCR assays for detection of Chlamydia pneumoniae in endarterectomy specimens. J Clin Microbiol 2001, 39(2), 519–524.
2. *Blasi F, Denti F, Erba M et al:* Detection of Chlamydia pneumoniae but not Helicobacter pylori in atherosclerotic plaques of aortic aneurysms. J Clin Microbiol 1996, 34, 2766–2769.
3. *Cook PJ, Honeybourne D, Lip GY et al:* Chlamydia pneumoniae antibody titers are significantly associated with acute stroke and transient cerebral ischemia: the West Birmingham Stroke Project. Stroke 1998, 29, 404–410.
4. *Davies MJ, Woolf N:* Atherosclerosis: what is it and why does it occur? Br Heart J 1993, 69, S3–S11.
5. *Davies MJ:* Aortic aneurysm formation. Lessons from human studies and experimental models. Circulation 1998, 98, 193–195.
6. *Dowell SF, Peeling RW, Boman J et al:* Standardizing Chlamydia pneumoniae assays: Recommendations from the Centers for Disease Control and Prevention (USA) and the Laboratory Centre for Disease Control (Canada). Clin Infect Dis 2001, 33, 492–503.
7. *Gaydos CA, Summersgill JT, Sahney NN et al:* Replication of Chlamydia pneumoniae in vitro in human macrophages, endothelial cells and aortic smooth muscle cells. Infect Immunol 1996, 64, 1614–1620.
8. *von Hertzen L, Alakärppä H, Koskinen R et al:* Chlamydia pneumoniae infection in patients with chronic obstructive pulmonary disease. Epidemiol Infect 1997, 118, 155-164.
9. *von Hertzen L, Töyrylä M, Gimishanov A et al:* Asthma, atopy and Chlamydia pneumoniae antibodies in adults. Clin Exp Allergy 1999, 29, 522–528.
10. *Juvonen J, Juvonen T, Laurila A et al:* Demonstration of Chlamydia pneumoniae in the walls of abdominal aortic aneurysms. J Vasc Surg 1997, 25, 499–505.
11. *Karlsson L, Gnarpe J, Naas J et al:* Detection of viable Chlamydia pneumoniae in abdominal aortic aneurysms. Eur J Vasc Endovasc Surg 2000, 19, 630–635.
12. *Kato A, Odamaki M, Takita T et al:* Association between interleukin-6 and carotid atherosclerosis in hemodialysis patients. Kidney International 2002, 61, 1143–1152.
13. *Kol A, Burcier T, Lichtman AH et al:* Chlamydial and human heat shock protein 60 activate human vascular endothelium, smooth muscle cells, and macrophages. J Clin Invest 1999, 103, 571–577.
14. *Kol A, Sukhova GK, Lichtman AH et al:* Chlamydial heat shock protein 60 localizes in human atheroma and regulates macrophage tumor necrosis-alpha and matrix metalloproteinase expression. Circulation 1998, 98, 300–307.
15. *Kuo CC, Campbell LA:* Detection of Chlamydia pneumoniae in arterial tissue. J Infect Dis 2000, 181(Suppl 3), S432–S436.
16. *Kuo CC, Jackson LA, Campbell LA et al:* Chlamydia pneumoniae (TWAR). Clin Microbiol Rev 1995, 8(4), 451–461.
17. *Laurila A, von Hertzen L, Saikku P:* Chlamydia pneumoniae and chronic lung diseases. Scand J Infect Dis Suppl 1997, 104, 34–36.
18. *Leinonen M, Saikku P:* Evidence for infectious agents in cardiovascular disease and atherosclerosis. Lancet Infect Dis 2002, 2, 11–17.
19. *Lindholt JS, Ashton HA, Scott RA:* Indicators of infection with Chlamydia pneumoniae are associated with expansion of abdominal aortic aneurysms. J Vasc Surg 2001, 34(2), 212–215.
20. *Lindholt JS, Juul S, Vammen S et al:* Immunoglobulin A antibodies against Chlamydia pneumoniae are associated with expansion of abdominal aortic aneurysm. Br J Surg 1999, 86, 634–638.
21. *Mac Sweeney STR, Powel JT, Geenhalgh RM:* Pathogenesis of abdominal aortic aneurysm. Br J Surg 1994, 81, 935–941.
22. *Mahony JB, Chong S, Coombes BK et al:* Analytical sensitivity, reproducibility of results, and clinical performance of five PCR assays for detecting Chlamydia pneumoniae DNA in peripheral blood mononuclear cells. J Clin Microbiol 2000, 38, 2622–2627.
23. *Mahony JB, Combes BK:* Chlamydia pneumoniae and atherosclerosis: does the evidence support a causal or contributory role? FEMS Microbiol Lett 2001, 197, 1–9.
24. *Meijer A, Roholl PJ, Gielis-Proper SK et al:* Chlamydia pneumoniae antigens, rather than viable bacteria, persist in atherosclerotic lesions. J Clin Pathol 2000, 53(12), 911–916.
25. *Mosorin M, Juvonen J, Biancari F et al:* Use of doxycycline to decrease the growth rate of abdominal aortic aneurysms: A randomized, double-blind, placebo-controlled pilot study. J Vasc Surg 2001, 34(4), 606–610.
26. *Noll G:* Pathogenesis of atherosclerosis: a possible relation to infection Atherosclerosis 1998, 140(Suppl 1), S3–S9.
27. *Ong G, Thomas BJ, Mansfield AO et al:* Detection and widespread distribution of Chlamydia pneumoniae in the vascular system and its possible implications. J Clin Pathol 1996, 49, 102–106.
28. *Orfila JJ:* Seroepidemiological evidence for an association between Chlamydia pneumoniae and atherosclerosis. Atherosclerosis 1998, 140(Suppl 1), S11.
29. *Patel M, Hardman DTA, Fischer CM et al:* Current view on the pathogenesis of abdominal aortic aneurysms. J Am Col Surg 1995, 181, 371–382.
30. *Peeling RW, Wang SP, Grayston JT et al:* Chlamydia pneumoniae serology: interlaboratory variation in microimmunofluorescence assay results. J Infect Dis 2000, 181(Suppl 3), S426–S429.
31. *Petersen E, Boman J, Persson K et al:* Chlamydia pneumoniae in human abdominal aortic aneurysms. Eur J Vasc Endovasc Surg 1998, 15, 138–144.
32. *Petersen E, Boman J, Wagberg F et al:* Presence of Chlamydia pneumoniae in abdominal aortic aneurysms is not associated with increased activity of matrix metalloproteinases. Eur J Vasc Endovasc Surg 2002, 24, 365–369.
33. *Petersen E, Boman J, Wagberg F et al:* In vitro degradation of aortic elastin by Chlamydia pneumoniae. Eur J Vasc Endovasc Surg 2001, 22, 443–447.
34. *Porqueddu M, Spirito R, Parolari A et al:* Lack of association between serum immunoreactivity and Chlamydia pneumoniae detection in the human aortic wall. Circulation 2002, 106(21), 2647–2648.
35. *Saikku P, Leinonen M, Mattila K et al:* Serological evidence of an association of a novel Chlamydia, TWAR, with chronic coronary heart disease and acute myocardial infarction. Lancet 1988, 2, 983–986.
36. *Saikku P, Leinonen M, Tenkanen L et al:* Chronic Chlamydia pneumoniae infection as a risk factor for coronary heart disease in the Helsinki Heart Study. Ann Intern Med 1992, 116, 273–278.

37. *Saikku P:* Problems in diagnosis of chronic Chlamydia pneumoniae infections. In: Rapid Methods and Automation in Microbiology and Immunology. Vaheri A, Tilton RC, Balows A, eds. Springer Verlag 1991, 309–313.
38. *Shah PK:* Inflammation, metalloproteinases, and increased proteolysis: an emerging pathological paradigm in aortic aneurysm. Circulation 1997, 96, 2115–2117.
39. *Tambiah J, Powell JT:* Chlamydia pneumoniae antigens facilitate experimental aortic dilatation: Prevention with azithromycin. J Vasc Surg 2002, 36, 1011–1017.
40. *Tuuminen T, Palomaki P, Paavonen J:* The use of serologic tests for the diagnosis of chlamydial infections. J Microbiol Methods 2000, 42, 265–279.
41. *Vammen S, Lindholt JS, Andersen PL et al:* Antibodies against Chlamydia pneumoniae predict the need for elective surgical intervention on small abdominal aortic aneurysms. Eur J Vasc Endovasc Surg 2001, 22, 165–168.
42. *Vammen S, Lindholt JS, Ostergaard L et al:* Randomized double- blind controlled trial of roxithromycin for prevention of abdominal aortic aneurysm expansion. Br J Surg 2001, 88, 1066–1072.
43. *Vammen S, Vorum H, Ostergaard L et al:* Immunoblotting analysis of abdominal aortic aneurysms using antibodies against Chlamydia pneumoniae recombinant MOMP. Eur J Vasc Endovasc Surg 2002, 24, 81–85.
44. *White JV, Haas K, Phillips S et al:* Adventitial elastolysis is a primary event in aneurysm formation. J Vasc Surg 1993, 17, 371–380.
45. *Wills A, Thompson MM, Crowther M et al:* Pathogenesis of abdominal aortic aneurysms – cellular and biochemical mechanisms. Eur J Vasc Endovasc Surg 1996, 12, 391–400.
46. *Wolski A, Niedźwiadek J, Mazur E et al:* Chlamydia pneumoniae infection in abdominal aortic aneurysm patients. Acta Angiol 2001, 7, 1–8.

Address for correspondence and reprint requests to:
Elżbieta Mazur M.D., Ph.D.
Department of Medical Microbiology
Chodźki 1, 20-093 Lublin
Tel: 081 7405837
Fax: 081 7405733
e-mail: elamazur@yahoo.com