Chlamydia pneumoniae Antibodies in Severe Essential Hypertension


Abstract—Several studies have implied an association between Chlamydia pneumoniae (C. pneumoniae) and cardiovascular disease. Our study was designed to determine whether this organism is associated with severe essential hypertension in a multiracial British population. Antibodies to C. pneumoniae were measured by microimmunofluorescence in 123 patients with chronic severe hypertension and 123 control subjects, matched for ethnic origin, age, sex, and smoking habit, admitted to the same hospital with various noncardiovascular, nonpulmonary disorders. Previous infection was defined by IgG 64 to 256, provided that there was no detectable IgM. Multiple regression analyses of matched and unmatched data were used to investigate the influences of antibody levels and potential confounding factors (ethnic origin, age, sex, smoking habit, diabetes mellitus, and social deprivation) on hypertension. A portion of the hypertensive patients underwent echocardiography, estimation of left ventricular mass index, and measurements of fibrinogen, D-dimer, and von Willebrand factor concentrations. Thirty-five percent of hypertensive patients and 17.9% of matched control subjects had antibody titers consistent with previous C. pneumoniae infection. The hypertensive patients differed significantly from their matched control subjects in their level of previous infection, with an odds ratio of 2.5 (95% confidence interval, 1.3 to 4.7). There were no significant differences in antibody levels between patients with left ventricular hypertrophy and those without it. Fibrinogen, D-dimer, and von Willebrand factor concentrations were not significantly associated with antibody levels. These data support an association of C. pneumoniae with severe essential hypertension. They provide no evidence of a predisposition to develop left ventricular hypertrophy in hypertensive patients with C. pneumoniae infection or of associations with hypercoagulability or endothelial dysfunction. (Hypertension. 1998;31:589-594.)

Key Words: hypertension, essential cardiovascular diseases infection

Chlamydia pneumoniae was first described in 1986.¹ Serological studies indicate that it is one of the most prevalent infectious agents worldwide,²³ with a wide range of clinical manifestations, including exacerbations of chronic obstructive pulmonary disease and chronic asthma.⁶ Several groups have demonstrated serological associations with coronary artery disease,⁷ strokes and transient cerebral ischemia,¹¹ and asymptomatic carotid atherosclerosis.¹²

Our study was designed to test the association of this organism with HT in the multiracial inner-city population of north and west Birmingham, UK, after adjustments for several potential confounding variables. We subjected the HT patients to echocardiography with estimation of LVMI. We also measured concentrations of fibrinogen, fibrin D-dimer, and von Willebrand factor in plasma and searched for associations of these factors with C. pneumoniae antibody levels.

Methods

Study Subjects and Investigations

Approval for this project was obtained from the Hospital Ethical Committee at the City Hospital, Birmingham. Subjects were patients admitted to this hospital via the Emergency Department, all of whom gave informed consent to participation. All patients were initially considered to be eligible: recruitment was prospective and continued at a steady rate, so that those who were eventually included in the HT and control groups were admitted consecutively throughout a 24-month period (March 1993 through March 1995). Exclusion criteria were known or suspected immunodeficiency, hypergammaglobulinaemia, connective tissue disease, and other autoimmune disease.

Patients in the HT group were recruited from outpatients attending the hypertension clinic at Birmingham City Hospital. All had had blood pressures greater than 160/90 on two or more occasions, and were taking antihypertensive medication. Predisposing conditions (such as Cushing’s and Conn’s syndromes, pheochromocytoma, and renal artery stenosis) had been excluded, where appropriate. Evidence of end-organ damage (renal failure or proteinuria, hypertensive retinopathy, or other active cardiovascular disease) was not required for recruitment.

Potential control subjects were selected randomly from all admissions to the Emergency Department with acute, nonpulmonary, noncardiovascular disorders, provided that there was no evidence (by the patients’ accounts and from the hospital’s records) of coexisting active cardiac, vascular, or pulmonary disease. Thus, there is no reason to suspect that they were predisposed to acquire C. pneumoniae infection.

At recruitment (on admission in the case of control patients), blood was taken for serology. The HT patients underwent further investigation, namely EKG, echocardiography, and determinations of levels of plasma fibrinogen, fibrin D-dimer, and von Willebrand factor. These investigations, which were ordered by the responsible physi-
Selected Abbreviations and Acronyms

- EKG = electrocardiography
- HT = chronic severe essential hypertension (study group)
- HT patients = patients enrolled in chronic severe essential hypertension study group
- LV = left ventricle
- LVH = left ventricular hypertrophy
- LVMI = left ventricular mass index
- MIF = microimmunofluorescence

avian, were not confined to any part of the study period or to any discernible subgroup of patients.

LVMI was estimated by echocardiography, using the formula of Devereux and the Penn conventions of measurement.

Demographic characteristics, medical history, smoking habit, drug therapy, and physical signs were recorded by the admitting medical staff; ethnic origin and smoking habit were later confirmed by postal questionnaires. Townsend scores of socioeconomic deprivation were assigned to those subjects who lived in the West Midlands conurbation, by linking their post-code sectors to census enumeration districts. These scores, which include items that reflect predominantly personal and family income levels, may be considered to reflect the socioeconomic status of people living within a particular locality. The higher the score (ie, the more strongly positive), the greater the degree of social deprivation.

Serological Testing

Two to five milliliters of serum was obtained within 6 hours of venipuncture and stored at –20°C until analysis. Each blood sample was labeled only with a serial number; thus, the investigator was blind to all patient data at the time of testing, and remained so until statistical analysis of the results.

Sera were tested by one investigator using Maxiscreen Chlamydia MIF slides (IO International Ltd) and fluorescein–conjugated anti-human immunoglobulins. Only an even pattern of elementary body fluorescence was regarded as positive. In every batch of slides tested, two control serum preparations known to be positive for this organism and two negative control samples were each applied to two slides. All sera were screened at a dilution of 1:8; thereafter, positive sera were tested at dilutions of 1:8 to 1:1024.

Because HT patients were not acutely ill when bled, we did not expect to find evidence of acute *C. pneumoniae* infection, and we were primarily interested in the frequency of previous infection. Titers indicating previous infection without recrudescence were presumed to be IgG 64 to 256, provided that IgM could not be detected. Titers of IgG ≥512 or IgM ≥8 were considered to indicate acute infection or reinfection just before entry into the study. Clearly, acute infections should have no bearing on long-standing hypertension, but because they could mask serological evidence of previous infection, patients with such titers were considered separately from previously infected and noninfected patients in the analysis of results.

Rheumatoid factor was assayed in patients in whom connective tissue diseases were suspected on clinical grounds, despite insufficient evidence to exclude them from the study. *C. pneumoniae* IgM antibodies were discounted for serological classification where it was present, because of its nonspecific IgM reactivity.

Analysis of Data

After recruitment was complete, we used a computer program to match each of the HT patients with one of the much larger number of potential control subjects for broad ethnic origin (Caucasian, Afro–Caribbean, or Asian), age (±10 years), sex, and smoking habit (current or previous versus never). We predicted (from our previous research and that of other published studies) that the prevalence of IgG anti-*C. pneumoniae* antibodies at titers of ≥64 would be approximately 15% to 20% in the population under study. With the nominal α or type I error level (two-tailed) set at .05, and the β or type II error level set at .20 (giving a power of 80%), and after we applied the z statistic for dichotomous variables, we estimated that 72 to 162 patients would be required in each of the matched groups to detect a difference of 15% to 20% in the frequencies of such titers.

A conditional logistic regression method for matched data, implemented in the EGRET statistical package, was used to explore possible associations of *C. pneumoniae* antibody levels and the matched variables with hypertension. We attempted to confirm the results of this analysis by applying a logistic regression modeling method, also from the EGRET package, to the unmatched HT and control groups, exploring the influences of ethnic origin, age, sex, and smoking habit on any association of *C. pneumoniae* antibody levels with hypertension. The latter method was also used to investigate possible influences of other characteristics for which matching was not achieved, ie, diabetes mellitus and Townsend score, on this association. Hence we derived an odds ratio (OR) expressing the association of previous *C. pneumoniae* infection with the presence or absence of hypertension, adjusted for potential confounding factors.

Finally, in the HT patients only, we performed general linear model ANOVAs of LVMI and concentrations of fibrinogen, fibrin D-dimer, and von Willebrand factor, stratifying data by all potentially associated factors. Because the fibrin D-dimer concentration had a very skewed distribution, we used its logarithmic (log10) transforms for these analyses, to stabilize variance.

Results

Study Subjects and Samples

Twenty-seven potential HT patients and 312 potential control subjects were excluded from this study by the criteria stated above, and 53 potential control subjects refused to participate. One thousand six hundred fifty-three subjects were recruited. One hundred thirty-five (8.2%) were in the HT group.

The remaining 1518 patients (92.8%) were assigned to the control group. In these patients, the primary indications for admission to the hospital were abdominal pain in 460, for which causes were established in 403 cases; chest pain, not suggestive of myocardial ischemia and for which no cause was found, in 91; malignant disease in 85; fractures of various bones in 77; urinary obstruction in 64; complications of diabetes mellitus in 44; and other miscellaneous medical and surgical diagnoses in 697.

We excluded all patients known or thought likely (by their admitting physicians) to have connective tissue disease or other autoimmune disease. After this, there remained in the study 5 HT patients and 133 control subjects in whom we suspected connective tissue diseases on clinical grounds, and whom we therefore tested for the presence of rheumatoid factor. Where this test was positive, in 2 HT patients and 24 control subjects, the finding of IgM antibodies was discounted for the diagnosis of acute *C. pneumoniae* infection.

Acute infection was diagnosed solely on the basis of IgM titers in 5 HT patients and 30 control subjects, of whom 5 HT patients and 22 control subjects would otherwise have been defined by their IgG titers as previously infected. Two HT patients and 11 control subjects were older than 60 years, and therefore were within the age group that has been associated with unsuspected rheumatoid factor production. Of these, 2 HT patients and 1 control subject subsequently took part in the
matched analysis. There were therefore 3 patients in the matched analysis who might conceivably have been identified wrongly as having acute infection.

The presence or absence of LVH was ascertained by echocardiography and EKG in 127 (94.1%) of the HT patients. Plasma fibrinogen, fibrin D-dimer, and von Willebrand factor were measured in 118 (87.4%), 115 (85.2%), and 120 (88.9%) of the HT patients, respectively.

Matching was achieved, on the basis of the criteria stated above, between 123 HT patients and 123 control subjects (Table). The following examination of C. pneumoniae antibody levels in hypertension relates only to the matched analysis.

C. pneumoniae and Hypertension

Of the matched HT patients, 43 (35.0%) had serological evidence of previous infection, 9 (7.3%) acute (re)infection, and 71 (57.7%) no infection. Of the matched control subjects, 22 (17.9%) had evidence of previous infection, 11 (8.9%) acute (re)infection, and 90 (73.2%) none.

Regression analysis suggested associations of HT with previous C. pneumoniae infection (OR, 2·5; 95% confidence interval [CI], 1.3 to 4.7) but not with acute (re)infection (OR, 1.0; 95% CI, 0.4 to 2.6). Among matched HT patients, the distribution of the three possible serological outcomes—acute (re)infection, previous infection, and none—differed from that among matched control subjects with a probability of $P<.05$, but this difference was entirely due to levels of previous infection. If we had omitted the 3 matched patients over 60 years of age, not tested for rheumatoid factor, in whom we detected acute C. pneumoniae infection, it would not have altered the lack of association between hypertension and acute (re)infection.

This analysis revealed no evidence of statistical interactions of ethnic origin, age, sex, or smoking habit with the C. pneumoniae–HT association. The analysis of unmatched groups was in agreement with the matched analysis, and it revealed no evidence of interactions of diabetes mellitus or Townsend score with the C. pneumoniae–HT association.

C. pneumoniae and LVH in Hypertension

Among 127 of the HT patients, EKG and echocardiography showed LVH in 92 and were normal in 35. Mean LVMI measurements were 173.5 and 124.1 g·m$^{-2}$, respectively. Serological results indicated previous infection, acute (re)infection, and no infection in 34.8%, 5.4%, and 59.8% of patients with LVH, and 31.4%, 2.9%, and 65.7% of those with no LVH. By $\chi^2$ analysis, there was no significant variation between the two categories in their distributions of previous infection, acute (re)infection, and no infection.

Fibrinogen, Fibrin D-Dimer, and von Willebrand Factor Levels

In HT patients with serological evidence of previous infection, acute (re)infection, and no infection (after adjustment by regression analysis for variations in ethnic origin, age, sex, and smoking habit), mean plasma concentrations of fibrinogen were 10.9 (95% CI, 9.4 to 12.6), 10.9 (8.8 to 12.9), and 11.5 (10.0 to 12.9) μmol/L; of fibrin D-dimer, 273 (184 to 405), 253 (150 to 424), and 228 (156 to 333) ng/dL; and of von Willebrand factor, 120 (110 to 131), 120 (101 to 140), and 125 (115 to 135) IU/dL, respectively. There were thus no significant differences in these variables between HT patients with previous infection, acute (re)infection, and no infection.

Discussion

C. pneumoniae and Hypertension

Hypertension exemplifies probably better than any other disorder the complexity of polygenic disease. In 95% of cases (so-called "essential hypertension"), no single cause can be identified; although factors such as consumption of alcohol and caffeine, salt intake, smoking, obesity, and physical inactivity may clearly contribute to increasing blood pressure. The notion that infections may predispose to hypertension is not new. For example, such a role has been proposed for Helicobacter pylori: of 33 patients in one urban general practice with unequivocal H. pylori gastritis, 42% had sustained hypertension compared with 12% of dyspeptic patients without H. pylori.20 Schreiber et al (1992) reported that intravenous injections of either live or heat-killed group B β-hemolytic streptococci in newborn lambs caused significant dose-dependent increases in systemic vascular resistance and mean systemic arterial pressure and that these effects were partly blocked by leukotriene D4 receptor antagonists, suggesting that leukotrienes might mediate hypertension in this infection.21 This is one of several immunologic changes that might be relevant to the infectious
origin of essential hypertension. Furthermore, chronic chlamydial infections have a marked propensity to cause fibrosis (as seen, for example, in the cicatricial scarring of the cornea that characterizes trachoma and in fibrosis of the Fallopian tubes in pelvic inflammatory disease due to *C. trachomatis*). It is therefore reasonable to speculate that *C. pneumoniae* within vascular endothelial cells might, by a similar process, lead to an increase in vascular resistance.

There are wide variations in the prevalence and incidence of hypertension in different parts of the world. Both in the United Kingdom and United States, it is more common among black people than in the white population. *C. pneumoniae* antibodies have been associated with Afro-Caribbean origin, raising the possibility that a genetic predisposition to this infection may contribute to the development of hypertension.

In a large Finnish study that showed a clear association of high titers of chlamydial IgG and IgA antibodies with chronic coronary artery disease and acute myocardial infarction, there was no correlation with other risk factors for these conditions, including hypertension. However, this lack of correlation may not be very significant, unless the research is focused on severe and sustained hypertension, and predisposing conditions (such as Cushing’s and Conn’s syndromes, pheochromocytoma, and renal artery stenosis) are excluded. The authors of the Finnish study did not specify the criteria for diagnosing hypertension in their report, and we suggest that our study of chronic severe hypertensive patients should have a higher probability of detecting such an association.

It is noteworthy that we found substantial levels of acute and previous infection in control subjects as well as HT patients, reflecting the high incidence of *C. pneumoniae* infection in our community. Nevertheless, the results of our study support an association between chronic severe essential hypertension and *C. pneumoniae* infection. We did not grade the HT patients according to the severity of their hypertension, but we found no significant differences in *C. pneumoniae* antibody levels between patients with and without serological evidence of LVH. We suggest that several factors may interact to produce this complication of hypertension.

Strategies to take into account potential confounding variables are essential in studies of *C. pneumoniae* antibodies, which have been associated with Afro-Caribbean origin, increasing age, male sex, and smoking. In this study, we found no noteworthy interactions (ie, effect modification) by these factors with the association between *C. pneumoniae* and hypertension. Subjects in the HT and control groups also differed in some other demographic characteristics whose influences on the risk of *C. pneumoniae* infection were unknown (Table). Using unmatched data, we found no interactions of diabetes mellitus or Townsend score with the association between *C. pneumoniae* and hypertension, although we believe that larger studies would be required to draw firm conclusions about these factors.

Fibrinogen, fibrin D-dimer, and von Willebrand factor may be regarded as markers for intravascular thrombogenesis and fibrin turnover, and for endothelial damage, respectively. Marked elevation of plasma fibrinogen has been reported in patients with severe hypertension; and in such patients, fibrinogen concentration is an independent predictor of blood pressure. Infections also lead to increases in prothrombotic factors, particularly fibrinogen, fibrin D-dimer (a fragment of cross-linked fibrin, hence a marker of thrombosis), and anti-cardiolipin antibodies. However, fibrinogen, fibrin D-dimer, and von Willebrand factor were not significantly associated with the presence of *C. pneumoniae* antibodies in our study.

### Serological Testing

Various techniques are available to detect *C. pneumoniae* antibodies. The best and most widely used is the MIF assay, which is time-consuming and subject to some operator variation but is sensitive and species-specific and reliably detects IgG, IgM, and IgA. The kit that we used has been used in several studies, and has a performance similar to other MIF assays.

IgG titers ≥256 may persist for many months and have generally been accepted (in a single serum specimen) as evidence of previous infection, provided that there is no rise in IgM antibodies. Titers ≥512 are generally regarded as indicating acute infection or reinfection. Many authors have used these serological criteria and a large study combining serology with an examination of pharyngeal swabs by polymerase chain reaction has provided evidence in support of them. We chose to reject IgG titers <64 to minimize the probability of false-positive results.

IgM is generally considered to signify acute primary infection. A threshold titer of ≥16 has been proposed, with titers of 8 indicating “probable acute infection,” but we found no difference in the proportions of patients with IgM antibodies at these two titers in a large pilot study, and therefore saw no merit in drawing a distinction between such low levels of antibody production provided that only an even distribution of definite elementary body fluorescence was accepted as positive. It has been suggested that rheumatoid factor may make the measurement of IgM antibodies unreliable, particularly in elderly patients. We therefore excluded from this study all patients known or thought likely to have connective tissue disease or other autoimmune disease, and measured rheumatoid factor in a further 138 cases in which such diseases were considered less probable, discounting IgM antibodies in the 26 cases in which it was present. As demonstrated above, ignoring IgM titers from the remaining matched patients over 60 years of age (3 patients) would not have significantly altered the relationships of HT to acute and chronic *C. pneumoniae* infections.

### Limitations of this Study

In the HT group, the presence or absence of LVH was ascertained in 94.1% of patients. There appears to be no association of *C. pneumoniae* infection with the development of LVH. Plasma fibrinogen, fibrin D-dimer, and von Willebrand factor levels were measured in fewer than 90% of the HT patients; in future studies, it will be important to measure these levels in larger numbers of patients, both with and without serological evidence of *C. pneumoniae* infection.

No attempt was made to control for travel history, ethanol consumption, or diet in this study. Although the last two
factors may influence the probability of systemic hypertension, we do not perceive them to be important factors in our population of severely hypertensive patients.

We believe that our strategy for preventing interference by rheumatoid factor in IgM measurement has allowed us to make a valid interpretation of the results. However, in the future we would recommend the routine absorption of sera with anti-human IgG, as advocated by Verkooyen et al.\textsuperscript{19}

Conclusions

These data support the associations of chronic severe hypertension with previous \textit{C. pneumoniae} infection. We believe that more research to elucidate the mechanism(s) of this association is warranted.

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P. J. Cook, G. Y. H. Lip, P. Davies, D. G. Beevers, R. Wise and D. Honeybourne

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