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Inflammatory markers and long-term risk of ischemic heart disease in men A 13-year follow-up of the Quebec Cardiovascular Study

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Abstract

We tested the hypothesis that elevated plasma interleukin-6 (IL-6), C-reactive protein (CRP) and fibrinogen concentrations are independent risk factors and interact in increasing the long-term risk of ischemic heart disease (IHD) in men. A total of 1982 IHD-free men from the Quebec Cardiovascular Study were followed over a period of 13 years during which 210 first fatal IHD events and non-fatal myocardial infarctions were recorded. Increased CRP levels (4th versus 1st quartile) were not associated with an increased risk of IHD after adjustment for non-lipid risk factors (age, body mass index, systolic blood pressure, diabetes, smoking and medication use at baseline), lipid risk factors (LDL and HDL cholesterol and triglyceride levels) and for IL-6 and fibrinogen (RR = 0.70, 95% CI = 0.43–1.13). High plasma IL-6 levels (4th versus 1st quartile) were associated with a 70% greater risk of IHD independent of confounding risk factors and of the other 2 inflammatory markers (RR = 1.71, 95% CI = 1.07–2.75). The relationship between high fibrinogen levels (4th versus 1st quartile) and IHD risk was borderline significant in multivariate analyses (RR = 1.53, 95% CI = 0.97–2.43). An inflammation score based on plasma IL-6 and fibrinogen levels improved the IHD risk predictive value of a multivariate model of traditional risk factors ($p = 0.03$). Including plasma CRP levels into the inflammatory score provided no additional predictive value. In conclusion, elevated plasma IL-6 concentrations are more strongly related to IHD risk than CRP and fibrinogen. An inflammation score based on high plasma IL-6 and fibrinogen levels used in combination with traditional risk factors may improve our ability to adequately identify high risk individuals.

Keywords: C-reactive protein; Interleukin-6; Fibrinogen; Inflammation; Ischemic heart disease

1. Introduction

A large body of evidence now supports the role of inflammation in the development and progression of atherosclerosis [1]. Inflammatory processes may contribute to plaque instability and thrombosis, thus enhancing the risk of acute ischemic heart disease (IHD) events [2]. Several studies have shown that elevated plasma interleukin-6 (IL-6), C-reactive protein (CRP) and fibrinogen levels are associated with an increased risk of IHD and severity of atherosclerosis [3–5]. IL-6 is a cytokine with a broad range of humoral and cellular response related to infection, inflammation, host defense and tissue injury [6]. IL-6 is also a major initiator of the acute phase response and a primary determinant for the hepatic production of CRP, a non-specific acute phase reactant, and fibrinogen, a coagulation factor [7]. While plasma IL-6, CRP and fibrinogen levels are physiologically linked, it remains unclear whether or not there is inter-dependence between these pro-inflammatory cytokines in modulating the risk of IHD and whether they have synergistic impact on IHD risk.

The aim of the present study was therefore to examine whether the IHD risk predictive value of plasma CRP, fibrinogen and IL-6 levels are independent of each other. We also

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investigated the synergistic impact of these pro-inflammatory cytokines on IHD risk among men of the Québec Cardiovascular Study followed over a 13-year period.

2. Methods

2.1. Study population and follow-up

The Québec Cardiovascular Study population has been previously reported [8,9]. Briefly, a random sample of 4635 Canadian men (aged 35–64 years), among whom 99% were from French decent and representing 65.5% of the population screened from seven towns in the Québec City metropolitan area, were randomly selected from provincial electoral lists in 1973 for the study of cardiovascular disease risk factors, namely plasma cholesterol levels and hypertension. This cohort was re-evaluated in 1975, 1980 and 1985, and data collected in 1985 on 2552 (55.1%) of the 4635 participants evaluated in 1974 were used as the baseline characteristics for the present prospective analyses. Electrocardiograms (ECG) and plasma lipid and lipoprotein levels were obtained in the 1985 participants. Among the 2552 participants, 102 volunteers were not part of the original random sample and 265 patients had a previous history of IHD prior to 1985. These men were excluded from the present prospective analyses. Diagnosis of diabetes was considered in men who self-reported the disease. Use of hypolipidemic drugs, mainly clofibrate and cholestyramine in 1985, was limited to 1% of men both with and without IHD. In 1990–1991 [8,9] and in 1998, participants were contacted by mail and invited to complete a short questionnaire, which provided history of cardiovascular diseases and type 2 diabetes. For those who reported such diseases and those who died, hospital charts were reviewed by cardiologists of the study who were blinded to the participants' risk profile. Over the 13-year follow-up period and among the 2185 subjects eligible for follow-up, only 8 subjects (0.4%) were lost to follow-up. Thirty-five subjects with available 5-year follow-up data could not be retraced in 1998. These participants were not excluded since we were able to use 5-year or time-to-event censoring in the survival analysis. Among the 2177 eligible men, plasma was no longer available in 195 subjects and measures of the various pro-inflammatory markers could not be performed. Thus, analyses were conducted in a sample of 1982 men with a full metabolic profile including inflammatory markers.

2.2. Definitions of IHD events

The occurrence of a first IHD event, which included coronary death and non-fatal myocardial infarction, was diagnosed as reported previously [9,10]. Among the cohort of 1982 middle-aged men initially free of clinical manifestations of IHD in 1985, a total of 210 first cases of IHD were recorded over the 13-year follow-up: 155 men had a first non-fatal myocardial infarction, and 55 a fatal coronary events.

2.3. Laboratory analyses

Twelve-hour fasting blood samples were obtained at the 1985 baseline evaluation and immediately used for all lipid and apolipoprotein measurements using methods that have been detailed in previous publications [9,11]. Cytokines, insulin and LDL size were measured on plasma obtained at the 1985 baseline evaluation and stored at −80 °C. Plasma CRP levels were measured using the Behring Latex-Enhanced highly sensitive CRP assay on a Behring Nephelometer BN-100 (Behring Diagnostic, Westwood, MA) and the calibrators (N Rheumatology Standards SL) provided by the manufacturer [12]. Plasma IL-6 levels were measured using the commercially available Quantikine HS Immunoassay ELISA kit (R&D Systems Inc., Minneapolis, MN) and calibrators (Diluent HD6F). Fibrinogen concentrations were determined by thrombin clotting time assay as described previously [13]. Fasting insulin concentrations were measured using a commercial kit as described previously [14] and LDL peak particle size and the proportion of small LDL ($LDL\%_{<255\ \text{Å}}$) were measured by polyacrylamide gradient gel electrophoresis [8].

2.4. Statistical analyses

Mean baseline characteristics of incident IHD cases and of IHD-free men during follow-up were compared by Student's *t*-test for parametric variables and by the Wilcoxon test for non-parametric variables. Differences in frequency data were tested by Chi-square analysis. The non-lipid and lipid risk variables in men classified according to number of inflammatory markers were compared using a general linear model (GLM) with the Tukey post hoc test to locate subgroup differences. An inflammatory score ranging from 0 to 2 was computed by attributing one point for a value greater than the median of the cohort samples for IL-6 and fibrinogen levels. Duration of follow-up was calculated in person-years by using the follow-up of each participant from the 1985 baseline evaluation until death, onset of IHD or the last contact. Cox proportional-hazards models were used to estimate risks of IHD events. For all Cox models, the proportional hazards assumptions were formally tested. Age, body mass index, systolic blood pressure, type 2 diabetes (presence versus absence), smoking habits (smokers of more than 20 cigarettes per day versus others), medication use (presence versus absence) at baseline, plasma triglyceride (natural log-transformed), LDL and HDL cholesterol levels were included as potential confounders where indicated. Receiver operating characteristic (ROC) curves were used to examine the additional predictive value of including individual inflammatory cytokines or an inflammatory score to a traditional risk factor model in discriminating subjects who did or did not suffer a first IHD event during follow-up. The areas under the ROC curve were the primary end point for these analyses and were compared using the likelihood ratio test. Statistical analyses were performed on SAS (SAS Institute, Cary, NC).