Atherosclerosis 221 (2012) 275–281

Contents lists available at SciVerse ScienceDirect

Atherosclerosis

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

White blood cell count is associated with carotid and femoral atherosclerosis

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ARTICLE INFO

Article history:
Received 17 August 2011
Received in revised form 6 December 2011
Accepted 23 December 2011
Available online 4 January 2012

Keywords:
White blood cells
Inflammation
Atherosclerosis
Intima-media-thickness
Carotid
Femoral
Plaque

ABSTRACT

Objective: Chronic low-grade inflammation is associated with atherosclerosis. Ultrasound imaging allows measurement of intima-media thickness (IMT) and plaque. We investigated the association between inflammatory markers and carotid and femoral atherosclerosis.
Methods: We studied 554 subjects with primary dyslipidemia (57% men, median age 49 years) and 246 age- and sex-matched normolipidemic subjects. Carotid and femoral arteries were imaged bilaterally with a standardized protocol. Mean and maximum common carotid IMT (CC-IMT and MaxCC-IMT) and common femoral IMT (F-IMT and MaxF-IMT), and carotid and femoral plaque were assessed. Carotid atherosclerosis was defined by CC-IMT and/or plaque height >75th percentile of a reference population. White blood cell count (WBCC) was measured in all subjects. High-sensitivity C-reactive protein (CRP) was measured in 330 dyslipidemic subjects.
Results: The age- and sex-adjusted probability of carotid atherosclerosis and femoral plaque increased by 20% (odds ratio [OR] 1.20; 95% CI, 1.10–1.31) and 25% (1.25; 1.13–1.38), respectively, for each 1000/mm^3 WBCC increment. WBCC was associated with age- and sex-adjusted CC-IMT and MaxCC-IMT ($p < 0.05$, both), and F-IMT and MaxF-IMT ($p < 0.001$, both). Adjustment for cardiovascular risk factors did not influence these associations. CRP was associated with CC-IMT and MaxCC-IMT ($p < 0.05$, both), but the associations disappeared after adjustment for body mass index. CRP was unrelated to carotid plaque or measures of femoral atherosclerosis.
Conclusions: WBCC, but not CRP, related to early and advanced measures of atherosclerosis independently of risk factors. Our findings support using the heretofore undervalued WBCC as an easy-to-measure, low-cost diagnostic marker of atherosclerosis.

1. Introduction

Carotid ultrasound for intima-media thickness (IMT) measurement and detection of atheroma plaques is a non-invasive, well standardized and validated imaging technique that is currently recommended by clinical guidelines for cardiovascular risk assessment [1]. Carotid IMT and plaque are associated with traditional cardiovascular risk factors [2], predict cardiovascular events independently of risk factors [3], can reclassify patients into different risk categories [4], and have been used for the evaluation of cardiovascular disease (CVD) in several conditions, including inflammatory diseases [5,6]. Ultrasound imaging allows for measurement of all stages of atherosclerotic disease as a continuous variable. Increased IMT and plaque represent early and advanced stages, respectively, of the natural history of atherosclerosis. However, even after accounting for traditional risk factors (age, sex, obesity, family history of premature CVD, lipids, blood pressure, smoking, and diabetes) most of the variance in IMT and, particularly, plaque remains largely unexplained [7]. It is therefore important to identify additional determinants of subclinical atherosclerosis to improve our knowledge of the disease and to better identify individuals at risk. Although genetics [8] and lifestyle, especially nutritional factors [9,10], certainly contribute to the unexplained proportion of the variance, other easily available clinical and laboratory variables are under investigation.

Chronic low-grade inflammation is associated with the development of CVD [11] and atherosclerosis is acknowledged to be an inflammatory condition on its own right [12]. Several

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doi:10.1016/j.atherosclerosis.2011.12.038

inflammatory markers such as high-sensitivity C-reactive protein (CRP), fibrinogen, Complement C3, or white blood cell count (WBCC) are prospectively associated with incident cardiovascular events [13–17]. There is, however, less information about the relationship between markers of inflammation and surrogate measures of CVD, such as IMT and plaque, and whether this association is independent of CVD risk factors that are also determinants of inflammation (especially, obesity and serum lipids), IMT, and plaque. In this study we investigated cross-sectional associations between well recognized markers of inflammation (WBCC and CRP) and atherosclerosis, as evaluated by IMT and plaque in the carotid and femoral arteries in dyslipidemic and normolipidemic individuals before and after taking into account traditional cardiovascular risk factors.

2. Subjects and methods

We evaluated 554 subjects with primary dyslipidemia (defined with untreated lipid values) from 2003 to the end of December 2010 in a Lipid Clinic located at Hospital Clínic, a university hospital in Barcelona. The clinic receives referrals from primary care physicians and specialty clinics for risk assessment, genetic diagnosis and treatment of severe hypercholesterolemia, and evaluation of difficult cases, including statin intolerance. Primary lipid disorders were diagnosed following standard lipid clinic criteria, as previously described [10]. In addition, we studied 246 sex- and age-matched normolipidemic subjects from our community whose carotid and femoral artery IMT and plaque measurements were used to set reference values, as reported [18,19]. Subjects with WBCC $\geq$ 14,000 cells/mm^3 (4 SD above the mean of the study group) and/or CRP $\geq$ 10 mg/L were excluded because of a high probability of acute inflammation. All subjects provided informed consent to a protocol approved by the local institutional review board.

Participants were assessed for clinical history, medication use, anthropometric characteristics, and standard cardiovascular risk factors. Fasting blood for biochemical profiles was drawn after 4–6 weeks without hypolipidemic drug treatment in subjects free of CVD. Baseline WBCC and lipid values were obtained from the medical history in subjects with prior CVD (53 patients). Off treatment CRP concentrations were available in none of the subjects in this subgroup, who were all receiving statin drugs. Furthermore, CRP was only available in 330 subjects with primary dyslipidemia since this determination was implemented at our biochemistry laboratory from February 2007 onwards. WBCC and CRP were determined with a hematology analyzer (ADVIA 2120, Siemens) and a turbidimetric immunoassay, respectively. For details of the procedures, please see Supplemental materials.

Supplementary material related to this article found, in the online version, at doi:10.1016/j.atherosclerosis.2011.12.038.

2.1. Carotid and femoral B-mode ultrasound imaging

Predefined and standardized imaging protocols to evaluate IMT and plaque presence in the carotids [10,18] and in femoral arteries [19,20] were performed. Plaques were visualized by using B-mode and color Doppler examinations in both longitudinal and transverse planes to take circumferential asymmetry into consideration. Carotid (bulb, internal, or common carotid) or femoral plaques were defined as focal intrusions into the lumen $\geq$1.2 mm thick. Mean common carotid (CC-IMT) and femoral (F-IMT), and maximum common carotid (MaxCC-IMT) and femoral IMT (MaxF-IMT) were recorded. When plaques were present, maximum IMT equaled the highest plaque height. Carotid atherosclerosis was defined by CC-IMT or carotid plaque height >75th percentile values of the sex- and age-specific reference population, as reported [21]. For more detailed description of the technique, please see Supplemental materials.

2.2. Statistical analyses

Data are presented as medians (Q1–Q3 quartiles) and percentages unless otherwise indicated. Non-normally distributed variables were log transformed to reduce skewness and then normality was re-evaluated. Between-group differences in anthropometric, laboratory and ultrasonographic variables were evaluated by the chi-square test, unpaired Student's *t*-test, or the Wilcoxon test as appropriate. These tests were also used to evaluate WBCC or CRP differences by gender, or in subjects with or without personal (or familial) history of CVD, hypertension, diabetes, or smoking habit. Spearman's correlation analysis was used to quantify cross-sectional relationships between anthropometric and laboratory variables and WBCC or CRP. Variables associated with WBCC or CRP were thereafter entered into multiple regression models to investigate independent determinates of inflammatory markers and to asses the contribution of each variable to WBCC or CRP variance.

To search for independent relationships between WBCC or CRP and atherosclerosis outcomes (dependent variables) we constructed multivariate models (logistic binary regression for carotid atherosclerosis or presence of plaque and linear regression for IMT outcomes), including sex and age as confounders in all models. To test whether the association of WBCC with IMT, carotid atherosclerosis, or plaque was modified by the lipid phenotype (dyslipidemia or normolipidemia), we introduced the interaction term WBCC $\times$ dyslipidemia in these models. Additionally, age- and sex-adjusted odds ratios (OR) and 95% confidence intervals (CI) for carotid atherosclerosis or presence of plaque were computed by using binary logistic regression. Furthermore, age- and sex-adjusted differences in IMT measures (and 95% CI of the mean values) between WBCC tertiles were investigated by analysis of covariance (ANCOVA). Finally, multivariable models were additionally adjusted for statin treatment (see below) and factors associated with atherosclerosis and inflammation, such as BMI, familial or personal history of CVD, diabetes, hypertension, smoking, dyslipidemia, total cholesterol, triglycerides, and HDL-cholesterol.

Given that many subjects had been treated with hypolipidemic drugs and high-dose statins may induce regression of IMT and plaque, we adjusted for statin treatment when evaluating associations with atherosclerosis outcomes. To this end, for each subject we calculated a statin score, an estimation of lifetime exposure to cholesterol lowering treatment, as the product of the duration of treatment in years by the average dose received of statin drugs standardized to simvastatin.

Carotid atherosclerois was the primary outcome variable. Other ultrasonographic variables were secondary and exploratory. The significance level was defined as $p \leq 0.05$. Analyses were performed with SAS software, v. 9.2 (SAS Institute Inc., Cary, NC).

3. Results

3.1. Subjects' characteristics by carotid atherosclerosis status

Characteristics of the study population are reported according to the main outcome variable: presence ($n = 411$) or absence ($n = 389$) of carotid atherosclerosis (Table 1). Expectedly, subjects with compared with those without carotid atherosclerosis were older and showed a higher load of cardiovascular risk factors, including a higher proportion of individuals with dyslipidemia (80% vs. 59%, $p < 0.001$, respectively).