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Plasma calprotectin and risk of cardiovascular disease: Findings from the PREVEND prospective cohort study

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ABSTRACT

Background and aims: We aimed to assess the association of circulating calprotectin, an inflammation-associated protein, with cardiovascular disease (CVD) risk and determine whether it improves risk prediction.
Methods: Plasma calprotectin measurements were made at baseline in 5290 participants in the PREVEND prospective study. Hazard ratios (95% confidence intervals [CI]) for CVD were calculated.
Results: After a median follow-up of 8.3 years, 339 first CVD events were recorded. Calprotectin concentration was correlated with several conventional risk factors as well as with high-sensitivity C-reactive protein (hsCRP) ($r = 0.42$). Calprotectin was log-linearly associated with CVD risk. The risk for CVD adjusted for conventional cardiovascular risk factors was 1.26 (95% CI, 1.13−1.41) per 1 standard deviation higher baseline $\log_e$ calprotectin, and was 1.24 (95% CI, 1.11−1.39) following further adjustment for triglycerides, body mass index, and other potential confounders. The association remained present after further adjustment for hsCRP 1.15 (95% CI, 1.02−1.30). Comparing extreme quartiles of plasma calprotectin levels, the corresponding adjusted HRs for CVD were 1.96 (1.37−2.82), 1.89 (1.31−2.72), and 1.56 (1.07−2.29). The association of calprotectin with CVD risk did not vary importantly in several relevant clinical subgroups. Adding calprotectin to the Framingham CVD Risk Score was associated with a C-index change (0.0016; p=0.42) difference in −2 log likelihood (p=0.038), IDI (0.0080; $p < 0.001$), and NRI (4.03%; p=0.024).
Conclusions: There is a log-linear association of calprotectin concentration with risk of CVD, which may be partly dependent on hsCRP. Adding calprotectin to conventional risk factors improves CVD risk assessment using measures of reclassification and −2 log likelihood.

1. Introduction

Though established risk factors such as a history of diabetes, blood pressure, blood lipids, and smoking status explain a large proportion of the risk of vascular disease [1], its pathogenesis is still not fully understood as it appears other additional factors may be involved. Accumulating evidence suggests that inflammatory processes may play an important role in the pathogenesis of coronary heart disease (CHD), which is the major manifestation of cardiovascular disease (CVD) [2,3]. The development of atherosclerosis is characterised by a chronic, low-grade inflammatory process [4]. As

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a result, there has been an increasing interest in investigating the role of several inflammatory markers in CVD development. Several epidemiological studies have reported on the associations of both "downstream" (e.g. C-reactive protein, fibrinogen) and "upstream" (e.g. interleukins, tumor necrosis factor-α) markers with risk of CVD [5–7]. Calprotectin, also known as S100A8/A9 complex or myeloid-related protein-8/14, is an inflammatory myeloid-related protein that is mainly secreted by neutrophils [8].

Calprotectin is considered as an acute phase protein, and elevated levels have been reported in several chronic inflammatory conditions such as rheumatoid arthritis, systemic lupus erythematosus, cystic fibrosis, psoriasis, and inflammatory bowel diseases [9–11]. In addition to its role in the modulation of inflammation, leukocyte trafficking, apoptosis, and immune response; calprotectin is used as a reliable marker for the diagnosis and follow-up of inflammatory bowel diseases [12,13]. Emerging evidence suggests that calprotectin may be implicated in the pathogenesis of CVD. A number of studies have demonstrated elevated levels of calprotectin in patients with acute coronary syndromes (ACS), both at the site of coronary occlusion and in the systemic circulation, as well as in atherosclerotic plaques [14–17]. However, because the evidence from these previous studies were based on cross-sectional evaluations, the temporal nature of the relationship between circulating calprotectin and CVD is not certain. A limited number of population-based prospective studies have reported associations between increased levels of calprotectin and increased risk of cardiovascular events. These previous reports however, were either not sufficiently powered, did not account adequately for potential confounders, or were conducted in selected populations with pre-existing CVD [15,18,19]. In addition, these previous studies did not assess the nature of the dose-response relationship between circulating calprotectin and CVD risk and did not evaluate whether the association is modified by relevant clinical characteristics. Given the uncertainties in the previous literature, we aimed to investigate in greater detail than ever before, the shape, nature, and magnitude of the prospective association between plasma calprotectin and risk of future CVD events using a population-based cohort of 5290 participants free from pre-existing CVD at baseline. We also assessed the consistency of the association in important clinical subgroups and investigated the extent to which calprotectin concentrations could improve the prediction of first-onset CVD when added to a conventional CVD risk prediction model.

2. Materials and methods

2.1. Study design and population

The STROBE (STrengthening the Reporting of OBservational studies in Epidemiology) guidelines for reporting observational studies in epidemiology was used to conduct the current study (Supplemental Data 1) [20]. The present analyses employed the Prevention of Renal and Vascular End-stage Disease (PREVEND) study, a prospective cohort study based in a general population and which was designed to evaluate the natural course of urinary albumin excretion and its associations with renal disease and CVD. The selection of the cohort, study design, and recruitment methods have been described in previous reports [21–25]. In brief, the PREVEND cohort study is based on a representative sample of men and women living in the city of Groningen located in the Netherlands. The cohort used for this analysis comprised of 6894 individuals aged 32–80 years who were invited for the second screening phase of the PREVEND study and had their baseline assessments performed between 2001 and 2003. For the current analysis, we used data of participants without pre-existing CVD, renal disease, or malignancy, which left a cohort of 5290 participants without missing information on plasma calprotectin, relevant confounders, and incident outcomes. The local ethics committee of the University Medical Center Groningen approved the PREVEND study protocol. Study procedures were conducted according to the Declaration of Helsinki and all participants provided written informed consent.

2.2. Assessment of calprotectin and risk markers

Study participants attended two outpatient visits during which baseline data were collected on sociodemographic characteristics, anthropometric measurements, medical history, and medication use. Additional information on medication use was collected from registries of all community pharmacies in the city of Groningen. This data source covers up-to-date information on medication use in 95% of PREVEND study participants [26]. Fasting plasma and serum venous samples were taken from participants after 15 min of rest prior to sample collection. Plasma samples were prepared by centrifugation at 4 °C and sera were stored at −80 °C until measurements were done. Plasma calprotectin levels were measured using Gentian Calprotectin turbidimetric immunoassay (Gentian, Moss, Norway) applied on a Mindray BS-400 analyser (Mindray, Shenzhen, China). HDL-C was measured by a homogeneous method (direct HDL, Aeroset System; Abbott Laboratories, Abbott Park, Illinois). Standard protocols were used to measure concentrations of total cholesterol, triglycerides, high sensitivity C-reactive protein (hsCRP), serum creatinine, and serum cystatin C and these have been described in previous reports [27–29]. Fasting plasma glucose (FPG) was measured using dry chemistry (Eastman Kodak, Rochester, New York). Estimated glomerular filtration rate (eGFR) was estimated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) combined creatinine-cystatin C equation [30]. Body mass index (BMI) was calculated as the ratio of the weight in kilograms to the square of height in meters.

2.3. Outcome ascertainment

The primary outcome for this analysis was first-onset composite CVD. Secondary outcomes were incident CHD and stroke events. We included all outcome events that occurred from study entry (2001–2003) to 1-1-2011. The source of data on hospitalization for incident CVD events was obtained from PRISMANT, which is the Dutch National Registry of hospital discharge diagnoses [31]. Cardiovascular deaths and their dates were ascertained by data linkage with the Dutch Central Bureau of Statistics. Outcome data were coded according to the *International Classification of Diseases*, Ninth Revision (ICD-9) until 1 January, 2009 and after this date, ICD-10 codes were used. First-onset composite CVD was defined as the combined outcomes of acute and subacute ischemic heart disease (IHD), acute myocardial infarction (AMI), coronary artery bypass grafting (CABG) or percutaneous transluminal coronary angioplasty (PTCA), occlusion or stenosis of the precerebral or cerebral arteries, subarachnoid hemorrhage, intracerebral hemorrhage, other intracranial hemorrhage, and other vascular interventions such as percutaneous transluminal angioplasty or bypass grafting of peripheral vessels and aorta. Coronary heart disease was defined as fatal or nonfatal IHD, fatal or nonfatal MI, CABG, and PTCA. Stroke events were defined as occlusion and stenosis of precerebral or cerebral arteries, and carotid obstruction, subarachnoid hemorrhage, intracerebral hemorrhage, other and unspecified intracranial hemorrhage.