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Prognostic value of multiple emerging biomarkers in cardiovascular risk prediction in patients with stable cardiovascular disease

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ABSTRACT

Background: Few studies have examined simultaneously the prognostic value of traditional and emerging biomarkers including atrial natriuretic peptide (ANP) and brain-type natriuretic peptide (BNP), for major cardiovascular disease (CVD) outcomes in patients with stable CVD, and results are equivocal.
Design: and Methods: Mid-regional pro-ANP (MR-proANP) and N-Terminal pro-BNP (NT-proBNP), CRP and homocysteine were measured in stable CVD patients (n = 1456; age: 61.8 y) at inclusion in the SU.FOL.OM3 cohort. Prospective association of biomarkers with risk of heart failure, major cardiovascular (non-fatal myocardial infarction, ischemic stroke or death from CVD) or overall cardiovascular event were examined with Cox proportional-hazards analyses. Increase in prediction risk upon addition of biomarker(s) to the traditional risk model was examined by change in C-statistic, NRI and IDI.
Results: During follow-up (median: 4.7 y), 40 heart failure, 145 major cardiovascular and 493 overall cardiovascular events were diagnosed. In models adjusted for age, sex, smoking, diabetes, serum creatinine and CVD inclusion criteria, NT-proBNP and CRP associated significantly with heart failure. Both natriuretic peptides predicted the risk of major cardiovascular events in adjusted models; Hazard ratio (HR) and 95% CI for each SD increase in MR-proANP and NT-proBNP were 1.24 (1.04–1.47), and 1.31 (1.09–1.57), respectively. The addition of NT-proBNP to a traditional risk model increased significantly the area-under-curve for heart failure and overall cardiovascular events (by 6 and 12%, respectively); addition of MR-proANP or homocysteine yielded modest (2%) but statistically significant increase for major cardiovascular events.
Conclusion: NT-proBNP consistently predicted CVD outcomes and may be useful singly or in combination with MR-proANP for risk-stratification in high-risk patients.

1. Introduction

New avenues for prevention of cardiovascular disease (CVD) including better diagnosis and risk evaluation are of increasing interest in high-risk groups. In this regard, novel biomarkers such as natriuretic peptides namely atrial natriuretic peptide (ANP) and brain-type natriuretic peptide (BNP) as well as C-reactive protein (CRP) and homocysteine are of interest.

ANP and BNP are vasoactive cardiac peptide hormones with natriuretic, diuretic, and vasodilator activity [1], that could be important diagnostic and prognostic tools for CVD and related mortality in general population and coronary heart disease (CHD) patients [2–8]. Few studies have simultaneously evaluated the prognostic value of both natriuretic peptides (proANP and proBNP) over and above conventional cardiovascular risk factors, beyond the period of hospitalization after an acute CVD event (MI, left ventricular systolic dysfunction and chronic heart failure) [9,10]. In addition, newer assays targeting more stable epitopes of ANP, such as mid-regional pro-ANP (MR-proANP) have become available that could offer more refined risk assessment [11]. In clinical practice established tests such as CRP and homocysteine are often considered in CVD risk assessment. CRP is commonly determined using routinely available assays as a systemic inflammatory marker [12]. Although the evidence linking elevated homocysteine and CVD risk

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is inconsistent [13,14], recent promising findings from NHANES III and Multi-Ethnic Study of Atherosclerosis (MESA) studies showing significant improvement in risk prediction for future CVD and CHD events in intermediate-risk patients upon addition of homocysteine to the Framingham risk model [15], has re-sparked interest in this marker for CVD risk assessment. The current study, thus, evaluated the comparative prognostic value of four biomarkers (natriuretic peptides MR-proANP and NT-proBNP, CRP and homocysteine) alone and in combination, in addition to conventional risk factors, in patients with stable CVD, in whom information on predictive risk has not been extensively evaluated using emerging biomarkers [9,16].

2. Methods

2.1. Study design

The SU.FOL.OM3 trial is a multicenter, double-blind randomized controlled trial (RCT) that evaluated the separate and combined effects of daily supplementation with B-vitamins, and/or n-3 polyunsaturated fatty acid for prevention of CVD [17]. Participants (45–80 y) were recruited via a nationwide network of 417 cardiologists, neurologists or other physicians. Those meeting the CVD inclusion criteria of a history of ischemic stroke or other coronary event i.e. acute coronary syndrome with or without MI with or without ST segment elevation within 1–12 months (mean: 4 months) were assessed at baseline, randomized to receive an active treatment or relevant placebo and followed (median: 4.7 y) [17]. Exclusion criteria included age (<45 years or>80 years), ill-defined diagnosis of cardiovascular disease, inability or unwillingness to comply with study treatment, and disease or treatment that might interfere with metabolism of homocysteine or omega 3 fatty acids, in particular methotrexate for treating cancer or rheumatoid arthritis and chronic renal failure (plasma creatinine concentration >200 μmol/l or creatinine clearance <40 ml/min).

The protocol was approved by the ethical committee "Comité Consultatif pour la Protection des Personnes se prêtant à la Recherche Biomédicale" (CCPPRB no. 1933) of Paris-Cochin, and the data protection board "Comité National Informatique et Liberté" (CNIL no. 901230). Participants provided written informed consent following protocols approved by these committees. The present trial was registered as ISRCTN41926726 in Current Controlled Trials (http://www.controlled-trials.com/ISRCTN41926726).

2.2. Measurements

In the current study, post-hoc analyses were performed on a subset of 1456 subjects, selected randomly from 2501 participants in the SU.FOL.OM3 study. Blood samples were obtained at baseline assessment, after a 5 to 12-h fast, processed, and plasma stored at −80 °C. All biochemical measurements were analyzed in the same laboratory following standard procedures. Plasma MR-proANP concentrations were determined with a commercial kit using a Kryptor Brahms fluorescence immunoassay (Thermo Fisher Scientific, Clichy, France). NT-proBNP assay was performed by a Roche electrochemiluminescence immunoassay (Modular E, Roche Diagnostics, Meylan, France) using manufacturer's reagents and controls. High-sensitivity CRP assay was performed by an ultrasensitive immune-technique on the Siemens BNII analyser calibrated using reagents and controls provided by the manufacturer (Siemens Healthcare Diagnostics, Saint-Denis, France). Plasma homocysteine was measured by a competitive immunoassay with direct chemiluminescence detection (Siemens Healthcare Diagnostics limited, Camberley, UK). The intra-assay and inter-assay coefficients of variation for all analytes were between 1 and 6%.

Fasting plasma concentrations of glucose, total cholesterol, high density lipoprotein cholesterol (HDL), calculated low density lipoprotein cholesterol (LDL), triglyceride and creatinine concentrations were determined using standard laboratory methods.

Data on age, smoking status (current smokers, former smokers and non smokers), and medication use were collected at inclusion via questionnaires [17].

Body Mass Index (BMI in kg/m^2) was calculated using height and weight measured at baseline by trained staff following standard protocols.

Blood pressure (BP) was recorded at baseline by trained staff in the sitting position, after a 5-min rest, with a semi-automatic device (Digital blood pressure monitor OMROM UA-787) using standardized protocols. Two measurements were taken. In the case of a BP difference greater than 10 mmHg for either systolic BP or diastolic BP, BP was measured again after a 5-min rest. The last two BP values were averaged to interpret hypertension status.

Diabetes mellitus was defined as fasting glucose $\geq$ 7 mmol/l and/or use of antidiabetic drugs or insulin.

Finally, our population was 61.8 $\pm$ 8.8 year old, 83% men, medically treated (92% were on antihypertensives, 94% antiplatelet agents, 87% hypolipemic agents and 12% antidiabetic treatment) patients in secondary cardiovascular prevention.

2.3. Outcomes

Three endpoints were examined in the present study: heart failure; major cardiovascular; and overall cardiovascular events. Heart failure events included heart failure and associated death. Major cardiovascular event was defined as a composite of non-fatal MI, ischemic stroke, or death from CVD. Overall cardiovascular events included any cardiovascular event including major cardiovascular events. The assessments of the outcomes are described in details [17]. Briefly, events were reported using questionnaires every six months, as well as information from the general practitioners, cardiologists, or neurologists treating the participants. Hospital discharge summaries and other clinical informations were gathered for all suspected cardiovascular and neurological events; and each event was discussed in a dedicated committee composed of three cardiologists or neurologists. All adjudications were performed by the committees, blinded to the treatment allocation.

2.4. Statistical analysis

Plasma MR-proANP, NT-proBNP, CRP, homocysteine as well as triglyceride were log transformed to achieve distributions consistent with normality. Baseline characteristics were compared between participants with and without cardiovascular endpoints using *t*-test or χ^2 test as appropriate. Cox proportional-hazards regression models were used to study the associations between the biomarkers at baseline (comparing tertiles for each marker) and the risk of specific endpoints examined i.e. heart failure, major cardiovascular events, and overall cardiovascular events. Models were rerun adjusting for significant variables (age, diabetes status, smoking status, and plasma creatinine at baseline) as well as randomization group, sex and CVD inclusion criteria. In addition, Cox proportional-hazards regression models were run considering each biomarker as a continuous variable; hazard ratios (HR) and 95% confidence intervals (CI) were obtained for 1-sd increase. Proportional-hazards assumptions were confirmed by Schoenfeld's tests. Finally, estimates of C-index (with 95% CI) for the Cox proportional-hazards regression models were calculated [18], as well as IDI and NRI [19]. Difference in C-index, indicating improvement in area-under-the ROC curve (AUC), upon addition of biomarker(s), singly or in combination, to a model containing