Chlamydia pneumoniae Seropositivity and Systemic and Renovascular Atherosclerotic Disease

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Background: Patients with hypertension may be vulnerable to vascular Chlamydia pneumoniae and/or cytomegalovirus (CMV) infection because of increased expression of adhesion molecules.

Objective: To determine whether C pneumoniae or CMV is associated with the presence of atherosclerotic lesions in hypertensive patients.

Methods: Ninety-six angiographic studies on 100 consecutive patients with clinical signs or symptoms suggestive of renovascular hypertension were reviewed for the presence or absence of atherosclerotic lesions at the level of the renal arteries as well and abdominal aorta. Also, the presence of a hemodynamically notable renal artery stenosis and antibodies to C pneumoniae (IgG and IgA) and CMV (IgG and IgM) was determined, and all classic risk factors were recorded.

Results: Atherosclerotic lesions were documented in 67 patients (70%), and in 49 patients (51%) such lesions were present at the level of the renal artery. In the univariate analysis, significant associations between IgG (odds ratio, 3.8; 95% confidence interval, 1.2-11.7; \( P = .02 \)) as well as IgA (odds ratio, 2.6; 95% confidence interval, 1.1-6.7; \( P = .03 \)) antibodies to C pneumoniae and the presence of atherosclerosis were found for both the aorta and the renal arteries. Seroprevalence (IgG) to C pneumoniae in the 23 patients with a hemodynamically notable renal artery stenosis was 100% and differed (\( P = .01 \)) from those without a notable renal artery stenosis (78%). In the multivariate analysis, IgG seropositivity to C pneumoniae was significantly associated with atherosclerosis (odds ratio, 6.0; 95% confidence interval, 1.3-27.5; \( P = .02 \)), and age. There was no association between CMV seropositivity and atherosclerosis.

Conclusion: The presence of antibodies to C pneumoniae was significantly associated with atherosclerosis and renovascular disease in hypertensive patients in whom a renal artery stenosis was strongly suspected.

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HIGH BLOOD pressure, elevated serum cholesterol level, and smoking are major risk factors for the development of atherosclerosis. Although still disputed, an increasing body of evidence suggests that Chlamydia pneumoniae and, to a lesser extent, cytomegalovirus (CMV) infection may also be involved in the pathogenesis of atherosclerosis.1-3 Some studies indicate that there is an interaction between these infections and the classical risk factors for atherosclerosis.4 For instance, C pneumoniae is more common in smokers5,6 and infections in general, and more specific C pneumoniae can induce proatherogenic changes in lipoprotein metabolism.7 The possible interaction between vascular infections and hypertension in the development of atherosclerotic disease has received little attention, although hypertension may favor a C pneumoniae or CMV infection. In hypertension, elevated levels of adhesion molecules have been reported,8 possibly induced by angiotensin.9 These adhesion molecules allow for the recruitment of monocytes to the vascular tree, supporting neointimal monocyte infiltration, which is a key step in atheroma formation. The recruitment of infected monocytes into the plaque, (thereby introducing an infection in the plaque) may significantly contribute to the process of premature atherosclerosis.

Angiographic studies have only visualized the coronary arteries and may have been biased toward the selection of patients with unstable plaques.10-14 In our hospital, an intra-arterial digital subtraction renal angiography is carried out in hypertensive patients in whom a renal artery stenosis is suspected. During this investigation, a large segment of the aorta below the diaphragma was visualized as well. Moreover, renal angiography is often carried out in relatively young patients, in whom symptoms of extensive vascular disease are mostly absent.

We speculated that in hypertensive patients a C pneumoniae and/or a CMV infection contribute to the development of
PARTICIPANTS AND METHODS

SUBJECTS

Between October 1998 and October 1999, we investigated 100 consecutive patients with hypertension of a suspected renovascular origin who attended the hypertension outpatient clinic of the University Hospital Maastricht, Maastricht, the Netherlands. The diagnosis of hypertension was made when office systolic blood pressure was above 140 mm Hg and/or diastolic blood pressure was above 90 mm Hg on at least 3 different occasions. If on the basis of clinical or ultrasound data renovascular disease was suspected, patients underwent digital subtraction angiography. Other major causes of secondary hypertension were excluded biochemically before the patients underwent angiography of the renal arteries. The following clinical criteria were used to select patients for angiography: treatment-resistant hypertension (ie, elevated blood pressure despite treatment with 2 or more antihypertensive drugs), renal dysfunction induced by an angiotensin-converting enzyme inhibitor, smoking and diastolic blood pressure above 110 mm Hg, malignant or accelerated hypertension, and extrarenal atherosclerosis in 2 or more organ systems. Demographic characteristics and risk factors such as smoking, diabetes mellitus, and lipid disorders were recorded. Hypercholesterolemia was defined as a serum total cholesterol level of above 251 mg/dL (6.5 mmol/L), and combined hyperlipidemia was diagnosed if triglyceride level was above 195 mg/dL (2.2 mmol/L) as well. At the time angiography was performed, blood was collected, and 2 mL of serum was obtained by centrifugation and stored at −80°C until serological analysis. Written informed consent was obtained from all patients, and the medical ethical committee of the Maastricht University hospital had approved the study protocol.

SEROLOGICAL TESTS AND RADIOLOGICAL EVALUATION

Serum IgG and IgA antibodies against C pneumoniae were detected using an enzyme-linked immunosorbent assay (C pneumoniae IgG and IgA detection kits; Labsystems, Helsinki, Finland). The presence or absence of IgG and IgA antibodies against C pneumoniae was determined by comparing the absorbance value of the sample with a cutoff value, as specified by the manufacturer. Specimens with values greater than the cutoff value (IgG >30 enzyme immunoassays and IgA >8 enzyme immunoassays) were positive. This assay shows good agreement with the microimmunofluorescence method,

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16,17 which was confirmed in our laboratory. When we tested 83 blood samples from healthy donors, a sensitivity of 95% and a specificity of 100% were recorded with the microimmunofluorescence assay as the gold standard. For the qualitative detection of IgM antibodies against CMV in serum, the ImX CMV assay (Abbott Laboratories, Abbott Park, Ill) was used, while IgG was measured semiquantitatively by the AXSym (Abbott Laboratories). Both tests are based on microparticle enzyme immunoassay technology. Specimens with values above the cutoff value (IgG >15 antibody units/mL and IgM index value >0.4 g/L) were considered positive.20 All serological studies were performed in a blinded fashion.

Digital subtraction angiographies were reviewed by 2 independent radiologists for the presence or absence of atherosclerotic lesions in both renal arteries and the aorta below the diaphragma. Results were given on the basis of consensus. If the results of the 2 radiologists did not agree, a third independent radiologist reevaluated the radiographs and defined the outcome of these cases. The presence of hemodynamically notable renal artery stenosis was recorded as well if there was more than a 50% stenosis in 1 or both of the renal arteries and/or an indication for renal artery angioplasty (venoarterial renin ratio >1.5).

STATISTICAL ANALYSIS

The association of atherosclerotic lesions with C pneumoniae and CMV antibodies, age, sex, smoking habits, diabetes, hyperlipidemia, and severity of hypertension was analyzed by univariate regression and expressed as odds ratios (ORs) with 95% confidence intervals (CIs). With a multivariate logistic regression model, ORs for the C pneumoniae and CMV antibodies and their 95% CIs were determined and adjusted for age, sex, smoking habits, diabetes, hyperlipidemia, and the severity of hypertension. Patients were divided according to age in quartiles as follows: quartile 1, 23 to 45 years; quartile 2, 46 to 52 years; quartile 3, 53 to 63 years, and quartile 4, 64 to 78 years. The χ² test or, when appropriate, Fisher exact test (both 2-sided), was used to test for significance. A P value of .05 was considered statistically significant. Statistical analysis was performed with SSPS software (SSPS Inc, Chicago, Ill) for personal computer.

atherosclerotic lesions, including those of the renal arteries. After all, renal artery stenoses are mostly considered only one facet of generalized atherosclerotic disease.15–17 The present study was designed to determine whether C pneumoniae or CMV seropositivity is associated with angiographically demonstrated atherosclerotic lesions in the renal artery and/or the aorta below the diaphragma in hypertensive patients.

RESULTS

Because of incomplete angiographic study results, the analyses were performed on data from 96 of the 100 patients who comprised our study. Demographic characteristics and the distribution of the classic risk factors for atherosclerosis in patients with and without angiographically demonstrated atherosclerotic lesions are presented in Table 1. Mean age and sex differed between patients with and without atherosclerosis. Patients with documented atherosclerosis were significantly older (58 years vs 45 years; P < .001) and more often men (68% vs 41%; P = .02) compared with those without atherosclerosis. Atherosclerotic lesions at the level of the renal artery were found in 49 patients (mean age, 58 ± 11 years), and in 23 patients (mean age, 61 ± 11 years) a hemodynamically notable renal artery stenosis was found. Fibromuscular dysplasia of the renal artery was documented in 9 patients, 8 of whom had IgG antibodies to C pneumoniae. The patients with fibromuscular dysplasia of the renal artery were evaluated for the presence or absence of an atherosclerotic lesion but were excluded from the evaluation if a hemodynamically notable renal artery stenosis was present. The presence or absence of an ath-
renal artery; and E, patients with a hemodynamically significant renal artery stenosis.

In 23 patients, IgG seropositivity was found more often than in those in whom no notable renal artery stenosis could be detected (23 [100%] vs 50 [78%]; \( P = .001 \)). The difference in seropositivity for IgA antibodies to \( C \) pneumoniae (19 [82%] vs 40 [62%]; \( P = .07 \)) did not reach statistical significance.

In 11 patients (mean age, 55 years) atherosclerotic lesions were found only at the level of the renal arteries and not in the aorta. All 11 patients were \( C \) pneumoniae seropositive, and 7 had a hemodynamically notable renal artery stenosis.

The univariate association of atherosclerosis with age, sex, smoking, diabetes mellitus, hyperlipidemia, severity of hypertension, and \( C \) pneumoniae and CMV antibodies is demonstrated in Table 3. Significant associations were found for age (\( P = .003 \)) and IgG (\( P = .02 \)) and IgA (\( P = .03 \)) antibodies to \( C \) pneumoniae. Smoking (even when analyzed separately for present smoking or a history of smoking), severity of hypertension, diabetes mellitus, and CMV antibodies were not associated with the presence of atherosclerosis. The association between atherosclerosis and hyperlipidemia did not reach significance.

In multivariate logistic regression analysis, the OR for \( C \) pneumoniae IgG seropositivity and atherosclerosis was 6.04 (95% CI, 1.33-27.5; \( P = .02 \)). Age was the other variable that contributed significantly to atherosclerosis: quartile 2 vs 1, OR, 2.89 (95% CI, 0.71-11.8; \( P = .1 \)); quartile 3 vs 1, OR, 10.1 (95% CI, 1.71-59.3; \( P = .01 \)); and quartile 4 vs 1, OR, 4.94 (95% CI, 0.99-24.6; \( P = .05 \)).
of the other variables showed an association with atherosclerosis in this multivariate regression. When the analysis was performed with IgA antibodies to \textit{C pneumoniae} (OR, 2.99; 95\% CI, 0.93-9.16; \(P = .06\)), only age remained significantly associated with atherosclerosis.

Because the seroprevalence of \textit{C pneumoniae} increases with age,\(^\text{19}\) we analyzed the cumulative frequency of atherosclerotic lesions in hypertensive patients who were \textit{C pneumoniae} IgG seropositive or seronegative (Figure and Table 4). A similar graph is obtained when analyzing the cumulative prevalence of renal atherosclerosis over time; all patients younger than 50 years with renal atherosclerotic disease were \textit{C pneumoniae} IgG seropositive. The mean age of \textit{C pneumoniae} seropositive patients (57 years), however, did not differ from that of the seronegative patients (59 years).

In this study we demonstrated that IgG antibodies to \textit{C pneumoniae} are significantly more present in hypertensive patients with angiographically demonstrated atherosclerotic lesions compared with those in whom no atherosclerosis could be detected. An association between \textit{C pneumoniae} and atherosclerosis was found for both the renal arteries and the abdominal aorta. To our knowledge, the association between angiographically demonstrated arterial disease and \textit{C pneumoniae} has been investigated only in coronary heart disease.\(^\text{12-16}\) Some studies found a significant association with ORs varying between 1.8 and 3.5, while others did not find an association. The endothelial surface that was investigated in the present study is large compared with the coronary system, and this factor, as well as there being a different selection of patients, may have contributed to the strong association. IgA antibodies to \textit{C pneumoniae} may indicate a persistent, chronic infection; however, contrary to IgG, these antibodies were only significantly associated with atherosclerosis in the univariate analysis, but not after adjusting for the classic risk factors in the multivariate analysis. This may indicate that \textit{C pneumoniae} contributes more to the initiation of atherosclerotic lesions than to their progression.

The prevalence of atherosclerosis increases with age, and we found that the hypertensive patients with atherosclerosis were significantly older than those without atherosclerosis (59 vs 49 years; \(P < .001\)). In the group of patients with documented atherosclerosis, those seropositive for \textit{C pneumoniae} were of similar age to the seronegative subjects. However, all patients younger than 50 years with atherosclerotic lesions were \textit{C pneumoniae} seropositive.

An atherosclerotic lesion of at least 1 of the renal arteries was found in 49 patients, 23 of whom were diagnosed as having a hemodynamically notable renal artery stenosis. The association between \textit{C pneumoniae} serology and a notable renal artery stenosis was significant. We found that a negative \textit{C pneumoniae} serology excluded the presence of a notable stenosis; IgG antibodies to \textit{C pneumoniae} had a negative predictive value of 100\%. The positive predictive value was 30\%, and it seems that IgG antibodies to \textit{C pneumoniae} have no great influence on the pretest probability of renal vascular hypertension. By using strict clinical criteria to perform renal angiography, renal vascular hypertension was detected in 23 (24\%) of our patients. However, our study was carried out in a selected group of hypertensive patients who

\begin{table}
\centering
\begin{tabular}{|c|c|c|c|}
\hline
Age Group, y & Patients Without Atherosclerosis & Patients With Atherosclerosis \\
\hline
20-29 & 1 & 4 & 0 & 0 \\
30-39 & 3 & 9 & 0 & 3 \\
40-49 & 5 & 10 & 0 & 12 \\
50-59 & 9 & 16 & 4 & 34 \\
60-69 & 9 & 20 & 6 & 52 \\
>69 & 9 & 20 & 7 & 60 \\
\hline
\end{tabular}
\caption{Age and the Cumulative Number of Hypertensive Patients With or Without Atherosclerosis and IgG Antibodies to \textit{Chlamydia pneumoniae}}
\end{table}
were referred to a hypertension outpatient clinic of a university hospital; the predictive value of C. pneumoniae serology as a diagnostic tool may be less in an unselected group of hypertensive patients.

Renal arterial atherosclerotic disease is often considered as only one facet of generalized atherosclerosis or of the aorta in particular. However, in 11 of the 49 patients with documented atherosclerotic lesions of at least 1 of the renal arteries, no atherosclerosis was detected at the level of the aorta. All 11 patients were C. pneumoniae IgG seropositive. Although these numbers are small, these data suggest that C. pneumoniae may be a risk factor for the development of atherosclerotic lesions limited to the renal artery in this highly selected group of patients.

Chlamydia pneumoniae infection of the renal artery may have hemodynamic consequences with worsening of hypertension, stimulation of atherogenesis, and possibly an increased susceptibility for vascular reinfection. This vicious circle may lead to serious vascular disease. Our study did not address the question whether C. pneumoniae is associated with hypertension. Unfortunately, previous studies addressing this question showed conflicting results. In a large Finnish study,21 focused on chronic coronary heart disease and acute myocardial infarction, no association was found between high titers of IgG and IgA antibodies to C. pneumoniae and hypertension, while another study specially designed for this purpose found more seropositivity in patients with severe essential hypertension compared with healthy controls.22 The authors of the latter study suggest that C. pneumoniae infection of blood vessels induces fibrosis, which may be a causal factor for hypertension. Our study indicates that a substantial number of hypertensive patients have C. pneumoniae–associated generalized atherosclerosis, which may contribute to high blood pressure by reducing vascular compliance.

Similar to C. pneumoniae, CMV can infect endothelial cells and blood mononuclear cells. In addition, after primo-infection, latency and reactivations occur, and the CMV seroprevalence increases with age. However, the association between CMV and atherosclerosis is weaker, and CMV can be detected in atherosclerotic and normal blood vessels.23 While C. pneumoniae is mostly detected in atherosclerotic vessels,24-26 In the present study, no association between CMV and atherosclerosis was found. The process of atherosclerosis is multifactorial. The other risk factor in our study included age. Unlike previous studies, smoking was not found to be a significant risk factor, possibly due to the small number of patients included. This may also explain why hyperlipidemia did not reach significance as a risk factor. In the multivariate analysis, only IgG antibodies to C. pneumoniae and age remained significant independent confounding factors.

In conclusion, the presence of antibodies to C. pneumoniae was significantly associated with atherosclerosis, renovascular disease, and a hemodynamically notable renal artery stenosis in hypertensive patients in whom a renal artery stenosis was strongly suspected. Possibly, C. pneumoniae contributes to the development of atherosclerosis in a direct way as well as an indirect way via deterioration of blood pressure.

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