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Atherosclerosis in dialysis patients: does *Chlamydia pneumoniae* infection contribute to cardiovascular damage?

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Abstract

Cardiovascular risk in the dialysis population is exceedingly high, and there is now convincing evidence that inflammation is strongly linked to atherosclerosis in this population. The source of inflammation in dialysis patients still remains undefined. Bacterial contamination during the extracorporeal circulation and bioincompatibility explain only a very small part of the high prevalence of inflammation [as defined by raised C-reactive protein (CRP)] in these patients. In the general population, several infectious agents have been implicated as likely culprits of atherosclerosis, and *Chlamydia pneumoniae* is the most suspected. In dialysis patients, the presence of a high titre of anti-*C. pneumoniae* antibodies is associated with the severity of atherosclerosis. The CREED database (Cardiovascular Risk Extended Evaluation in Dialysis patients) has on file 278 patients tested for *C. pneumoniae* and followed-up for 4 years. Interestingly, in this database, the risk for cardiovascular death is ~4 times higher in the group of patients ($n = 50$) seropositive for *Chlamydia* and with raised CRP than in those with no evidence of *Chlamydia* infection and normal CRP. Yet seropositivity to *Chlamydia* did not significantly increase the risk associated with raised CRP. These data suggest that raised CRP and *C. pneumoniae* seropositivity is a high risk situation, but it remains very uncertain as to whether *Chlamydia* infection *per se* contributes to the high cardiovascular risk of dialysis patients.

Keywords: atherosclerosis; *Chlamydia pneumoniae*; cardiovascular risk; cardiovascular disease; dialysis; end-stage renal disease; uraemia

Cardiovascular risk, atherosclerosis and inflammation in end-stage renal disease

It is now known that the cardiovascular risk in the dialysis population is exceedingly high. Traditional or Framingham risk factors (Table 1), are over-represented in dialysis patients, and play an important role in determining such a high risk. The risk difference is smaller in the older cohorts, being only ~5 times higher in dialysis patients aged over 85 years than in the control population. However, looking at the other end of the age distribution, i.e. the youngest cohort, the risk difference is astonishingly high, 500 times higher [1]. Since Framingham risk factors are relatively less important in the young, other factors should be sought to explain such a huge difference. Other factors which are peculiar to renal failure (hyperphosphataemia, hyperparathyroidism and anaemia) and factors such as mild hyperhomocysteinaemia and inflammation should be taken into account.

Generally speaking, when attention is focused on atherosclerosis, the lipid core of the atherosclerotic plaque is considered to be the major, if not the only, problem, and for this reason atherosclerosis is considered as a purely metabolic complication. Yet if one examines the shoulder of the plaque beneath the endothelial layer, one can see a dense cell infiltrate which is composed mainly of macrophages. These macrophages are metabolically active and this activity is so strong that it causes a local increase in temperature which can be detected by intravascular thermistors [2]. The importance of inflammation in the pathogenesis of atherosclerosis is now well established [3], and C-reactive protein (CRP) has emerged as a solid indicator of this inflammatory process. CRP in dialysis patients is markedly increased and exceeds the upper limit of the normal range in ~50% of cases. Raised CRP in these patients is strongly linked to cardiovascular risk. In 1998, we showed that the relative risk of atherosclerosis was four times higher in patients in the upper CRP quartile than in those in the lower CRP quartile of the dialysis population [4,5]. More importantly, Zimmermann showed that the 2-year

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Table 1. Traditional and non-traditional risk factors in the dialysis population

Framingham risk factors	**Factors peculiar to ESRD**
Age	Hyperphosphataemia
Sex	Hyperparathyroidism
Smoking	Anaemia
Hypertension	
LVH	**Emerging risk factors**
Dyslipidaemia	Hyperhomocysteinaemia
Diabetes	Inflammation (↑CRP)

cardiovascular mortality rate was five times higher in dialysis patients in the fourth CRP quartile than in those in the first quartile [6].

The source of inflammation in dialysis patients is still undefined. Bacterial contamination during the extracorporeal circulation and bioincompatibility have been the prime suspects. However, there must be factors more important than dialysis treatment because as many as 33% of patients on conservative treatment have raised CRP [7]. In the last decade, a great effort has been made to identify new cardiovascular risk factors in the general population. Looking again at the vulnerable atherosclerotic plaque, several infectious agents have been identified as likely culprits of atherosclerosis. The most widely implicated of these agents is *Chlamydia pneumoniae* [8]. In this review, we will focus on the role of this bacterium in dialysis patients.

Chlamydia pneumoniae infection and atherosclerosis: fulfilling the Koch postulates

Implicating *C. pneumoniae* in the aetiology of atherosclerosis is quite a complicated process because the Koch postulates ought to be fulfilled before concluding that this pathogen is causally involved in atherosclerosis in man.

The Koch postulates hold that:

(i) There must be evidence that the infectious agent is present in affected people and the infectious agent must be isolated from the diseased host and grown in pure culture.
(ii) The specific disease must be reproduced when a pure culture of the pathogen is inoculated into a healthy susceptible host.
(iii) The pathogen should be present in the host before the appearance of the disease.
(iv) The eradication of the infection should be associated with healing of the disease.

The first postulate: demonstration, isolation and growth of a pure culture of the infectious agent

A successful epidemiological and microbiological investigation on an unknown disease usually ends with the identification of a new agent. For example, *Legionella* was isolated in the air-conditioning system and eventually identified as the culprit of the severe bronchopulmonary disease which affected American veterans who convened in a hotel in Philadelphia in the 1970s [9]. The involvement of *Chlamydia* in atherosclerosis is a more elliptical story. This agent initially was considered as responsible for a bronchopulmonary syndrome. Serological tests were developed and extensively applied. A spin-off of the extensive use of serological studies was the emergence of new associations between this bacterium and other diseases. In 1988, Saikku *et al.* observed that the relative risk of *Chlamydia* infection in patients with myocardial infarction was four times higher compared with the general population [10]. The infectious hypothesis of coronary heart disease was most appealing, and this paper was followed by several cross-sectional and follow-up studies exploring the relationship between *Chlamydia* and atherosclerostic complications. Observational studies on the association of *C. pneumoniae* and cardiovascular disease have been reviewed recently by Danesh in a comprehensive meta-analysis [8]. Overall, the risk for coronary heart disease associated with *Chlamydia* infection was much less (only 15%) than that which emerged from the initial cross-sectional study by Saikku *et al.*

The presence of *Chlamydia* in atherosclerotic plaques has been demonstrated by a variety of techniques, including electron microscopy, immunocytochemistry employing monoclonal antibodies highly specific to *Chlamydia* antigens, and *Chlamydia* DNA amplification techniques. In a systematic review of 16 pathology studies including 320 patients without atherosclerosis and 697 patients with evidence of atherosclerosis, which updated a previous systematic review by Danesh *et al.* [11], we estimated the relative risk for *Chlamydia* being detected in the atherosclerotic lesions to be 17 times higher than in uninvolved arterial tissue. On the other hand, it was found that *C. pneumoniae* can be grown in 16% of atherosclerotic lesions [12]. Yet the possibility remains that *Chlamydia* is an 'innocent bystander', a spectator rather than a protagonist in the atherosclerotic plaque.

The second postulate: the animal model

Laitinen *et al.* [13] in the New Zealand rabbit, an animal model that does not develop atherosclerosis with a 'normal diet', were able to produce clear-cut atherosclerotic lesions by nasal inoculation of *C. pneumoniae* in the majority of experimental animals (66%). By the same token, it was found that in the low-density lipoprotein (LDL) receptor null mice, a transgenic knock-out model that develops atherosclerosis only when fed a high cholesterol diet, *Chlamydia* infection substantially amplifies the severity of atherosclerosis. Thus observations in this model indicate that there may be an interaction between cholesterol and *Chlamydia* in determining arterial damage [14].

The third postulate: the presence of the infection should precede the disease

Davidson *et al*. [15], in a collaborative study involving local coroners in Alaska, were able to study 60 subjects who died from non-cardiovascular causes (mainly violent deaths) in whom a serum sample collected 1–26 years before death was available. Furthermore, in all these cases, the degree of atherosclerosis could be ascertained accurately by autopsy. Interestingly, when these subjects were classified on the basis of the presence of a high titre of anti-*C. pneumoniae* antibodies in the serum sample, the probability of having atherosclerotic lesions at death was six times higher in those with a high anti-*Chlamydia* titre than in those without, and the risk was even greater after adjustment for possible confounders including smoking habits [15]. Thus there is evidence that the association between *Chlamydia* and atherosclerosis may be a causal one, and the validity of the findings we have just examined applies to the general and the uraemic population alike. However, before moving to the fourth postulate, it is important to know whether there is direct evidence of a relationship between *Chlamydia* infection and atherosclerosis in uraemic patients.

Chlamydia pneumoniae infection and atherosclerosis in uraemic patients

An interesting finding in our echo-colour Doppler study was that in men on chronic dialysis, IgG anti-*Chlamydia* titre was an independent correlate, though a slight one ($P=0.03$), of the number of atherosclerotic plaques [4]. Importantly, the interaction term CRP–anti-*Chlamydia* antibodies was an even stronger and independent predictor. Stenvinkel *et al*., in uraemic patients maintained on conservative treatment, showed IgA anti-*Chlamydia* antibodies to be related independently to intima media thickness [7]. In line with these observations, a recent study has reported that *Chlamydia* is strongly linked to survival in patients on peritoneal dialysis [16]. Over a 7-year follow-up, survival was 90% in *Chlamydia*-negative patients and 40% in *Chlamydia*-positive patients, a 50% difference. A limitation in this study was the small number of patients (only 34) and the small number of events (only 12), which precluded statistical adjustment for confounders which, as we will see in the analysis of our cohort, is very important.

Chlamydia pneumoniae *infection in the CREED database*

The CREED database has on file 278 patients tested for *Chlamydia*; the follow-up is 4 years and 58 cardiovascular deaths have been registered. In this database, taking as a reference a group that is composed by patients negative for *Chlamydia*, the unadjusted hazard ratio for all-cause death was 3.24 higher in patients with a titre $>1:256$ than in the reference group ($P=0.007$), and there was a linear risk in patients having intermediate titres (from 1 : 36 to 1 : 128). However, the anti-*Chlamydia* antibody titre was related to other risk factors: directly to age ($r=0.21$, $P=0.0001$), sex ($r=0.20$, $P=0.001$), smoking ($r=0.24$, $P=0.0001$) and diabetes ($r=0.18$, $P=0.003$), and inversely to serum cholesterol ($r=-0.13$, $P=0.03$). When we adjusted the analysis for these confounders, the relationship between *Chlamydia* and the risk for all-cause death became much weaker and not significant. Yet our finding in the echo-colour Doppler study [5] that *Chlamydia* interacted with CRP in predicting the number of atherosclerotic plaques suggested that *Chlamydia* infection might be of relevance in this particular subgroup. For this reason, we tested the combined influence of CRP and seropositivity to *Chlamydia* in a multivariate Cox model. Patients were divided into three groups. The first was a reference group comprising patients negative both for *Chlamydia* and CRP and the third was the group including patients with raised CRP and positive for *Chlamydia*. Remarkably, the risk for cardiovascular death was 3.82 times higher (95% confidence interval 1.35–10.77, $P=0.01$) in this group than in the reference group. In this model, the combined effect was adjusted for age, sex, previous cardiovascular events, diabetes, cholesterol and homocysteine, and did not change substantially, even after forcing into the model other non-significant factors such as treatment modality, calcium, phosphate and duration of dialysis treatment. Yet, seropositivity to *Chlamydia* did not significantly increase the risk associated with raised CRP. Thus raised CRP and *C. pneumoniae* seropositivity seem to be a high risk situation, but it remains very uncertain whether *Chlamydia* infection *per se* contributes to the high cardiovascular risk in dialysis patients.

The unfulfilled Koch postulate: eradication of infection

Eradication of the disease after resolution of the infectious process is the most convincing proof that the suspected pathogen is causally linked to the disease under investigation. Notwithstanding the importance of the problem, few attempts have been made to see whether eradication of *Chlamydia* infection may reduce cardiovascular risk in patients with coronary heart disease. Studies performed so far have gathered a small number of patients (only 584) and a very low number of cardiovascular events (only 67) (Table 2). Two studies are positive and one negative. Overall, the evidence that the use of antibiotics is useful to treat coronary heart disease remains rather weak. Until now, there have been no studies in patients with end-stage renal disease (ESRD). Probably, we will have the much awaited answers in the next few years because there are five ongoing trials, three in the USA and two in the UK (Table 3). The number of patients enrolled in these studies is high, $\sim$14 000, and these

Table 2. Published studies

Study	No. of patients	Antibiotic	Event	Duration	No. of events	Absolute risk reduction	
Gupta [17]	80	Azithromycin	MI	18 months	29	−20%	+
Gurfinkel [18]	202	Roxithromycin	Acute MI, Unstable angina	1 month	22	−4%	+
Anderson [19]	302	Azithromycin	Stable CHD	6 months	16	+1.4%	−
Total	584				67		

Table 3. Ongoing trials

Trial	Location	Size	Entry criteria	Drugs/duration (months)	Years
ACES	USA	4000	Previous MI or coronary revascularization	Azithromycin/12	4
PROVEIT	USA	4000	Acute coronary syndrome	Gatifloxacin/18	1.5
WIZARD	USA	3800	Previous MI or coronary revascularization	Azithromycin/3	3
MARBLE	UK	1300	Waiting for coronary artery bypass graft surgery	Azithromycin/3	1
Stamina	UK	600	Previous MI	Azithromycin + drugs against *Helicobacter pylori*	1.5

studies deal with both acute and stabilized coronary conditions. Four trials are based on azithromycin, and one on gatifloxacin, a fluoroquinolone antibiotic. Regrettably, no trial has been planned in patients with ESRD.

In conclusion, inflammation in dialysis patients is triggered mainly by factors unrelated to dialysis treatment. It is still undecided whether *Chlamydia* infection is a causal risk factor in the general as well as in the dialysis population. Ongoing trials with macrolide antibiotics will soon tell us whether this risk factor is modifiable.

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