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Interleukin-6 is an independent predictor of progressive atherosclerosis in the carotid artery: The Tromsø Study

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ABSTRACT

Background and aims: Novel biomarkers are linked to cardiovascular disease (CVD). The aim of the present study was to investigate the association between 28 blood biomarkers and the formation and progression of carotid plaque.

Methods: In a nested case control study with 703 participants from the population based Tromsø Study, a large biomarker panel was measured in blood obtained at baseline. Carotid ultrasound was assessed both at baseline and at 6 years of follow-up. Four groups were defined: Group 1: no plaque at baseline or at follow-up (reference group); Group 2: novel plaque at follow-up; Group 3: stable plaque at follow-up; Group 4: progression of plaque at follow-up. By multinomial logistic regression analyses, we assessed the risk of being in the different plaque groups with regard to traditional cardiovascular risk factors and levels of biomarkers at baseline.

Results: Adjusted for traditional risk factors, interleukin-6 (IL-6) was an independent predictor of plaque progression (OR 1.44, 95% CI 1.12−1.85 per SD increase in IL-6 level). This result remained significant after inclusion of other novel biomarkers to the model, and when subjects with former CVD were excluded. Neopterin was protective of novel plaque formation (OR 0.73, 95% CI 0.57−0.93). Myeloperoxidase and Caspase-1 were independent predictors of plaque progression, but this effect disappeared when excluding subjects with former CVD.

Conclusions: IL-6 is an independent predictor of plaque progression, suggesting that it may be a marker of progressive atherosclerosis in the general population and that its central role in CVD may be related to promotion of plaque growth.

1. Introduction

Increasing evidence suggests that inflammation plays a pivotal role in the formation, progression and rupture of atherosclerotic plaques [1]. Clinical endpoints such as myocardial infarction (MI), stroke or sudden death may be triggered by an extensive inflammatory reaction at the site of the plaque [2], causing plaque rupture and subsequent thrombosis.

Progression of carotid atherosclerosis evaluated by total plaque area (TPA) [3], plaque volume [4] and degree of stenosis [5] is related to higher risk of vascular events compared to atherosclerosis that remain stable over time. Unstable plaques share some distinctive features, such as a thinner fibrous cap overlying a large necrotic core, a strong intra-plaque inflammatory reaction, a more rapidly progression and an echolucent appearance on ultrasonography [6,7]. Thus, the pathogenesis and subsequent release of certain biomarkers in the bloodstream might differ between stable and unstable plaques. Identification of biomarkers associated with the atherosclerotic process is of interest both for singling out individuals at risk, for understanding the pathophysiological mechanisms involved and subsequently for the development of

Abbreviations: TRFs, traditional cardiovascular risk factors; TPA, total plaque area; FDR, false discovery rates; WBC, white blood cells; DDM, D-dimer; PCT, procalcitonin; MPO, myeloperoxidase; Cu/Zn SOD, copper/zinc superoxide dismutase; BNP, brain natriuretic peptide; CtproAVP, copeptin; MRproADM, midregional proadrenomedullin; MRproANP, midregional proatrial natriuretic peptide.

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preventive therapies [8]. Several markers of inflammation, metabolism, hemodynamic stress, oxidative stress and vascular remodeling circulating in the bloodstream have been associated to atherosclerosis and cardiovascular events in experimental and epidemiological studies [8,9].

The Tromsø study, with its high participation rate and comprehensiveness of clinical examinations including repeated carotid ultrasound assessments, provides a unique opportunity for assessing the association between potential blood based biomarkers and atherosclerosis in a prospective population based setting. The objective of the present study was to identify circulating protein biomarkers associated with formation and progression of carotid plaque. At baseline, we examined 28 novel biomarkers representing different pathophysiological pathways in blood from 703 subjects nested from the large population based Tromsø Study [10]. We studied the association between novel biomarkers and plaque-status at follow-up 6 years later, and assessed whether this association was independent of traditional risk factors (TRFs).

2. Materials and methods

2.1. Subjects

The Tromsø Study is a longitudinal population-based study with repeated health surveys [10]. In the 4th survey in 1994/1995 (baseline), all subjects aged 55–74 years and random 5%–10% samples in other age groups >24 years, were invited to ultrasound scanning of the right carotid artery. Ultrasound assessment was performed in 6727 subjects (76% of the eligible). Subjects who did not consent to medical research (n = 40) were excluded. In the 5th survey in 2000/2001 (follow-up), all subjects who were scanned in 1994 and who were still registered as inhabitants of Tromsø were invited to a second ultrasound examination. 4858 subjects were rescanned at follow-up. Of these, four groups were randomly selected on the basis of carotid ultrasound findings at baseline and follow-up. There were originally 200 subjects in each group, matched on age and sex. A panel of 28 biomarkers was measured in blood obtained at baseline. We excluded 95 subjects due to missing baseline blood samples, and 2 subjects were excluded due to low quality of the ultrasound measurements, leaving 703 subjects to be included in four groups: *1) No plaque group*: Study participants who had no plaque at baseline nor follow-up (n = 126); *2) Novel plaque group*: Participants with no plaque at baseline and novel plaque at follow-up (n = 187); *3) Stable plaque group*: Participants with prevalent plaque at baseline and no increase in total plaque area (TPA) between baseline and follow-up (n = 194); *4) Plaque progression group:* Subjects with plaque at baseline and increase in TPA at follow-up (n = 196). Written informed consent was obtained from each participant included in the study, the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Regional Committee for Medical Health and Research Ethics.

2.2. Cardiovascular risk factors

Information about smoking, diabetes mellitus, MI, stroke, and use of antihypertensive- and lipid-lowering medication was obtained from self-administered questionnaires. At baseline, standardized measurements of height and weight were taken, non-fasting blood samples for analyses of serum lipids and glucose were collected. Serum total cholesterol was analyzed by an enzymatic colorimetric method using a commercially available kit (CHOD-PAP, Boehringer-Mannheim, Mannheim, Germany). Serum high density lipoprotein (HDL) cholesterol was measured after precipitation of lower-density lipoproteins with heparin and manganese chloride. Determination of glycosylated hemoglobin (HbA1c) in EDTA whole blood was based on an immunoturbidometric assay (UNIMATES, F. Hoffmann-La Roche AG). The HbA1c percent value was calculated from the HbA1c/Hb ratio. Specially trained personnel recorded blood pressure with an automatic device (Dinamap Vital Signs Monitor, Tampa, Fla). Three readings were recorded with 1-min intervals, and the average of the final 2 readings was used in the analyses. Hypertension was defined as systolic blood pressure >140 mmHg and/or diastolic blood pressure >90 mmHg and/or use of antihypertensive medication. Cardiovascular disease (CVD) was defined as previous MI or stroke. Diabetes mellitus was defined as self-reported diabetes and/or regular use of insulin and/or oral antidiabetic medication and/or HbA1c > 6.5.

2.3. Biomarkers

A panel of 28 novel biomarkers that previously have shown promising results on the association with CVD were selected and analyzed in blood obtained at baseline. The selected biomarkers have proposed links to atherosclerosis through different pathophysiological mechanisms: inflammatory markers (C-reactive protein (CRP), fibrinogen, white blood cells (WBC), monocyte count, neopterin, interleukin-6 (IL-6), interleukin-18 (IL-18), soluble intercellular adhesion molecule 1 (ICAM-1), soluble vascular adhesion molecule 1 (VCAM-1), Caspase-1, matrix metallopeptidase 9 (MMP-9), tissue inhibitor of metalloproteinase 1 (TIMP-1), D-dimer (DDM), procalcitonin (PCT), protein S-100); markers of oxidative stress (myeloperoxidase (MPO), copper/zinc superoxide dismutase (Cu/Zn SOD); metabolic markers (adiponectin, leptin, apolipoprotein A1 (ApoA1), apolipoprotein B100 (ApoB100), ApoB100/ApoA1 ratio); markers of hemodynamic stress (brain natriuretic peptide (BNP), copeptin (CtproAVP), midregional proadrenomedullin (MRproADM), midregional proatrial natriuretic peptide (MRproANP); and markers of renal function (creatinine, cystatin-C). The study blood samples underwent no more than three freeze/thaw cycles from time of receipt to protein data production. All samples were kept at 4 °C between sample dilutions, and were otherwise stored at −70 °C until assay production. Fibrinogen, creatinine, and WBC were measured at the Department of Clinical Chemistry, University Hospital of North Norway, Tromsø. Fibrinogen was measured using the PT-Fibrinogen reagent (Instrumentation Laboratory), plasma creatinine was analyzed by a modified Jaffe reaction and WBC counts with automated cell counters by standard techniques. All other biochemical analyses were performed at the Mainz Biomarker Laboratory, Johannes Gutenberg University, Mainz by Biosite stroke panel (protein S-100, DDM, BNP), Biosite MPO panel (MPO), ELISA R&D (IL-6, ICAM-1, VCAM-1, leptin, adiponectin, Caspase-1, MMP-9, TIMP-1), ELISA MBL (IL-18), Bnprospects nephelometry Dade Behring (ApoA1, ApoB100, CRP, cystatin-C) and B.R.A.H.M.S. Cryptor (CtproAVP, MRproADM, MRproANP), ELISA B.R.A.H.M.S. (neopterin), B.R.A.H.M.S. PCT sensitive LIA (PCT) and Ransod test kit, Randox (Cu/Zn SOD). According to manufacturers all inter- and intra-assay coefficients of variation were below 10%, except inter assay coefficients for Adiponectin, IL-18 and PCT which ranged between 10 and 20%.

2.4. Ultrasonography

High-resolution B-mode ultrasonography of the right carotid artery was performed at baseline and follow-up with a duplex scanner (Acuson Xp10 128, ART-upgraded) equipped with a 7.5-MHz linear array transducer and followed the same scanning, reading procedures and reproducibility as published previously