International Journal of Cardiology 168 (2013) 484–489

Contents lists available at ScienceDirect

International Journal of Cardiology

journal homepage: www.elsevier.com/locate/ijcard

Antibodies to malondialdehyde oxidized low-density lipoproteins predict long term cardiovascular mortality in high risk patients

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ARTICLE INFO

Article history:
Received 3 May 2012
Received in revised form 3 September 2012
Accepted 25 September 2012
Available online 25 October 2012

Keywords:
Coronary artery disease
Anti-LDL-oxidized antibodies
Prognosis
Atherosclerosis

ABSTRACT

Aims: Antibodies to oxidized low-density lipoproteins (oxLDLAbs) are detectable in the serum of patients with and without atherosclerosis, but it is unclear if they play a pathogenic or a protective role in atherogenesis or if they are simply a marker of atherosclerosis. Therefore, in a prospective cohort study we investigated if oxLDLAbs titer predicts cardiovascular (CV) events in high-risk coronary artery disease patients.
Methods and results: The titer of IgG antibodies to malondialdehyde modified oxidized low-density lipoproteins was measured in 748 randomly selected patients of the GENICA study who underwent coronary angiography and assessment of incident CV events at follow-up. Patients were classified by oxLDLAbs into a low and a high titer group, corresponding to the first three and the last quartile, respectively. Cardiovascular event-free survival was compared between oxLDLAbs groups by Kaplan–Meier and multivariate technique including propensity score matching analysis. During long-term follow-up (median 7.2 years) CV deaths were observed in 65 patients (11.6%), more commonly in the high than in the low oxLDLAbs group (patients free from CV death 83.1% vs. 89% respectively, $p = 0.025$). The incidence of CV events was also higher in the former than in latter (event-free survival 69.2% vs. 77.7% respectively, $p = 0.030$).
Conclusions: An oxLDLAbs titer above the 75th percentile is a marker of LDL oxidation which predicts a worse CV prognosis at long term follow-up in high-risk Caucasian patients referred for coronary angiography.

1. Introduction

Accumulation of low-density lipoproteins (LDLs) in the subendothelial extracellular space promotes recruitment of monocytes and their differentiation into macrophages. The latter produce reactive nitrogen and oxygen species (ROS) that induce oxidation of LDLs thus generating oxidized LDLs (oxLDLs). OxLDLs are cytotoxic to endothelial cells, chemotactic for monocytes, and mitogenic for macrophages and smooth muscle cells [1–3]. Moreover, their binding to the scavenger receptors is followed by internalization with ensuing foam cell formation. Hence, they have multiple effects that are key for atherogenesis [4].

Oxidation of LDLs induces immunogenic epitopes in their molecule [5] with ensuing generation of antibodies against oxLDLs (oxLDLAbs) [6] that are detectable in the sera of the majority of patients with advanced atherosclerotic lesions [7]. These autoantibodies can therefore be viewed as an in vivo marker of LDL oxidation, but their biological role remains puzzling [6] since they were found also in children without atherosclerosis [8]. It has therefore been contended that oxLDLAbs protect from atherosclerosis by neutralizing the aforementioned pathogenic actions of oxLDL [9,10]. Along this line a reduced atherosclerosis that correlated with the levels of immunoglobulin G (IgG) oxLDLAbs was found in rodents immunized with oxLDL [9–12]. Moreover, a monoclonal IgM anti-oxLDL antibody (EO6) derived from apoE-deficient mouse was shown to block the oxLDL binding to the macrophage scavenger receptors CD36 and SR-B1. Thus, oxLDLAbs and the innate immune response to oxLDL may protect from atherosclerosis via the macrophages [13].

Notwithstanding all these premises and the wealth of experimental data, longitudinal studies on the prognostic value of oxLDLAbs have given controversial results [6]: about half of the studies showed an association of oxLDLAbs with cardiovascular (CV) events, but the rest showed no association. Only one study in end-stage renal disease patients, e.g. highly selected populations, suggested that a low oxLDLAbs titer could predict CV mortality [14], but results were opposite in another study [15]. Moreover, precautions to avoid the confounding effect of an unbalanced distribution of risk factors across

Abbreviations: BP, blood pressure; CAD, coronary artery disease; HDL, high-density lipoprotein; LDL, low density lipoprotein; MI, myocardial infarction; MDA, malondialdehyde; OxLDL, oxidized low density lipoprotein; OxLDLAbs, antibodies anti-oxidized low density lipoprotein.

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http://dx.doi.org/10.1016/j.ijcard.2012.09.165

the oxLDLAbs groups were adopted in no studies thus far. Therefore, in the prospective branch of the GENICA (Genetic and Environmental factors In Coronary Artery disease) study we sought to test the hypothesis that oxLDLAbs predict CV events using a statistical analysis that minimizes the impact of potential confounders.

2. Methods

2.1. Study participants

The protocol of the GENICA study will be briefly recalled as it was previously detailed [16,17]. The study enrolled consecutive Caucasian patients referred for coronary angiography to investigate chest pain and/or suspected CAD between 1999 and 2001. All signed a consent form to participate in this study and the Medical Ethics Committee approved the protocol. Refusal to participate and previous inclusion in the study were the only exclusion criteria. Among the population potentially eligible (no. 1273) only two patients refused participation to the study. Information on medical history, smoking habits, presence/absence of arterial hypertension, diabetes mellitus, dyslipidemia, and current medications was gathered with a staff administered questionnaire [17]. Definitions for body mass index (BMI), smoking status, diabetes mellitus, impaired glucose tolerance, hypercholesterolemia, and hypertriglyceridemia were already reported [16,17]. Blood pressure was measured by mercury sphygmomanometer using Korotkoff phase V for diastolic, according to the WHO guidelines. Hypertension was defined as systolic blood pressure ≥ 140 mm Hg and/or diastolic blood pressure ≥ 90 mm Hg and/or use of antihypertensive drugs.

2.2. Coronary angiography

Angiography and measurement of left ventricular ejection fraction (LVEF) and the grading of the CAD burden with the modified Duke Prognostic Index score were carried out as described [18]. This score considers major epicardial coronary arteries with ≥50% diameter stenosis and goes from 0 (all major coronary arteries with lesions <50% diameter stenosis) to 100 (≥95% left main stenosis). It was reported to accurately predict five-year mortality of medically treated patients [19].

2.3. Laboratory measurements

Patients were studied between 8.30 a.m. and noon. Blood samples were taken immediately before coronary angiography, put on ice, and centrifuged at 3000×*g* (at 4 °C for 10 min). Total cholesterol, HDL-cholesterol, triglycerides, glycemia, sodium, potassium, blood urea nitrogen, and creatinine levels were measured with conventional methods. IgG autoantibodies against malondialdehyde (MDA)-modified LDL (IgG oxLDLAbs) were assayed by ELISA with a commercially available kit (Anti-oxLDL Antibody ELISA KIT; IMMCO Diagnostic Inc.), according to manufacturer's specifications. The intra-assay and inter-assay coefficients of variation of this method were 9% and 15%, respectively, in our hands [16].

2.4. Follow-up data

Information on the long-term outcome of the patients was gathered blindly to their biochemical profile with a predefined form through review of medical charts for the patients regularly seen at referring hospitals, and through telephone interviews of family doctors, and/or patients, and/or first-degree relatives for those not attending regular follow-up visits. Predetermined primary endpoints were CV events, including acute coronary syndromes, stroke, and CV deaths. The latter comprised sudden death or due to congestive heart failure, acute coronary syndromes, or stroke according to the Syst-Eur Trial criteria [20]. All events were validated by the adjudication committee (GPR, MZ, and GM) blinded to patients' biochemical profile.

2.5. Statistical analysis

Serum triglycerides, HDL- and LDL-cholesterol, age, creatinine, CAD Duke Index score, LVEF, and oxLDLAbs were examined after log or square root transformation to achieve a Gaussian distribution. A random sample comprising 748 of the CAD patients originally recruited in the GENICA study was obtained with single random number generation through SPSS. Patients in this sample were selected for the oxLDLAbs titer measurement, which was performed blindly with respect to clinical and anthropometric data (see Fig. 1).

Standardized z scores were calculated to identify univariate outliers and exclusion of cases with z scores exceeding |3.29| that corresponded to a $p<0.001$, was decided a priori. Mahalanobis distance was assessed by regression analysis to identify multivariate outliers; cases with χ^2 in excess of 32.909 (12 df at $\alpha = 0.001$) were considered outliers and removed from further analysis (14 patients) according to the technique of Tabachnick and Fidell [21].

Comparison of quantitative variables across groups was done by ANOVA followed by Bonferroni's post hoc test. Chi-square analysis was used to compare the frequencies of categorical CAD risk factors.

Standard multiple regression analysis was used beforehand to verify the assumption that cases lost at follow-up did not differ significantly from those available for survival analysis. Propensity score was calculated with logistic regression analysis including all available variables (including gender, age, BMI, LDL- and HDL-cholesterol, triglycerides, serum creatinine, homocysteine, glycemia, serum sodium concentration, heart rate, arterial hypertension, smoking habit, LVEF, the Duke Prognostic Index of coronary atherosclerotic burden, length of follow-up, history, and treatment variables) that are known to potentially affect the outcomes. To correct for the imbalanced distribution of variables between the patients with low and high OxLDLAbs we did a greedy matching without replacement using a caliper of 0.2 standard deviations of the logit of the propensity score [22].

The distribution of measured baseline covariates was then compared between low and high OxLDLAbs groups in the matched samples, assessing the balance in measured variables with standardized differences (see Supplemental table) [23]. We plotted the Kaplan–Meier curve and compared the event-free survival for the matched set with the test proposed by Klein and Moeschberger [24]. Statistical significance was defined as $p<0.05$. SPSS 18 for Windows (SPSS Italy Inc., Bologna, Italy) was used for all analyses.

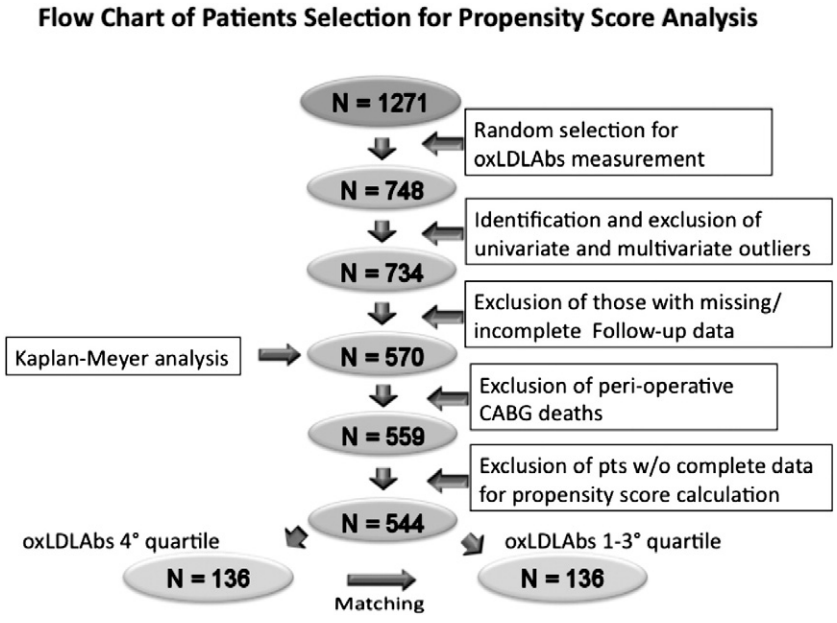

Fig. 1. Data analysis flow chart. Flow chart showing the selection process by which the patients were submitted to statistical analysis.