Clinica Chimica Acta 486 (2018) 66–75

Contents lists available at ScienceDirect

Clinica Chimica Acta

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

Plasma neutrophil gelatinase-associated lipocalin and risk of cardiovascular disease: Findings from the PREVEND prospective cohort study

Setor K. Kunutsor[a,b,*], José L. Flores-Guerrero[c], Lyanne M. Kieneker[c], Tom Nilsen[d], Clara Hidden[d], Erling Sundrehagen[d], Samuel Seidu[e,f], Robin P.F. Dullaart[c], Stephan J.L. Bakker[c]

[a] Translational Health Sciences, Bristol Medical School, University of Bristol, Southmead Hospital, Learning & Research Building (Level 1), Bristol, UK
[b] National Institute for Health Research Bristol Biomedical Research Centre, University Hospitals Bristol NHS Foundation Trust and University of Bristol, Bristol, UK
[c] Department of Internal Medicine, University of Groningen and University Medical Center Groningen, Groningen, The Netherlands
[d] Gentian, Moss, Norway
[e] Leicester Diabetes Centre, Leicester General Hospital, Gwendolen Road, Leicester LE5 4WP, UK
[f] Diabetes Research Centre, University of Leicester, Leicester General Hospital, Gwendolen Road, Leicester LE5 4WP, UK

ARTICLE INFO

Keywords:
Neutrophil gelatinase-associated lipocalin
Cardiovascular disease
Risk factor
Cohort study

ABSTRACT

Background: Neutrophil gelatinase-associated lipocalin (NGAL), a novel biomarker of acute kidney injury, might play a role in the development of atherosclerotic cardiovascular disease (CVD). We aimed to assess the association of circulating NGAL with CVD risk.

Materials and methods: Plasma NGAL concentrations were measured at baseline in 5275 participants in the PREVEND prospective study. Hazard ratios (95% confidence intervals [CI]) for CVD were estimated.

Results: After a median follow-up of 8.3 years, 338 participants developed first CVD events. Plasma NGAL was weakly to moderately correlated with several CVD risk markers. There was a non-linear relationship between NGAL and CVD risk. In analyses adjusted for established risk factors, the hazard ratio (95% CI) for CVD in a comparison of the top quartile versus bottom quartiles 1–2 of NGAL values was 1.35 (1.05–1.75; $P = 0.022$), which was abrogated after additional adjustment for other potential confounders (mainly attributed to high sensitivity C-reactive protein) 1.20 (0.92–1.57; $P = 0.176$). The association was considerably attenuated following further adjustment for renal function 1.05 (0.79–1.40; $P = 0.745$). The association between NGAL and CVD risk did not vary importantly in relevant clinical subgroups.

Conclusion: Evidence suggests a non-linear association between NGAL and CVD risk, which is dependent on inflammation and renal function.

1. Introduction

Neutrophil gelatinase-associated lipocalin (NGAL), also known as lipocalin-2 or oncogene 24p3, is a human protein that is mainly expressed by neutrophils and in low levels by various epithelial cells [1–3]. The physiological functions of NGAL include involvement in the innate immune response to bacterial infection [4] and it also functions as a growth factor [5]. NGAL has emerged as a precise and sensitive novel biomarker for acute kidney injury (AKI) [6, 7]; within two hours of an AKI, high levels of NGAL are secreted into the blood and urine [6]. NGAL measurements also have potential relevance for chronic kidney disease (CKD), nephropathy, and kidney transplant [8, 9]. Beyond the kidney, [10]emerging evidence suggests that NGAL may be implicated in the pathogenesis of atherosclerotic cardiovascular disease (CVD). Recent data from both human studies and animal models have demonstrated NGAL to be highly expressed in thrombi and atherosclerotic

Abbreviation: AKI, acute kidney injury; AMI, acute myocardial infarction; BMI, body mass index; CABG, coronary artery bypass grafting; CHD, coronary heart disease; CHS, Copenhagen Heart Study; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; CI, confidence interval (CI); CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; FAR, floating absolute risk; FPG, fasting plasma glucose; HDL-C, high density lipoprotein cholesterol; HR, hazard ratio; hsCRP, high sensitivity C-reactive protein; IHD, ischemic heart disease; IQR, interquartile range; MMP, matrix metalloproteinase; NGAL, Neutrophil gelatinase-associated lipocalin; PREVEND, Prevention of Renal and Vascular End-stage Disease; PTCA, percutaneous transluminal coronary angioplasty; RBS, Rancho Bernardo Study; SD, standard deviation; SBP, systolic blood pressure; UAE, urine albumin excretion

* Corresponding author at: Translational Health Sciences, Bristol Medical School, University of Bristol, Southmead Hospital, Learning & Research Building (Level 1), Bristol, UK.
E-mail address: skk31@cantab.net (S.K. Kunutsor).

https://doi.org/10.1016/j.cca.2018.07.034
Received 30 May 2018; Received in revised form 10 July 2018; Accepted 19 July 2018
Available online 20 July 2018

plaques [11–14]. NGAL is also highly expressed in the heart [13] and elevated levels have been demonstrated in patients with heart failure, coronary heart disease (CHD) or syndromes, and stroke [15–17]. A number of studies have also reported associations between elevated NGAL levels and poor outcomes (e.g., mortality, hospital re-admissions, heart failure, major adverse cardiac events) in patients with pre-existing CVD or kidney disease [18–23]. The overall evidence suggests that NGAL may be involved in CVD development. However, because the existing studies were (i) mostly cross-sectional evaluations of clinical studies; (ii) conducted in animal models or in patients with pre-existing disease; or (iii) either not sufficiently powered or did not account adequately for potential confounders; the temporal nature of the relationship between circulating NGAL and risk of CVD is not certain. A limited number of population-based prospective studies have however reported associations between increased circulating levels of NGAL and increased risk of first cardiovascular events in the general population [24, 25]. Though these previous studies were elegantly analyzed, they had a number of limitations which included not assessing the nature of the dose-response relationship between circulating NGAL and CVD risk and whether the association is modified by relevant clinical characteristics. In this context, we aimed to investigate in greater detail than ever before, aspects of the association such as the shape, nature, magnitude, and consistency of the prospective association between plasma NGAL and risk of first-onset CVD events using a population-based cohort of 5275 participants who were free from pre-existing CVD at baseline.

2. Materials and methods

2.1. Study design and population

We conducted the current study according to STROBE (STrengthening the Reporting of OBservational studies in Epidemiology) guidelines for reporting observational studies in epidemiology (Supplementary material 1) [26]. We employed the Prevention of Renal and Vascular End-stage Disease (PREVEND) study for this analysis. PREVEND is a general population-based prospective cohort study which was designed to explore the natural course of urinary albumin excretion (UAE) and its association with kidney disease and CVD. The study design and recruitment processes have been described previously [27–29]. The PREVEND cohort consists of a representative sample of men and women living in the city of Groningen in the Netherlands. The current cohort involved 6894 individuals aged 32–80 years who were invited for the second screening phase of the PREVEND study and who had baseline assessments performed between 2001 and 2003. For this analysis, we included participants without pre-existing CVD, renal disease, or malignancy at baseline. The current analysis involved 5275 participants with non-missing information on plasma NGAL, relevant covariates, and cardiovascular outcomes. The local ethics committee of the University Medical Center Groningen approved the study protocol. The study procedures were conducted according to the Declaration of Helsinki and written informed consent was obtained from all study participants.

2.2. Measurement of NGAL and risk markers

Baseline data on demographics, anthropometric measurements, lifestyle factors, and other cardiovascular risk markers were collected during two outpatient visits by study participants. Venous blood samples were obtained from study participants after an overnight fast and 15 min of rest prior to sample collection. Plasma samples were prepared by centrifugation at 4 °C and samples stored at −80 °C until analysis. Plasma NGAL concentrations were measured using Gentian NGAL turbidimetric immunoassay (Gentian, Moss, Norway) applied on a Mindray BS-400 analyzer (Mindray, Shenzhen, China). The Gentian NGAL assay was calibrated to the commercially available Bioporto NGAL Test, using the value transfer protocol as described by Blirup-Jensen et al. [30] In addition, the Gentian NGAL calibrator has been validated according to the Clinical and Laboratory Standards Institute guidelines, with external validation performed by individual laboratories [31].

Fasting plasma glucose (FPG) was measured by dry chemistry (Eastman Kodak, Rochester, New York). Total cholesterol, high density lipoprotein cholesterol (HDL-C), triglycerides, and high sensitivity C-reactive protein (hsCRP) were measured using standard protocols previously described [32–36]. Serum creatinine was determined by Kodak Ektachem dry chemistry (Eastman Kodak, Rochester, New York). Serum cystatin C concentrations were measured by Gentian Cystatin C Immunoassay (Gentian AS, Moss, Norway) on a Modular analyzer (Roche Diagnostics). Cystatin C was calibrated directly using the standard supplied by the manufacturer (traceable to the International Federation of Clinical Chemistry Working Group for Standardization of Serum Cystatin C) [37]. The intra- and inter-assay coefficients of variation were < 4.1% and < 3.3%, respectively. UAE was estimated as the mean of two 24-h urine collections and the concentration was determined by nephelometry (BNII; Dade Behring Diagnostic, Marburg, Germany). The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) combined creatinine-cystatin C equation was used to calculate estimated glomerular filtration rate (eGFR) [38]. Type 2 diabetes was defined as a FPG level of ≥7.0 mmol/l, a nonfasting glucose level of ≥11.1 mmol/l or use of antidiabetic medication according to self-reports or to pharmacy data [39].

2.3. Ascertainment of outcomes

We included first-onset cardiovascular outcomes that occurred from study enrollment through to 01 January 2011. Composite CVD was the primary outcome for this analysis, with CHD and stroke endpoints as subsidiary outcomes. The sources of information on deaths were ascertained by computerized data linkage with the Dutch Central Bureau of Statistics. The Dutch national registry of hospital discharge diagnoses (PRISMANT) was the source of data on cardiovascular morbidity hospitalizations [40]. Outcome data were coded according to the *International Classification of Diseases*, Ninth Revision (ICD-9) until 01 January 2009; after which ICD-10 codes were used. We defined composite CVD as the combined outcomes of acute and subacute ischemic heart disease (IHD), acute myocardial infarction (AMI), percutaneous transluminal coronary angioplasty (PTCA) or coronary artery bypass grafting (CABG), intracerebral hemorrhage, other intracranial hemorrhage, subarachnoid hemorrhage, stenosis or occlusion of the precerebral or cerebral arteries, and other vascular interventions such as percutaneous transluminal angioplasty or bypass grafting of aorta. CHD was defined as fatal or nonfatal IHD, fatal or nonfatal MI, PTCA, and CABG. Stroke events were defined as intracerebral hemorrhage, other intracranial hemorrhage, subarachnoid hemorrhage, stenosis or occlusion of the precerebral or cerebral arteries, and carotid obstruction.

2.4. Statistical analyses

We log-transformed values of skewed variables to achieve approximately symmetrical distributions. Baseline characteristics were presented as means (standard deviation, SD) or median (interquartile range, IQR) for continuous variables and percentages for categorical variables. We calculated partial correlation coefficients (adjusted for age and sex) to evaluate the cross-sectional associations of plasma NGAL levels with CVD risk markers. Cox proportional hazards regression models were used to assess the associations of plasma NGAL with risk of CVD, after confirming no major departure from hazards proportionality assumptions using Schoenfeld residuals [41]. We plotted cumulative Kaplan-Meier curves for CVD during follow-up according to categories of NGAL. We assessed the shape of the association of plasma NGAL with CVD risk by plotting hazard ratios (HRs) calculated within