Infection and venous thrombosis

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Key Words

Deep vein thrombosis, pulmonary embolism, venous thromboembolism, chlamydia pneumoniae, thrombosis.

Summary

Chlamydia pneumoniae infection has been linked to atherosclerosis, but a possible relationship with venous thromboembolism (VTE) had not been sought. We determined circulating anti-C. pneumoniae antibody levels in patients with VTE. Fifty-four percent of the cases and 15.9% of the controls had specific IgG titers of at least 256 ($p < 0.0001$). The crude odds ratio for VTE was 6.2 (95% CI, 3.8-10.1), and rose to 7.7 (4.5-13.2) after excluding subjects carrying the factor V Arg 506 Gln or factor II G 20210 A mutations. Other studies did not confirm such an association or found a lower odds ratio. This association remains to be confirmed in other case-control or prospective studies.

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Venous thromboembolic disease (VTE) is a multifactorial disorder with both genetic and acquired risk factors. Several gene mutations have been linked to thrombophilia, but after

taking into account conventional acquired risk factors and genetic predisposition, at least one-third of thrombotic episodes remain unexplained. At the beginning of this century, deep vein thrombosis was believed to be mainly of infectious origin, but infections are now only implicated in specific clinical contexts such as pelvic vein thrombosis in the puerperium, use of venous access devices, and intravenous narcotic use (1). Nevertheless, during the last decade evidence has accumulated linking chronic infection to atherosclerosis and thrombosis (2-5). The infectious agent most strongly implicated in atherosclerosis is Chlamydia pneumoniae, one of four species of the genus Chlamydia. C. pneumoniae is an obligate intracellular parasite and infects epithelial cells of the upper respiratory tract, and the vascular system. C. pneumoniae has been detected in arteries (mainly in atherosclerosis plaque), but also in an iliac vein by means of polymerase chain reaction amplification (PCR), suggesting that it might play a role not only in arterial thrombosis but also in venous thrombosis.

Methods

Subjects

To identify a possible link between C. pneumoniae infection and venous thrombosis, we measured circulating antibodies to C. pneumoniae in 176 patients with VTE and 197 age- and sex-matched healthy controls in the Paris Thrombosis Study (PATHROS), a case-control study designed to examine genetic risk factors for VTE, has been described in detail else-

where (1). Frozen plasma from 176 patients (86%) remained available; samples obtained less than three months after the onset of acute venous thromboembolic disease were available in 87 cases (median 1 day; interquartile range 0-16), while the remaining 89 samples were obtained more than 3 months after onset (median 12 months; interquartile range 6.5-36). Healthy age- and sex-matched controls were recruited from May to September 1996 from a health care center to which they had been referred for a routine check-up. None of these 197 controls had a history of VTE, stroke, myocardial infarction or peripheral vascular disease, on the basis of a medical questionnaire.

Laboratory assays

For serological testing, each case and control sample was labeled with a randomly assigned number and analyzed blindly in the microbiology department. *C. pneumoniae* serological status was determined by microimmunofluorescence (MIF) assay with the SeroFIA IgG Chlamydia kit (Savyon Diagnostics LTD, Ashdod, Israel), which has a sensitivity of 100% and a specificity of 96.7%. Briefly, purified *C. pneumoniae* elementary bodies (strain IOL 207) were used to detect IgG antibodies.

Results

The cases and controls did not differ in terms of age or the sex ratio. Half the patients were recruited within three months after the acute episode of VTE. The characteristics of this subgroup were not statistically different from those of the entire case population (data not shown). The prevalence of the factor V Arg 506 Gln and factor II G 20210 A mutations was within the expected range in Caucasians (21.9% of cases and 5.1% of controls, $p < 0.0001$; and 10.2% and 4.1%, $p = 0.02$, respectively).

C. pneumoniae-specific IgG titers tended to be higher in the cases than in the controls (Table). Significantly more patients than controls had *C. pneumoniae* IgG titers of 256 or more (54% and 15.9%, respectively; $P < 0.0001$). The crude odds ratio for VTE associated with IgG titers of 256 or more was 6.2 (95% CI, 3.8-10.1). After adjusting for age, sex, and factor V Leiden and factor II G 20210 A mutations, the odds ratio was 7.5 (95% CI, 4.4-12.5). In the subset of patients with blood samples taken less than three months after VTE the adjusted odds ratio among the subjects with IgG titers > 256 was 6.7 (95% CI, 3.6-12.2). Moreover, the odds ratio for VTE increased with the IgG titer: for titers of 256, 512 and 1024, the adjusted odds ratios were 2.1 (95% CI, 1.1-4.1), 5.3 (2.7-10.6) and 33.0 (4.4-248.4), respectively (Table 3). A larger proportion of seropositive controls had a (low) IgG titer of 128 (54.9% and 21%, respectively). Similar odds ratios were obtained in the subgroup of cases tested within 3 months after VTE.

These results did not change when the six cases with a his-

tory of arterial disease were excluded from the analysis. To discriminate acute from chronic infection, we screened for circulating anti-*C. pneumoniae* IgM antibodies in the 95 cases and 31 controls who had IgG titers above 256. Only one subject, a case patient, was IgM-positive.

Discussion

We demonstrated a link between *C. pneumoniae* serological status and venous thrombosis. The crude odds ratio for VTE associated with circulating titers of anti-*C. pneumoniae* IgG antibodies of 256 or more was 6.2 (95% CI; 3.8-10.1), and remained high after adjusting for sex, age and the factor V Leiden and factor II 20210 A mutations (odds ratio 7.5; 95% CI: 4.4-12.5).

Several hypothesis could explain the link between high levels of anti-*C. pneumoniae* IgG and VTE (1). Chronic chlamydial infection of a vein is unlikely to occur in the absence of vessel injury, although *C. pneumoniae* has been found in a vein. Should this occur, induction of tissue factor (TF), a major coagulation activator, could switch the natural anticoagulant phenotype of endothelial cells to a procoagulant phenotype. Chlamydia may also induce the expression of adhesion molecules (E-selectin, ICAM-1 and VCAM-1), and the production of a pro-inflammatory cytokine, interleukin-6, by endothelial cells, smooth muscle cells and macrophages.

Endothelial cells might also be affected indirectly through an immune mechanism, in which anti-Chlamydia IgG antibodies recognize epitope(s) expressed on endothelial cells. Another possible explanation for the link between *C. pneumoniae* and VTE involves increased basal activation of the coagulation system due to persistent infection of circulating leukocytes that have encountered and ingested *C. pneumoniae* in the lungs. Infected monocytes can be induced to express tissue factor (TF), and the importance of TF-containing leukocytes as a source of circulating TF potentially involved in thrombosis has recently been emphasized. Finally, high levels of factor VIII, an independent risk factor for venous thrombosis, could also be induced by *C. pneumoniae* infection, through an acute-phase reaction.

A serological link between *C. pneumoniae* and VTE does not establish a causal relationship, as it fails to show whether *C. pneumoniae* infection precedes the disease or whether the microorganism is present within the vessel wall.

The association between *C. pneumoniae* infection and deep vein thrombosis, was not confirmed in the Leiden Thrombophilia study (6). But, in this study controls were mainly partners of cases and lived in the same environment. This situation could explain the lack of association found. Another study found a weak association between proximal deep vein thrombosis and positive anti-*C. pneumoniae* antibodies (unpublished).

This association, which remains to be confirmed in other studies, opens a new field of research into risk factors of VTE. If the link is confirmed, antibiotic therapy might prove helpful in reducing the high rate of recurrences after venous thrombo-

sis, a strategy similar to that based on macrolides for secondary prevention of coronary heart disease. Only properly designed prospective studies could establish a causative link between *C. pneumoniae* infection and VTE.

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