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# Autoantibodies against oxidized low-density lipoprotein and lipid profile in patients with chronic periaortitis: case-control study.

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## **Short title:**

Anti-OxLDL Ab in chronic periaortitis.

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## Abstract

**Objective:** Chronic periaortitis is thought to result from an autoallergic reaction to oxidized low-density lipoprotein (OxLDL). No data exist on lipid profile and atherosclerotic biomarkers. We investigated circulating levels of OxLDL and of anti-OxLDL (aOxLDL) antibodies in patients with chronic periaortitis.

**Methods:** Cross-sectional case-control study on 20 patients with chronic periaortitis. Patients were compared to 20 age- and sex-matched controls. aOxLDL antibodies were measured by ELISA and expressed as mean optical density values at 450 nm from duplicate measurements (OD<sub>450</sub>).

**Results:** aOxLDL antibody titres (median [interquartile range]) did not differ significantly between patients and controls (aOxLDL-IgM: 0.70 [0.24-1.08] vs. 0.54 [0.25-0.73] OD<sub>450</sub>; aOxLDL-IgG: 0.59 [0.38-0.75] vs. 0.41[0.33-0.63]OD<sub>450</sub>). Female patients had higher aOxLDL-IgM levels than male patients (1.02 [0.46-1.38] vs. 0.29 [0.22-0.84] OD<sub>450</sub> ;  $P=0.05$ ). aOxLDL-IgM titres were lower in patients with cardiovascular disease (CVD) than in patients without CVD (0.22 [0.16-0.37] vs. 0.92 [0.70-1.30] OD<sub>450</sub>;  $P=0.003$ ) and correlated positively with HDL-cholesterol ( $r = 0.47$ , 95%CI 0.02 to 0.69;  $P=0.03$ ) and inversely with diastolic blood pressure ( $r = -0.46$ , 95%CI -0.75 to -0.01;  $P=0.03$ ) and OxLDL/apoB ratio ( $r = -0.41$ , 95%CI -0.73 to 0.04;  $P=0.06$ ). No differences or associations were found between aOxLDL-IgG titres and other variables between or within patients and/or controls. In patients, OxLDL levels correlated with smoking pack-years ( $r = 0.58$ , 95%CI 0.17 to 0.81;  $P=0.007$ ).

**Conclusion:** Data suggest a differing innate immune response to OxLDL in patients with chronic periaortitis compared to controls. Whether this response is causally related to chronic periaortitis development remains to be clarified.

## Key words:

Chronic periaortitis; Autoantibodies; Oxidized low-density lipoprotein; Apolipoprotein B; Atherosclerosis.

## Introduction

The term chronic periaortitis refers to a group of rare chronic inflammatory disorders sharing similar clinical and histopathological findings (1,2). These include idiopathic retroperitoneal and mediastinal fibrosis, peri-aneurysmal fibrosis and inflammatory abdominal aortic aneurysm (1,2). All show severe adventitial lymphocyte and plasma cell infiltration with fibrosis around atherosclerotic plaques. This periaortic inflammation is found only where advanced atherosclerosis breaches or attenuates the media, thereby allowing contact of atheromatous material with the adventitia (1-3).

These findings led to the hypothesis that chronic periaortitis may be due to an autoallergic reaction to a component of the atherosclerotic plaque (1-3). The possibility that ceroid, an oxidized lipoprotein byproduct, might be antigenic was first suspected when human immunoglobulin IgG and to lesser extent IgM was found to localize to ceroid in atherosclerotic plaques from patients with chronic periaortitis (4). Subsequently, circulating autoantibodies to ceroid and oxidized low-density lipoprotein (OxLDL) were demonstrated in patients with chronic periaortitis but also, albeit to lesser extent, in elderly persons with minimal atherosclerosis and in patients with ischemic heart disease (5). Ceroid and OxLDL showed strong cross-reactivity (5). To date, these preliminary findings have not been verified.

Although the presence of these antibodies may be an epiphenomenon of little pathogenetic significance, the postulated pathogenesis of chronic periaortitis as being the result of an autoallergic reaction to OxLDL has since been adopted by many investigators (6-8). The described association with autoimmune disorders and the often observed beneficial response to immunosuppressive treatment would further fit the proposed hypothesis (9,10).

However, atherosclerosis *per se* is considered a chronic inflammatory process of the arterial wall associated with systemic and local immune responses to various antigens, notably OxLDL (11). No specific data exist on lipid profile and atherosclerotic biomarkers in patients with chronic periaortitis. In addition, aneurysm exclusion does not always lead to regression of periaortic fibrosis in aneurysmal chronic periaortitis (12).

We performed a case-control study comparing serum aOxLDL antibodies and lipid profile in patients with chronic periaortitis with those in age- and sex-matched control subjects.

## Material and methods

### *Subjects*

In a cross-sectional case-control study, 20 study patients were randomly selected from our cohort of patients with a definite diagnosis of chronic periaortitis followed at the out-patient department of internal medicine of the Albert Schweitzer hospital, Dordrecht. Chronic periaortitis was diagnosed on the basis of clinicoradiological and/or histopathological criteria and encompasses patients with idiopathic RPF and patients with peri-aneurysmal fibrosis ('inflammatory aneurysm')(1,10,12,14). Median time interval since diagnosis of chronic periaortitis was 19.5 (IQR 10-33) months. Except for 4 patients who were treated with immunosuppressants, first-line treatment consisted of tamoxifen 20 mg two-times daily. Study patients were compared to 20 age- and sex-matched control subjects. These were predominantly recruited from partners (n = 18), thereby eliminating possible confounding by environmental factors. Written informed consent was obtained from all participants. The study was approved by the ethics committee of our institution. Information of smoking habits, history of cardiovascular disease and use of antihypertensive and/or lipid-lowering medication was obtained from all subjects. Clinical examination included measurement of weight and height for calculation of body mass index and measurement of resting arm blood pressure. Hypertension was defined as on-going use of antihypertensive medication and/or a resting arm blood pressure of > 140 mm Hg systolic and/or > 90 mm Hg diastolic. Cardiovascular disease was defined as established coronary vascular and/or cerebrovascular disease and/or peripheral arterial occlusive disease requiring intervention. Life-time amount of smoking was registered in pack-years by dividing the life-time number of cigarettes by 20 x 365. Blood samples from patients and controls were obtained under fasting conditions and placed in K<sub>2</sub>ethylenediaminetetra-acetate(1.8 mg/ml)-containing vials and in vials without anticoagulant at 4°C. Serum samples were prepared from blood allowed to clot by centrifugation, and lipids were determined freshly. Plasma samples were prepared within 30 minutes of collection, supplemented with sacharose (0.6%, w/v) to prevent formation of LDL aggregates, and stored at -80 °C until analysis of OxLDL.

### *Antibodies against oxidized LDL*

Circulating autoantibodies to OxLDL, differentiated by IgG and IgM subclass, were measured as described previously (18). In short, samples were incubated in wells of microtiter plates precoated with native or oxidized LDL, and bound antibodies were detected using peroxidase-

conjugated antibodies from goat specific for human IgG or IgM (Sigma-Aldrich, Steinheim, Germany). The results were expressed as the mean optical density values at 450 nm from duplicate measurements, and the antibody titer to oxidized LDL was calculated by subtracting the binding to native LDL from the binding to oxidized LDL. With this subtraction method the assay not only corrects for specific binding to native LDL but also for nonspecific binding of each sample to the microtiter wells. All determinations of autoantibodies to oxidized LDL were performed on 1 day using the same batch of native and oxidized LDL and the same batch of reagents.

#### *Other methods*

For measurement of OxLDL we used a commercially available non-competitive ELISA (Mercodia, Uppsala, Sweden). In this assay the immobilized monoclonal antibody 4E6 specifically captures oxidized apolipoprotein B from the sample, which is subsequently detected with an antibody to apolipoprotein B. Intra- and interassay coefficients of variation amounted to 6.0% and 7.0%, respectively. Total plasma apoB concentrations were determined by immunonephelometry. Intra- and interassay coefficients of variation amounted to 2.4 and 6.0%, respectively. High-sensitivity C-reactive protein (hsCRP) was measured using a commercially available enzyme immunoassay according to the instructions of the manufacturer (Hyphen BioMed, Neuville-sur-Oise, France; and Dako, Glostrup, Denmark, respectively). Intra- and interassay coefficients of variation amounted to 1.4% and to 3.8% and to 6.0% and 9.7%, respectively. Plasma cholesterol and triglycerides were measured with an enzymatic colorimetric assay system (Boehringer-Mannheim automatic analyses for Hitachi 717; Diagnostica). High-density lipoprotein (HDL)-cholesterol was measured after lipoproteins that contained apoB were precipitated with phosphotungstate-magnesium chloride. Low-density lipoprotein (LDL)-cholesterol was calculated using the Friedewald formula.

## Statistical analysis

Because of skewed distribution of several variables, all continuous variables were reported as median and interquartile range (25th to 75th percentile). In addition, all analyses were done with non-parametric tests. Differences between continuous variables were analyzed by using Mann-Whitney test. Categorical variables were expressed as proportions and compared with Fisher exact test. Spearman rank correlation coefficient was used to test associations between variables. All reported *P*-values are two-sided. A *P*-value of  $< 0.05$  was considered significant. All statistical analyses were performed with SPSS software (version 11.0.1; SPSS inc., Chicago, IL).

## Results

Demographic and clinical characteristics of study patients and control subjects are shown in Table 1. There were only 3 non-smokers in the patient group (15%) and 4 non-smokers (20%) in the control group, but patients tended to have more smoking pack-years. Use of antihypertensive medication was more frequent among study patients than among control subjects. Otherwise, there were no significant differences in demographic or clinical characteristics between groups. Lipid profile and hsCRP levels of study patients and control subjects are also shown in Table 1. Study patients had significantly lower LDL-cholesterol levels than control subjects. As plasma apoB concentrations were similar, this probably reflects higher VLDL levels in study patients. HsCRP levels tended to be higher in study patients. Titres of aOxLDL-IgM and IgG, OxLDL and ratio OxLDL/apoB of study patients and control subjects are shown in Table 2. Titres of aOxLDL-IgM and IgG did not differ significantly between study patients and control subjects. When cases and controls were analyzed together ( $n = 40$ ), aOxLDL-IgM titres were significantly higher in females than in males (Fig 1). This difference in aOxLDL-IgM titres between females and males was observed in the patient group as well as in the control group, albeit only statistically significant in the patient group. Titres of aOxLDL-IgG did not differ between males and females in the total group nor in the subgroup of patients or control subjects (Fig 1). Compared to male patients, female patients had higher levels of total cholesterol and HDL-cholesterol. Otherwise, there were no significant differences between males and females in the patient group (Table 3). In study patients, aOxLDL-IgM titres were significantly lower in those with established cardiovascular disease compared to those without cardiovascular disease (Fig 2). This difference could not be explained by differences in the number of males among study patients with or without cardiovascular disease (5/8 males [62%] vs. 7/12 males [58%]). In study patients, aOxLDL-IgM titres correlated inversely with diastolic blood pressure and with the OxLDL/apoB ratio (Fig 3). aOxLDL-IgM titres in patients also correlated positively with total cholesterol ( $r = 0.56$ , 95%CI 0.14 to 0.80;  $P = 0.009$ ), LDL-cholesterol ( $r = 0.64$ , 95%CI 0.25 to 0.85;  $P = 0.003$ ) and HDL-cholesterol (Fig 3), respectively. No correlation was found between titres of aOxLDL-IgM in patients and other variables (data not shown). In control subjects, no correlations were found between aOxLDL-IgM titres and other variables (data not shown). With respect to titres of aOxLDL-IgG, no correlation with other variables was found in both the patient group and control group (data not shown). There were no differences between study patients and control subjects in OxLDL

levels and OxLDL/apoB ratio (Table 2). Circulating plasma OxLDL levels correlated significantly with total cholesterol, LDL-cholesterol and apoB levels in both the patient group and the control group (data not shown). In study patients but not in control subjects, plasma OxLDL concentrations correlated negatively with age (Spearman  $r = -0.62$  [95%CI -0.84 to -0.24];  $P < 0.01$ ) and positively with smoking pack-years (Spearman  $r = 0.58$  (95%CI 0.17 to 0.81];  $P < 0.01$ ).

## Discussion

This study was undertaken to evaluate the role of aOxLDL antibodies in patients with chronic periaortitis and to distinguish them from age- and sex-matched controls. Chronic periaortitis typically occurs in patients at increased cardiovascular risk (19). In addition, aOxLDL antibodies are prevalent among patients with atherosclerotic vascular disease (15-17). Except for a tendency of more smoking pack-years and higher hs-CRP levels, patients and controls were comparable in terms of cardiovascular risk. We observed no significant differences in aOxLDL IgG or IgM antibody levels between patients and controls.

Parums *et al* found aOxLDL antibodies, undifferentiated by subclass IgG or IgM, to be more frequently present in patients with chronic periaortitis compared to elderly ‘controls’ (i.e., subjects without chronic periaortitis and only minimal atherosclerosis) and compared to patients with angina pectoris (5). Data were not adjusted for age and sex. Indeed, elderly ‘controls’ were older (mean age 66.7 y vs. 62.7 y) and more often of female sex (40% vs. 15%). aOxLDL antibody levels are inversely proportional to aging of people (23). Moreover, aOxLDL antibodies in elderly individuals were determined in stored serum samples obtained before death. It may be that age of serum samples and storage conditions influence aOxLDL antibody levels. All these factors may have biased results. In addition, complete absence of clinical and laboratory data, including medication use, in this study makes direct comparison of patients and ‘controls’ difficult (5).

The frequent presence of aOxLDL antibodies in subjects without chronic periaortitis in Parum’s study and the non-significant difference in aOxLDL antibody titres between study patients and controls may suggest that antigenic exposure with subsequent formation of aOxLDL antibodies is common, whereas clinical periaortitis disease is rare. This may reflect inter-individual differences in response to this antigenic stimulus, or that antibody formation is no more than an epiphenomenon seen in patients with (subclinical) atherosclerosis of no clinical significance. Of note, most patients with chronic periaortitis in our study were (being) treated with tamoxifen. Tamoxifen is associated with beneficial effects on lipid profile, C-reactive protein levels and on atherosclerotic lesions (20-22), and as such, may reduce aOxLDL antibody levels. Although one can not exclude the possibility that a significant difference in aOxLDL antibody titres between patients and controls is masked by such mechanisms, no data exist substantiating this possible confounding effect.

Many studies have been conducted to understand the relevance of aOxLDL antibodies in atherosclerotic vascular disease, but its clinical significance is not clear (17-19). aOxLDL

antibodies are also present in various chronic inflammatory and auto-immune disorders such as systemic lupus erythematosus, gout, rheumatoid arthritis and Behcet's disease as well as in other conditions such as diabetes mellitus and pre-eclampsia (17-19,24-26). One thing they have in common is that all these diseases are thought to be prone for atherosclerosis development (17-19). The same holds true for patients with chronic periaortitis (1,2,16).

IgM and IgG antibodies to OxLDL may play a different part in the pathogenesis of atherosclerotic vascular disease, with aOxLDL-IgM having a protective role whereas aOxLDL-IgG enhances plaque formation (17,18,27). aOxLDL antibody level may decline over the years, thereby possibly explaining the higher incidence of cardiovascular disease at older age (23). In hypertensive subjects, high aOxLDL-IgM levels predicted a favorable outcome in the development of carotid atherosclerosis (28). In women, low aOxLDL-IgM titres were associated with the presence of diabetes mellitus and/or myocardial infarction (27). Low aOxLDL-IgM titres were associated with the presence of established CVD in our patients. These findings might further suggest a protective role of aOxLDL-IgM. Increased consumption of aOxLDL-IgM in advancing atherosclerosis may be an alternative explanation (29). Interestingly, patients with low aOxLDL-IgM titres tended to have a high diastolic blood pressure, low HDL-cholesterol level and high OxLDL/apoB ratio. A higher OxLDL/apoB ratio indicates increased oxidative stress in the vascular wall (30,31). All these factors may contribute to the increased risk of CVD in patients with low aOxLDL-IgM.

As we tested sera from patients with stable chronic periaortitis, aOxLDL antibody titres reflect basal values. This may account for the noted associations. In various states of active vascular inflammation, aOxLDL antibody titres may be temporarily reduced or increased and can therefore not be used to ascertain basal values (17-19,24,25,32). However, it is remarkable that aOxLDL-IgM but not aOxLDL-IgG antibody titres were associated with several other cardiovascular variables in our study. Contrary to aOxLDL-IgG, aOxLDL-IgM is probably largely confined to the intravascular compartment (17). Therefore, one might speculate that these serum levels are of more clinical relevance than serum levels of aOxLDL-IgG, which is in large part confined to the vascular wall (4,17).

As in other studies (27,28), females tended to have higher aOxLDL-IgM titres than males in our study. A possible explanation is that postmenopausal women have lower estrogen levels, which may lead to uninhibited oxidation of LDL (34,35). The risk of a primary immune response increases with the formation of aOxLDL antibodies. Postmenopausal hormone replacement therapy may (33) or may not reduce aOxLDL levels (34,36). However, none of our female patients or controls used hormonal replacement therapy.

Circulating OxLDL was not associated with aOxLDL-IgG or IgM antibody titres. Consistent with the literature (17-19,30,37,38), OxLDL levels were positively associated with total cholesterol, LDL-cholesterol and apoB levels in patients and controls. Smoking was not associated with aOxLDL antibody titres in this and other studies (39,40). However, smoking was positively correlated with OxLDL levels in patients but not in controls. Others also noted no correlation between OxLDL levels and smoking in clinically healthy, middle-aged men from the general population (37). Smoking is a well-known risk factor for atherosclerotic aortic aneurysm, and an even stronger risk factor for the inflammatory variant (9,39). We recently observed an independent association between smoking and infrarenal aortic diameter in patients with chronic periaortitis (19). Cessation of smoking should therefore be strongly encouraged in all cases of chronic periaortitis, irrespective of the presence of aneurysmal dilation.

The exact pathway by which chronic periaortitis occurs remains unknown. Although it seems clear that not aneurysm formation *per se* but advanced atherosclerosis is the prerequisite for development of typical chronic periaortitis, it remains an enigma why so few patients with advanced atherosclerosis develop this chronic fibrotic inflammatory disorder. Indeed, only 5% of atherosclerotic abdominal aortic aneurysms are of the inflammatory variant (12, 39). Deranged immunological self-tolerance, possibly by genetic predisposition, may play a role (8,10,16). An association between chronic periaortitis and HLA-DRB1\*03, an allele linked to several autoimmune conditions, was recently noted (42). This might explain the simultaneous occurrence of autoimmune disorders (e.g., lupus erythematosus, Wegener's granulomatosis, polymyalgia rheumatica) in some patients with chronic periaortitis (9,10,16) and the often observed beneficial response to immunosuppressive treatment (13). One might speculate that in predisposed patients, an exaggerated and persistent inflammatory response with subsequent development of chronic periaortitis results from the same stimuli and mechanisms that are involved in atherosclerosis development *per se*. In addition, as this disorder may occur in patients without atherosclerosis, chronic periaortitis as a primary autoimmune disease should also be considered (10).

Our study also has limitations. As sample size was rather small, our study may not have enough power to detect significant differences. However, given the low annual incidence of chronic periaortitis of 1.3 per 100.000 (16), it will be difficult to recruit a large number of patients. As noted, the cross-sectional nature of our study may have influenced results as some patients were still receiving tamoxifen. Unfortunately, we did not have serum samples of study

patients before the start of treatment for chronic periaortitis to determine sequential aOxLDL antibody titres before and during treatment.

In conclusion, this first case-control study showed no significant difference in aOxLDL antibody titres between patients with stable chronic periaortitis and control subjects. However, distinctive differences and associations of aOxLDL-IgM antibodies and of circulating OxLDL levels were observed within the study but not within the control group. Data suggest a differing innate immune response to OxLDL in patients with chronic periaortitis compared to controls. Whether this response is causally related to chronic periaortitis development remains to be clarified.

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**Conflict of interest:** No.

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## References

1. Mitchinson MJ. Retroperitoneal fibrosis revisited. *Arch Pathol Lab Med* 1986;110:784-786.
2. Parums DV. The spectrum of chronic periaortitis. *Histopathology* 1990;16:423-431.
3. Parums DV, Choudhury RP, Shields SA, Davies AH. Characterisation of inflammatory cells associated with 'idiopathic retroperitoneal fibrosis'. *Br J Urol* 1991;67:564-568.
4. Parums DV, Chadwick DR, Mitchinson MJ. The localisation of immunoglobulin in chronic periaortitis. *Atherosclerosis* 1986; 61: 117-123.
5. Parums DV, Brown DL, Mitchinson MJ. Serum antibodies to oxidized low-density lipoprotein and ceroid in chronic periaortitis. *Arch Pathol Lab Med* 1990;114:383-387.
6. Bullock N. Idiopathic retroperitoneal fibrosis. *BMJ* 1988;297:240-241.
7. Baker LRI. Auto-allergic periaortitis (idiopathic retroperitoneal fibrosis). *BJU* 2003;92:663-665 (Editorial comment).
8. Meier P, Vogt B, Blanc E. Rethinking the triggering inflammatory processes of chronic periaortitis. *Nephron Exp Nephrol* 2007;105:e17-e23.
9. Haug ES, Skomsvoll JF, Jacobsen G, Halvorsen TB, Saether OD, Myhre HO. Inflammatory aortic aneurysm is associated with increased incidence of autoimmune disease. *J Vasc Surg* 2003;38:492-497.
10. Vaglio A, Corradi D, Manenti L, Ferretti S, Garini G, Buzio C. Evidence of autoimmunity in chronic periaortitis: a prospective study. *Am J Med* 2003;114:454-462.
11. Galkina E, Ley K. Immune and inflammatory mechanisms of atherosclerosis. *Annu Rev Immunol*. 2009;27:165-97.
12. Van Bommel EFH, van der Veer SJ, Hendriksz TR, Bleumink GS. Persistent chronic periaortitis ('inflammatory aneurysm') after abdominal aortic aneurysm repair: systematic review of the literature. *Vasc Med* 2008;13:293-303.
13. Van Bommel, Hendriksz TR, Huiskes AW, Zeegers AG. Brief communication: tamoxifen therapy for idiopathic retroperitoneal fibrosis. *Ann Intern Med* 2006;144:101-106.
14. Van Bommel EF, Siemes C, Hak LE, van der Veer SJ, Hendriksz TR. Long-term renal and patient outcome in idiopathic retroperitoneal fibrosis treated with prednisone. *Am J Kidney Dis* 2007;49:615-25.
15. Tits LJH, van, Waart F de, Hak-Lemmers HLM, et al. Effects of alfa-Tocopherol on superoxide production and plasma intercellular adhesion molecule-1 and antibodies to oxidized LDL in chronic smokers. *Free Radical Biol Med* 2001;30:1122-1129.

16. Virella G, Lopes-Virella MF. Lipoprotein autoantibodies: measurement and significance. *Clin Diagnostic Lab Immunology* 2003;10:499-505.
17. Shoenfeld Y, Wu R, Dearing LD, Matsuura E. Are anti-oxidized low-density lipoprotein antibodies pathogenic or protective? *Circulation*. 2004;110(17):2552-8.
18. Tsimikas S, Willerson JT, Ridker PM. C-reactive protein and other emerging blood biomarkers to optimize risk stratification of vulnerable patients. *J Am Coll Cardiol* 2006;47:C19-31.
19. Van Bommel EFH, Jansen I, Hendriksz TR, Aarnoudse ALHJ. Idiopathic retroperitoneal fibrosis: prospective evaluation of incidence and clinicoradiological presentation. *Medicine* 2009;88:193-201.
20. Bonanni B, Johansson H, Gandini S, et al. Effect of tamoxifen at low doses on ultrasensitive C-reactive protein in healthy women. *J Thromb Haemost* 2003;1:2149-2152.
21. De Medina P, Payre BL, Bernad J, et al. Tamoxifen is a potent inhibitor of cholesterol esterification and prevents the formation of foam cells. *J Pharmacol* 2004;308:1165-1173.
22. Grainger DJ, Schofield PM. Tamoxifen for the prevention of myocardial infarction in humans: preclinical and clinical evidence. *Circulation* 2005;112:3018-3024.
23. Tinahones FJ, Gomez-Zumaquero JM, Garrido-Sanchez L, et al. Influence of age and sex on levels of anti-oxidized LDL antibodies and anti-LDL immune complexes in the general population. *J Lipid Res* 2005;46:452-457.
24. Gomez-Zumaquero JM, Tinahones FJ, Ramón E de, Camps M, Garrido L, Soriguer FJ. Association of biological markers of activity of lupus erythematosus with levels of anti-oxidized low-density lipoprotein antibodies. *Rheumatology* 2004;43:510-513.
25. Tsutsumi Z, Moriwaki Y, Takahashi S, Ka, T, Yamamoto T. Oxidized low-density lipoprotein autoantibodies in patients with primary gout: effect of urate-lowering therapy. *Clin Chim Acta* 2004;339:117-122.
26. Winyard PG, Tatzber F, Esterbauer H, Kus ML, Blake DR, Morris CJ. Presence of foam cells containing oxidised low density lipoprotein in the synovial membrane from patient with rheumatoid arthritis. *Ann Rheum Dis* 1993;52:677-680.
27. Dotevall A, Hulthe J, Rosengren A, Wiklund O, Wilhelmsen L. Autoantibodies against oxidized low-density lipoprotein and C-reactive protein are associated with diabetes and myocardial infarction in women. *Clin Science* 2001;101:523-531.

28. Georgiades SJ, Wu R, Thulin T, Frostegard J. Antibodies of IgM subclass to phosphorycholine and oxidized LDL are protective factors for atherosclerosis in patients with hypertension. *Atherosclerosis* 2006;188:160-166.
29. Orchard TJ, Virella G, Forrest KY, Evans RW, Becker DJ, Lopes-Virella MF. Antibodies to oxidized LDL predict coronary artery disease in type 1 diabetes: a nested case-control study from the Pittsburgh Epidemiology of Diabetes Complications Study. *Diabetes* 1999;
30. Van Tits LHJ, Himbergen TM, Lemmers HLM, de Graaf J, Stalenhoef AFH. Proportion of oxidized LDL relative to plasma apolipoprotein B does not change during statin therapy in patients with heterozygous familial hypercholesterolemia. *Atherosclerosis* 2006;185:307-31248:1454-1458.
31. Van Tits LHJ, De Graaf J, Hak-Lemmers H, Bredie S, Demacker P, Holvoet P, Stalenhoef AFH. Increased levels of low-density lipoprotein oxidation in patients with familial hypercholesterolemia and in end-stage renal disease patients on hemodialysis. *Lab Invest* 2003;83:13-21,2003.
32. Swets BP, Brouwer DAJ, Cohen Tervaert JW. Patients with systemic vasculitis have increased levels of autoantibodies against oxidized LDL. *Clin Exp Immunol* 2001;124:163–167.
33. Heikkinen AM, Niskanen L, Ylä-Herttuala S, et al. Postmenopausal hormone replacement therapy and autoantibodies against oxidized LDL. *Maturitas* 1998;29:155-161.
34. Bureau I, Laporte F, Favier M, et al. No antioxidant effect of combined HRT on LDL oxidizability and oxidative stress biomarkers in treated post-menopausal women. *J Am Coll Nutr* 2002;21:333-338.
35. Hoogerbrugge N, Zillekens MC, Jansen H, et al. Estrogen replacement decreased the levels of antibodies against oxidized low density lipoprotein in postmenopausal women with coronary heart disease. *Metabolism* 1998;47:675-680.
36. Uint L, Gebara OCE, Pinto LB, et al. Hormone replacement therapy increases levels of antibodies against heat shock protein 65 and certain species of oxidized low density lipoprotein. *Braz J Med Biol Research* 2003;36:491-494.
37. Meisinger C, Baumert J, Khuseyinova N, Loewel H, Koenig W. Plasma oxidized low-density lipoprotein, a strong predictor for acute coronary heart disease events in apparently healthy, middle-aged men from the general population. *Circulation* 2005;112:651-657.

38. Hulthe J, Fagerberg B. Circulating oxidized LDL is associated with subclinical atherosclerosis development and inflammatory cytokines (AIR study). *Arterioscler Thromb Vasc Biol* 2002;22:1162-1167.
39. Nitecki SS, Hallett JW, Stanson AW, Ilstrup DM, Bower TC, Cherry KJ, Gloviczki P, Pairello PC. Inflammatory abdominal aneurysms: a case-control study. *J Vasc Surg* 1996;23:860-869.
40. Frostegard J, Ruihua WU, Lemne C, Thulin T, Witzum JL, de Faire U. Circulating oxidized low-density lipoprotein is increased in hypertension. *Clin Science* 2003;105:615-620.
41. Bergmark C, Wu R, de Faire U, Lefvert AK, Swedenborg J. Patients with early-onset peripheral vascular disease have increased levels of autoantibodies against oxidized LDL. *Arterioscler Thromb Vasc Biol* 1995;15:441-445.
42. Martorana D, Vaglio A, Greco P, Zanetti A, Moroni G, Salvarani C, Savi M, Buzio C, Neri TM. Chronic periaortitis and HLA-DRB1\*03: another clue to an autoimmune origin. *Arthritis Rheum* 2006;55:126-30.

## Legends to the figures

### Figure 1:

aOxLDL antibody titres, differentiated by subclass IgM and IgG, according to sex in the overall group, patients and controls. Female patients had higher aOxLDL titres than male patients. A similar trend, albeit not significant, was seen for aOxLDL-IgM in the control group.

### Figure 2:

aOxLDL-IgM titres in study patients with established cardiovascular disease (CVD) and in study patients with no established CVD. aOxLDL-IgM titres were significantly lower in patients with established CVD.

### Figure 3:

aOxLDL-IgM titres in study patients were inversely correlated with diastolic blood pressure (Spearman  $r = -0.46$ , 95%CI -0.75 to -0.01) and ratio OxLDL/apoB (Spearman  $r = -0.41$ , 95%CI -0.73 to 0.04), and positively correlated with HDL-cholesterol levels (Spearman  $r = 0.47$ , 95%CI 0.02 to 0.69). Lines depict regression line of y on x and 95% confidence intervals for regression line.

**Table 1. Demographics, Clinical Characteristics, Lipid Profile and High-Sensitivity C-reactive**

**Protein Level of Study Patients and Control Subjects.**

| Variable                                             | Patients<br><i>n</i> = 20 | Controls<br><i>n</i> = 20 | <i>P</i> -value |
|------------------------------------------------------|---------------------------|---------------------------|-----------------|
| Age (years)                                          | 61 (53 – 71)              | 59 (53 - 69)              | 0.59            |
| Male sex, <i>n</i> (%)                               | 11 (55)                   | 10 (50)                   | 0.79            |
| BMI (kg/m <sup>2</sup> )                             | 27.5 (23.8 – 28.9)        | 26.2 (23.2 – 30.8)        | 0.96            |
| Smoking pack-years (years)                           | 27.3 (14.6 - 39.5)        | 16.2 (3.05 – 26.5)        | 0.12            |
| Systolic BP (mm Hg)                                  | 140 (125 – 153)           | 146 (134 – 156)           | 0.25            |
| Diastolic BP (mm Hg)                                 | 81 (74 – 86)              | 85 (80 – 91)              | 0.08            |
| CVD*, <i>n</i> (%)                                   | 8 (40)                    | 5 (25)                    | 0.41            |
| Diabetes mellitus, <i>n</i> (%)                      | 4 (20)                    | 3 (15)                    | 0.78            |
| Patients using antihypertensive agents, <i>n</i> (%) | 15 (75)                   | 8 (40)                    | 0.06            |
| Patients using statins, <i>n</i> (%)                 | 8 (40)                    | 5 (25)                    | 0.35            |
| Total cholesterol (mmol/L)                           | 5.2 (4.4 – 5.8)           | 5.6 (4.7 – 6.4)           | 0.12            |
| Triglycerides (mmol/L)                               | 1.8 (1.3 – 2.4)           | 1.7 (1.2 – 1.9)           | 0.29            |
| HDL-cholesterol (mmol/L)                             | 1.2 (0.9 – 1.4)           | 1.3 (1.1 – 1.5)           | 0.19            |

|                          |                  |                   |      |
|--------------------------|------------------|-------------------|------|
| LDL-cholesterol (mmol/L) | 2.7 (1.9 – 3.7)  | 3.7 (2.7 – 4.2)   | 0.02 |
| Apolipoprotein B (mg/L)  | 969 (775 – 1108) | 1013 (863 – 1164) | 0.30 |
| hsCRP (mg/L)             | 5.2 (1.4 – 11.0) | 2.4 (1.0 – 6.9)   | 0.20 |

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Values are median and interquartile range or number and percentage, where appropriate.

Abbreviations: BMI, body mass index; BP, blood pressure; CVD, cardiovascular disease.

\*: Defined as established coronary vascular, cerebrovascular or peripheral vascular disease, requiring intervention.

**Table 2. Levels of aOxLDL antibodies, OxLDL and Ratio OxLDL/apoB in Study Patients and Control Subjects.**

|                             | <b>Ig Class</b> | <b>Patients<br/><i>n</i> = 20</b> | <b>Controls<br/><i>n</i> = 20</b> | <b><i>P</i>-value</b> |
|-----------------------------|-----------------|-----------------------------------|-----------------------------------|-----------------------|
| aOxLDL (OD <sub>450</sub> ) | IgM             | 0.70 (0.24 – 1.08)                | 0.54 (0.25 – 0.73)                | 0.31                  |
|                             | IgG             | 0.59 (0.38 – 0.75)                | 0.41 (0.33 – 0.64)                | 0.23                  |
| OxLDL (U/L)                 |                 | 62.0 (51.9 – 66.0)                | 65.5 (54.6 – 80.4)                | 0.15                  |
| Ratio OxLDL/apoB            |                 | 0.06 (0.05 – 0.07)                | 0.06 (0.06 – 07)                  | 0.90                  |

Values are median and interquartile range.

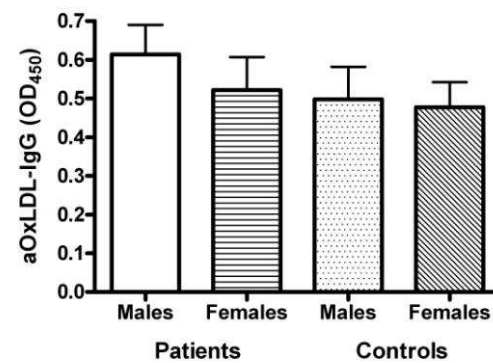
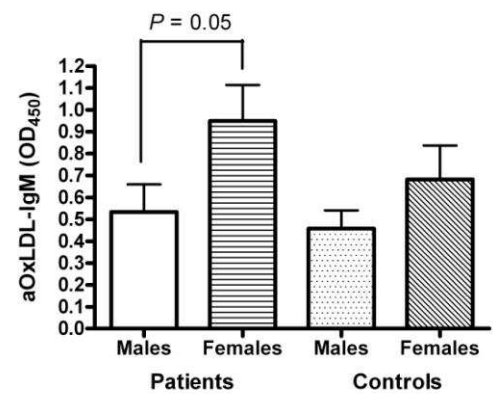
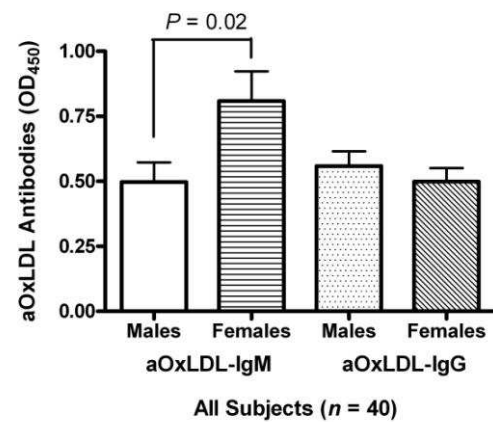
**Table 3. Characteristics of Male and Female Patients with Chronic Periaortitis.**

| Variable                   | Study Patients     |                    | P-value |
|----------------------------|--------------------|--------------------|---------|
|                            | Males<br>n = 11    | Females<br>n = 9   |         |
| Age (years)                | 60 (52 - 73)       | 63 (55 - 72)       | 0.81    |
| BMI (kg/m <sup>2</sup> )   | 28.7 (24.2 - 30.9) | 24.2 (22.4 - 28.2) | 0.07    |
| Smoking pack-years (years) | 26.5 (15.0 - 40.0) | 31.5 (5.4 - 40.8)  | 1.0     |
| Systolic BP (mm Hg)        | 127 (116 - 156)    | 144 (134 - 151)    | 0.21    |
| Diastolic BP (mm Hg)       | 80 (72 - 85)       | 81 (75 - 86)       | 0.84    |
| Total cholesterol (mmol/L) | 4.4 (3.4 - 5.5)    | 5.7 (5.0 - 6.2)    | 0.01    |
| Triglycerides (mmol/L)     | 2.0 (1.0 - 2.5)    | 1.7 (1.5 - 2.3)    | 0.79    |
| HDL-cholesterol (mmol/L)   | 1.1 (0.82 - 1.3)   | 1.4 (1.2 - 1.6)    | 0.03    |
| LDL-cholesterol (mmol/L)   | 2.3 (1.8 - 3.7)    | 3.4 (2.7 - 3.7)    | 0.11    |
| Apolipoprotein B (mg/L)    | 929 (659 - 1053)   | 1002 (828 - 1210)  | 0.12    |
| hsCRP (mg/L)               | 4.4 (1.2 - 11.0)   | 6.0 (1.5 - 27.0)   | 0.59    |
| OxLDL (U/L)                | 62.0 (46.8 - 66.8) | 60.4 (53.4 - 69.8) | 0.64    |
| Ratio OxLDL/apoB           | 0.06 (0.06 - 0.07) | 0.06 (0.05 - 0.07) | 0.63    |

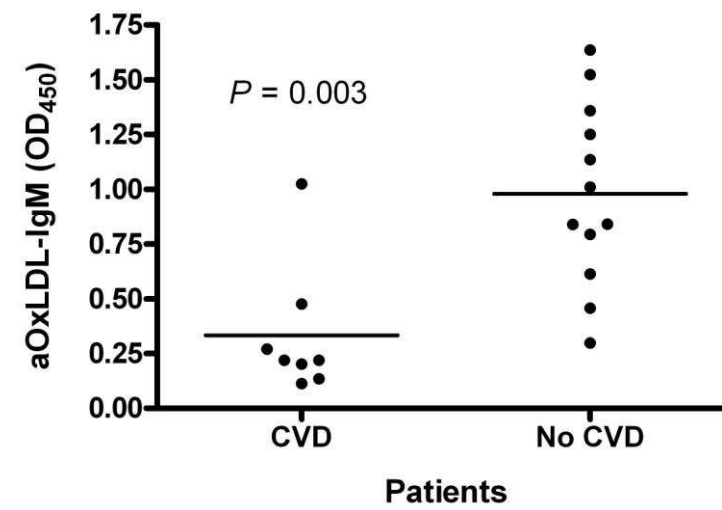
Values are median and interquartile range.

Abbreviations: BMI, body mass index; BP, blood pressure.

Figure



Figure



Figure

