Natriuretic Peptides, Diagnostic and Prognostic Biomarkers

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A prudent question is one half of wisdom
Francis Bacon, 1561-1626

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General introduction: Pro-B-type natriuretic peptide derived molecules, markers of cardiovascular disease

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INTRODUCTION

The natriuretic peptide family

In humans, the natriuretic peptide family consists of three different types of peptides: atrial natriuretic peptide (synonym: atrial natriuretic factor), B-type natriuretic peptide (synonym: brain natriuretic peptide) and C-natriuretic peptide.[1] Atrial natriuretic peptide (ANP) was the first natriuretic peptide to be discovered and in humans ANP is predominantly formed in the cardiomyocytes of the atria.[2] B-type natriuretic peptide (BNP) was first discovered in porcine brain hence its original name brain natriuretic peptide.[3] In humans, the majority of BNP is formed in ventricular cardiomyocytes, and mRNA levels in brain are negligible. Therefore, brain natriuretic peptide is now recognized as B-type natriuretic peptide. C-type natriuretic peptide (CNP) is basically a neuropeptide, found in brain and vascular endothelial cells.

Genes and structure of natriuretic peptides

The ANP (natriuretic peptide precursor type A; NPPA) and BNP (natriuretic peptide precursor type B; NPPB) genes are localized in tandem in the same vicinity on the distal short arm of chromosome 1.[4] Location of ANP gene 1p36.21; Location of BNP gene 1p36.2. The CNP gene (natriuretic peptide precursor type C; NPPC) is present on chromosome 2 (2q24-qter).[5] All natriuretic peptide genes consist of three exons intervened by two introns, indicating that all peptides are derived from one common ancestral gene.[6]

All three peptides have a central intramolecular ring structure (Figure 1) of 17 amino acid residues. This ring structure is also found in natriuretic peptides of other species and has been conserved throughout evolution. The C-terminal sequence varies between the three peptides consisting of 5 residues for ANP, 6 for BNP and none for CNP. The C-terminal sequence appears to be a major determinant of the biologic activity of each peptide.

Pro-peptides

ANP arises from a single precursor, the preproANP molecule which is translated from ANP mRNA.[6] From this 151 amino acid peptide, 25 amino acids are removed forming proANP. Upon release in the circulation, proANP is cleaved into ANP and the amino-terminal end, NT-proANP. BNP and CNP are formed in a similar way.[1] ProBNP is cleaved off from preproBNP, which is cleaved to form BNP and amino-terminal pro-B-type natriuretic peptide (NT-proBNP).

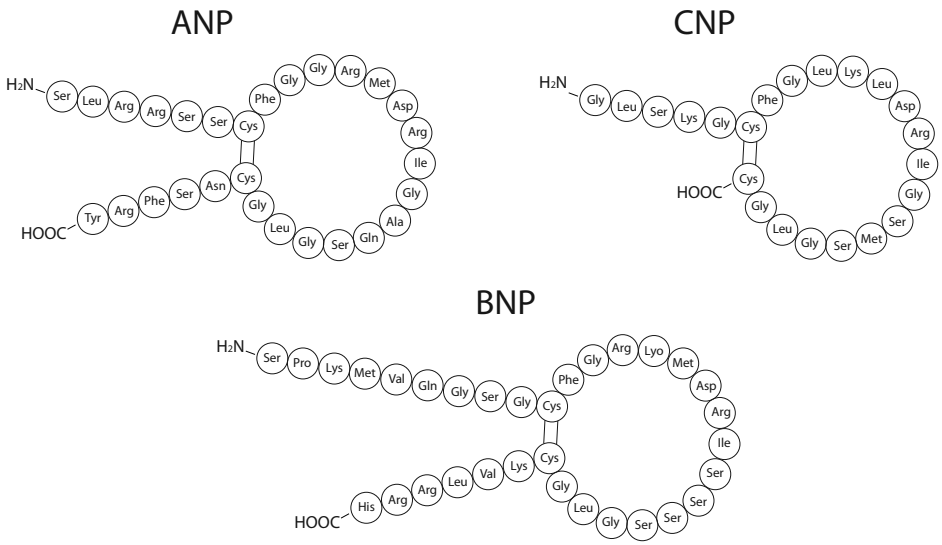

Figure 1. Peptide-structure of atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP) and C-type natriuretic peptide (CNP).

Within the kidney, the ANP prohormone's posttranslational processing is different from that of other tissues, resulting in an additional four amino acids added to the NH_2 terminus of ANP (urodilatin).[7]

Natriuretic peptide receptors

Two classes of natriuretic peptide receptors are known: membrane bound guanylyl cyclases and a clearance receptor. The membrane bound guanylyl cyclases consist of an extracellular ligand-binding domain and an intracellular kinase-like and guanylyl cyclase-domain. Binding of the receptor results in activation of the intracellular guanylyl cyclase, leading to an increase in intracellular cyclic guanosine monophosphate. Two types of guanylyl cyclase-coupled receptors have been identified, the natriuretic peptide receptor A and B.[8] Natriuretic peptide receptor A has the highest ligand selectivity for ANP and BNP, whereas natriuretic peptide receptor B predominantly binds CNP.[9] The natriuretic peptide receptor A is most abundantly expressed in the glomerulus of the kidney, the adrenal zona glomerulosa, the pituitary, the cerebellum and the heart, while natriuretic peptide receptor B is detected in the adrenal medulla, the pituitary and the cerebellum.[10]

The natriuretic peptide receptor C, named after its clearance (C) function, is a truncated form of natriuretic peptide receptor, lacking a kinase-like

and a guanylyl cyclase-domain. The natriuretic peptide receptor C has a high affinity for all natriuretic peptides and rapidly internalizes bound peptides.[9] It is the most abundant receptor making up 90% of the total natriuretic peptide receptor population and is present in almost all tissues.[11] Natriuretic peptide receptor C is expressed on endothelial cells explaining its high density in lung and glomerulus.[11]

REGULATION AND BIOLOGIC ACTION OF BNP AND NT-PROBNP

Regulation of gene expression of BNP

In contrast to ANP, BNP is not stored intracellularly and the mechanisms responsible for mRNA production and preservation determine directly the peptide levels in the circulation.[12] In vitro application of direct mechanical strain to cardiomyocytes results in an increase in BNP gene expression through a number of regulatory elements in the BNP promoter; proximal GATA elements, activator protein-1 transcription factor and shear stress response elements.[13] It has been suggested that the mechanical force is translated into a biochemical stimulus via the extracellular matrix protein cell surface receptor, integrin.[14, 15] Besides cardiomyocyte stretch, BNP transcription is induced by neurohormones such as endothelin, angiotensin II, and adrenergic agonists.[16-18] Furthermore, both pro-inflammatory cytokines IL-1β and TNF-α and lipopolysaccharides from Gram-negative bacteria can increase BNP secretion.[19, 20] Lastly, the thyroid hormones T3 and T4 stimulate BNP secretion in vitro and vivo.[21]

Biologic properties of BNP

ANP and BNP play a complementary role in the regulation of renal and cardiovascular homeostasis through the natriuretic peptide receptor A. NT-proBNP does not bind to the natriuretic peptide receptors and has no biologic action. Stimulation of the natriuretic peptide receptor A at the level of the glomerulus causes afferent dilation together with efferent arteriolar vasoconstriction, thereby increasing the glomerular filtration rate.[22] In the collecting duct, natriuretic peptide receptor A stimulation decreases sodium reabsorption, thereby increasing sodium excretion.[23] Finally, the secretion of renin and aldosterone is inhibited.[24]

To investigate the properties of ANP and BNP, transgenic mice have been generated with a targeted disruption of the natriuretic peptide receptor A, ANP and BNP.[25-27] Mice lacking the natriuretic peptide receptor A have salt-resistant hypertension and ventricular hypertrophy with cardiac fibrosis.[26] Mice lacking ANP have salt sensitive hypertension and ventricular hypertrophy without cardiac fibrosis.[25] Mice lacking BNP exhibit cardiac fibrosis with no signs of hypertension and ventricular hypertrophy.[27] These findings suggest that BNP plays a different role than ANP in acute ventricular overload, with BNP preventing cardiac fibrosis during ventricular pressure overload *in vivo*. Overexpression of BNP in transgenic mice results in lower blood pressure due to a reduced peripheral vascular resistance.[28]

Clearance of BNP and NT-proBNP

BNP is actively cleared from the circulation through two different pathways: enzymatic degradation by neutral endopeptidase and receptor-mediated endocytosis followed by lysosomal degradation after binding to the natriuretic peptide clearance receptor.[9] Neutral endopeptidase, a zinc metallopeptidase, is present on the surface of endothelial cells, smooth-muscle cells, cardiomyocytes, fibroblasts and brush-border membranes in the proximal tubule of the kidney.[29] NT-proBNP does not bind to the natriuretic peptide clearance receptor and renal clearance is regarded as its main mechanism for removal from the circulation. In patients undergoing renal vein sampling we showed that renal extraction ratio of NT-proBNP is 0.17 compared to 0.11 for BNP.[30] Both BNP and NT-proBNP (molecular weight: 3.5 and 8.5 kDa, respectively) are freely filtered by the glomeruli and catabolized in the brush border of tubular epithelial cells.[31] As BNP is also cleared by its clearance receptor and neutral endopeptidase, the half-life time of BNP is only 22 minutes compared to 120 minutes for NT-proBNP.

ASSAYS

BNP as well as NT-proBNP concentrations can presently be measured using fully automated commercial methods. Rapid tests for "on-site" and "point-of-care" diagnosis are now available for both biomarkers. The available assays have reasonable to high precision. Before the introduction of the commercial electrochemiluminescence immunoassays, natriuretic peptides were deter-

mined using radioimmunoassays. Plasma concentrations are expressed as pmol/l or pg/ml. The conversion factor for BNP is: 1 pg/ml = 0.29 pmol/l, and for NT-proBNP: 1 pg/ml = 0.12 pmol/l. Values for BNP obtained with different assay methods can not always be compared directly. There is no clear-cut conversion factor for comparing BNP with NT-proBNP values. In ethylenediaminetetraacetic acid blood at room temperature BNP is stable for at least 24 hours and NT-proBNP for at least 72 hours.

DIAGNOSTIC AND PROGNOSTIC PROPERTIES OF BNP AND NT-PROBNP

Screening for heart failure in the general population

The prevalence of heart failure in the western world is increasing due to the ageing population and the much-improved survival after myocardial infarction.[32] Timely diagnosis would make it possible to start in an early phase with drug treatment with proven favorable effect on morbidity and mortality, such as ACE-inhibitors and beta-blockers.[33] In the past years, a number of studies has been published on the test characteristics of BNP and NT-proBNP for diagnosis and exclusion of heart failure in the general population and subjects with a high risk for heart failure.[34-37] Golden standard in these studies was measurement of left ventricular ejection fraction by echocardiography or magnetic resonance imaging, with or without addition of clinical characteristics. From these studies it can be concluded that a normal concentration excludes the presence of heart failure with a high degree of certainty. In a recently published nomogram, for instance, the *a priori* estimated chance of heart failure of 20% decreased *a posteriori* to 2.9% with a rapid BNP test.[38] The reverse however is not true, because BNP and NT-proBNP values are influenced by a great number of factors and can also be increased in other diseases such as renal insufficiency and chronic obstructive lung disease. The chance of heart failure can therefore be as low 2% with an elevated abnormal test value. Because of the low positive-predictive value, screening for heart failure in the general population with BNP or NT-proBNP can not be recommended.

Diagnosis of heart failure in general practice

In case of clinical suspicion of heart failure, a general practitioner can request a plasma BNP or NT-proBNP measurement, and use the outcome to decide on echocardiographic investigation or referral to a cardiologist.[39] The diagnostic value of BNP and NT-proBNP in first-line patients suspected of heart failure has been investigated.[40-43] These studies all show a high negative-predictive value (88% – 98 %). The positive-predictive value is much lower and highly variable (32% – 70 %). Since a normal electrocardiogram also makes the diagnosis of heart failure very improbable, some studies have evaluated the diagnostic accuracy of electrocardiogram compared to BNP, and investigated whether an electrocardiogram plus BNP has greater accuracy than an electrocardiogram alone.[42] Not unexpectedly, no significant difference was found between the sensitivity of an electrocardiogram and BNP measurement, although BNP was more specific (less false-positives) than an electrocardiogram for the diagnosis of heart failure. Addition of BNP measurement to the electrocardiogram does not increase the sensitivity. In conclusion, in case of suspicion of heart failure in patients presenting in general practice, either an electrocardiogram or BNP, but not both, should be performed.

Diagnosis of heart failure at the emergency room

Acute shortness of breath is an important reason for referral to an emergency room. For fast and efficacious treatment it is imperative to know whether the shortness of breath is due to heart failure. A number of studies have investigated whether this distinction can be made by using a rapid "point-of-care" BNP or NT-proBNP test.[44-47] The final diagnosis of heart failure was made in these studies by a panel of cardiologists using all patient's data, including patient's history, imaging techniques, and laboratory data, with the exception of the natriuretic peptide value. A rapid "point-of-care" BNP or NT-proBNP test can more accurately determine heart failure to be the cause of the shortness of breath than clinical judgment. Cutoff values for BNP and NT-proBNP have been defined to this end.

Diagnosis of diastolic heart failure

In 40% – 60% of patients with heart failure systolic function is not compromised, but the underlying problem is diastolic dysfunction. This implies a problem with the filling of the heart, for instance because of fibrosis or hypertrophy. In diastolic heart failure plasma BNP and NT-proBNP concentra-

tions are elevated, although less than with systolic heart failure.[48, 49] BNP and NT-proBNP tests can thus, in contrast to echocardiography, not distinguish between systolic and diastolic heart failure.

Natriuretic peptide guided treatment of heart failure

Patients hospitalized for acute heart failure have high plasma concentrations of BNP and NT-proBNP. Various studies have shown that successful treatment leads to a decrease in the elevated filling pressures of the heart, with concomitant decrease in natriuretic peptide concentrations.[50, 51] It has been suggested, that repeated measurements of BNP, may be useful for monitoring treatment, and, that patients should not be discharged until a threshold value of BNP is reached.[52] It has been shown that the patients with a decrease in BNP or NT-proBNP during clinical treatment are less frequently readmitted for heart failure, and have less mortality, than the patients with no decrease, or even an increase, in BNP or NT-proBNP.[53, 54] In multivariable Cox-regression analysis, edema and changes in NT-proBNP were the only two independent determinants for heart failure related readmission and mortality.[53, 54] However, the results of the Can Pro-Brain-Natriuretic Peptide Guided Therapy of Chronic Heart Failure Improve Heart Failure Morbidity and Mortality? (PRIMA) study showed that individually tailored therapy in patients hospitalized for heart failure does not help to avoid hospitalization or extend survival over the long term.[55]

In the outpatient setting, the first prospectively randomized study (n = 69) showed that titration of treatment of heart failure to a specified BNP target value resulted in less admissions for acute heart failure and lower mortality.[56] This was confirmed in a larger study of relative young patients (n = 220, mean age 65 years).[57] In the largest most recent study, including a typically elderly heart failure cohort (n = 499, mean age 77 years), no effect of BNP-guided therapy was seen on readmission rates in the total group, however in the younger participants a reduction in readmissions was seen.[58] In summary, natriuretic peptide-guided patient management can only be advocated in relatively young heart failure patients in the outpatient setting. The disappointing results in other patient groups can partly be explained by the sometimes substantial variation in plasma BNP and NT-proBNP concentrations in patients with stable chronic heart failure. One study in 6 patients, followed for 6 weeks with weekly natriuretic peptide measurement, showed an average intra-individual variation of 41 % (4% – 232 %) for BNP and 35

% (8% – 103 %) for NT-proBNP.[59] Only a substantial reduction in natriuretic peptide levels during therapy (> 80%) is related to an improved outcome.[60]

Prognostic aspects

BNP and NT-proBNP plasma concentrations correlate reasonably well with the severity of heart failure as based on clinical and/or echocardiographic parameters, and thus not surprisingly both biomarkers predict heart failure-related morbidity and mortality.[45, 46, 51, 61-64] In a large study (3916 patients with symptomatic heart failure) BNP and NT-proBNP were found to be equally predictive of total mortality and heart failure-related morbidity and mortality.[65] BNP and NT-proBNP are independently associated with cardiovascular morbidity and mortality after correction for traditional risk factors for heart and vascular diseases.[62-64] Whether NT-proBNP has incremental value for the prediction of cardiovascular disease in individuals without a history of cardiovascular disease is unknown.

CONCLUSION

In the years following the discovery of the natriuretic peptides, the structure, receptors and biological function of ANP, BNP and CNP and its amino-terminal counterparts have been carefully delineated. ANP and BNP increase natriuresis and counteract the renin-angiotensin system in response to an increase in cardiac load.[22, 24] Humans inherited this cardiac endocrine system from the salt-water fish, that excrete the surplus of ingested sea-salt through their gills in response to an increase in ventricular natriuretic peptide.[1]

The increase in plasma levels of BNP and NT-proBNP seen in humans with an increase in ventricular load resulted in a number of trials in which BNP and NT-proBNP were tested as diagnostic markers for acute heart failure in the emergency department setting. Both BNP and NT-proBNP proved to be excellent markers to exclude cardiac disease.[44-47] Positive predictive value was somewhat lower as also other conditions, such as increased neurohormone levels and septicemia, can result in an increase of natriuretic peptides.[16-20] It was expected that BNP and NT-proBNP would not only be outstanding markers for the diagnosis of heart failure, but that natriuretic peptide guided treatment of patients admitted or followed in an outpatient clinic for heart failure would improve prognosis. Nevertheless, recent studies have shown

that BNP guided treatment has no advantage over treatment guided by clinical judgment in the elderly heart failure patient.[58] However, in the group of patients younger than 65 years an advantage was seen in the outpatient clinic setting.[57, 58]

Both BNP and NT-proBNP are accurate diagnostic markers of acute heart failure and may help to guide treatment of young patients with heart failure in the outpatient clinic. Due to their clear association with not only heart failure related morbidity and mortality but also with cardiovascular disease in general, BNP and NT-proBNP may have incremental predictive value for cardiovascular disease.

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2

Aim of the thesis

As described in the introduction of this thesis, measurements of natriuretic peptides are applied as diagnostic and prognostic biomarkers for heart failure. The diagnostic accuracy of BNP and NT-proBNP for heart failure in the emergency department setting has been demonstrated by independent research groups, but the important question whether the introduction of NT-proBNP in the emergency department improves daily clinical practice as reflected by improved patient care without additional costs still remains to be addressed.

In contrast to the high diagnostic accuracy in the emergency department setting, the value of BNP and NT-proBNP for diagnosing heart failure in the general population is disappointingly low. This is likely in part related to the low prevalence of this condition in the general population. It would be worthwhile to investigate, whether in a population with a higher prevalence of cardiac dysfunction, as for instance in the elderly, the accuracy of B-type natriuretic peptides for diagnosing heart failure or cardiac dysfunction is considerably higher.

An inevitable consequence of advancing age is stiffening of large arteries. Consequences of this increase in large arterial stiffness are an increase in central systolic and a decrease in diastolic arterial pressure. Large artery stiffness therefore has negative effects on cardiac structure and function by simultaneously increasing cardiac load and decreasing cardiac perfusion. Whether the increase in large arterial stiffness provides an explanation for the observed increase in the plasma concentration of B-type natriuretic peptides with advancing age is an interesting hypothesis that requires further investigation.

Plasma levels of natriuretic peptides are increased in stable and unstable heart failure and in patients with this condition plasma levels are correlated with and predictive of cardiac morbidity and mortality and hence survival. Patients with renal failure, who are candidates of renal replacement therapy are extremely prone to cardiovascular disease and baseline values of natriuretic peptides in these patients may be prognostic for survival as well. Finally, it could be that natriuretic peptides are also useful as predictors of risk for cardiovascular disease and mortality in the general population, but whether this predictive value is still present after accounting for the classical and some other new cardiovascular risk factors or indicators remains to be established.

To address the questions as mentioned, a number of clinical and epidemiological studies has been conducted of which the aims were as follows:

1. to assess the impact of introduction of NT-proBNP measurement on clinical decision making in patients presenting to the emergency department of the Erasmus MC with acute dyspnea (chapter 3).

2. to investigate the correlation of natriuretic peptide plasma levels with echocardiographic abnormalities in a geriatric outpatient cohort (chapter 4).

3. to determine the influence of increasing large artery stiffness on natriuretic peptide levels (chapter 5).

4. to investigate the prognostic value of the natriuretic peptides in patients with end-stage renal disease (chapter 6).

5. to investigate the independent predictive value of NT-proBNP for cardiovascular morbidity and mortality in older subjects without a history of cardiovascular disease and compare its predictive value with commonly used other risk markers (chapter 7 and 8).

3

Amino-terminal pro-B-type natriuretic peptide testing in the emergency department: Beneficial effects on hospitalization, costs and outcome

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ABSTRACT

Background NT-proBNP is an established biomarker for heart failure. Assessment of this biomarker in patients with acute dyspnea presenting to the emergency department may aid diagnostic decision-making resulting in improved patient care and reduced costs.

Methods In a prospective clinical trial, patients presenting with acute dyspnea to the emergency department of the Erasmus MC, Rotterdam, the Netherlands, were randomized for either rapid measurement or no measurement of NT-proBNP. For ruling out heart failure, cutoff values of 11 pmol/l in male and 17 pmol/l in female patients were used, and for ruling in heart failure a cutoff value of 120 pmol/l. Time to discharge from the hospital and costs related to hospital admission were primary end-points. Bootstrap analysis was used for comparison of costs and 30-day mortality between the NT-proBNP and control group.

Results A total of 477 patients (54% male) was enrolled. Mean age was 59 years, with 44% of patients having a history of cardiac disease. Median time to discharge from the hospital was 1.9 (interquartile range 0.12 to 8.4) days in the NT-proBNP group (n = 236) compared with 3.9 (interquartile range 0.16 to 11.0) days in the control group (n = 241) (p = 0.04). Introduction of NT-proBNP testing resulted in a trend towards reduction in costs related to hospital admission and diagnostic investigations of 1096 euro per patient (95% confidence interval: –197 to 2574 euro), whereas 30-day mortality was similar (15 patients in the NT-proBNP and 18 patients in the control group).

Conclusions Introduction of NT-proBNP testing for heart failure in the Emergency Department setting reduces the time to discharge and is associated with a trend towards cost reduction.

INTRODUCTION

In patients with acute dyspnea presenting to the emergency department fast and accurate diagnosis is essential for the delivery of proper medical care. Diagnostic decision making can be difficult, especially because heart failure often coincides with other conditions, such as chronic obstructive pulmonary disease.[1-3] BNP and NT-proBNP testing has been evaluated for the diagnosis of heart failure in patients with complaints of acute dyspnea.[4-16] Based on these findings BNP or NT-proBNP testing has been recommended for diagnostic evaluation of patients suspected for heart failure.[17, 18]

A crucial question is whether natriuretic peptide testing improves the management of care and saves costs of patients presenting with acute dyspnea to the emergency department. So far, a few studies have addressed this question.[19-22] These studies indicate that introduction of rapid point of care BNP or NT-proBNP testing in the emergency department is associated with cost savings without adversely affecting short-term outcome.

Obviously, the effect of introduction of natriuretic peptide testing on clinical outcome and cost savings strongly depends on the way medical care is organized and delivered. Hence, findings may vary from region to region and, therefore, are not necessarily interchangeable. The objective of our study was to investigate whether introduction of rapid NT-proBNP testing in the emergency department of our hospital associates with improved diagnostic decision making as reflected in cost savings without compromising clinical outcome.

METHODS

Protocol

This study was conducted as a prospective, randomized, controlled trial. From December 2004 to February 2006, patients presenting with dyspnea to the emergency department of the Erasmus MC, Rotterdam, the Netherlands were asked to participate. Adult patients were eligible if they presented with acute dyspnea as their most prominent complaint. Patients with acute dyspnea due to a trauma or cardiogenic shock and patients with renal failure requiring hemodialysis or peritoneal dialysis were excluded. The initial assessment of patients for eligibility to participate was performed by the

nurses in the emergency department. In patients assigned to the NT-proBNP group, the NT-proBNP plasma level was determined at admission. In patients of the control group the physicians were blinded for the NT-proBNP levels. To rule out heart failure in the NT-proBNP group, the attending physician was provided with the following cutoff values: 11 pmol/l for males and 17 pmol/l for females. To rule in heart failure, a cutoff value of 120 pmol/l was provided. The results of NT-proBNP testing were used in combination with the findings of clinical history taking, physical examination and regularly performed laboratory testing, chest x-ray, electrocardiogram and in some patients computed tomography. Echocardiography and pulmonary function tests were mostly performed during hospitalization or on an outpatient basis.

The severity of shortness of breath was scored according to the New York Heart Association Functional Classification. For each patient the attending physician indicated the likelihood of heart failure on a visual analogue scale, ranging from 1 to 100 %, before the sample was sent to the laboratory. Heart failure was considered to be highly unlikely if the visual analogue score was equal or below 25%, very likely if the visual analogue scale score was above 75% and intermediate if the visual analogue scale score was between 25 and 75%. The study was approved by the Erasmus MC Ethics Committee. Written informed consent was obtained from all participants. The study was registered at http://www.controlled-trials.com, ISRCTN28653133.

Assignment and masking

Patients were randomized 1:1 without stratification, according to a computer-generated scheme. The attending physician was unaware of the allocation of an included subject as the randomization was performed by sealed, non-see-through envelopes. Upon arrival of the blood sample at the laboratory, the attending technician assigned the patients to either the NT-proBNP group or control group according to the instructions in a sealed envelope accompanying the blood sample.

Participant flow and follow-up

Physicians of several specialties examined the patients presenting to the emergency department. If a patient was referred by a general practitioner, evaluation was performed by a resident in internal medicine, pulmonology or cardiology. The residents consulted a senior specialist before deciding on final diagnosis, therapy, discharge or hospital admission. If a patient came to

the hospital without prior consultation of a general practitioner, a resident directly assigned to the emergency department performed the first evaluation. Primary endpoints were time to discharge and costs of treatment. Costs per patient included the costs of stay on the general ward and/or the intensive or coronary care unit, the costs of cardiopulmonary investigations (chest X-ray, electrocardiography, echocardiography, chest computed tomography, coronary angiography, right heart catheterization, pulmonary function tests, bronchoscopy, myocardial perfusion scintigraphy, bicycle ergometry) and the costs of NT-proBNP measurements. The all-inclusive price of one NT-proBNP measurement was 27 euro. This price may vary from 24 to 32 euro between various centers due to differences in personnel costs. The costs were calculated per patient. Costs for hospital admission were based on national prices for admission to a general ward (490 euro per day) or intensive care unit (1733 euro per day) of a university hospital. For diagnostic investigations the prices as charged to health insurance companies were used. If a patient was transferred to another hospital, only the time to discharge and costs generated in the Erasmus MC were used in the calculation of the primary endpoints. Secondary endpoints were duration of stay at the emergency department, the proportion of patients admitted to the hospital and the proportion of patients admitted to an intensive or coronary care unit. Thirty-day outcome was assessed by review of electronic hospital records. If no records were present, patients were contacted by telephone or mail. If unsuccessful, their general practitioner was contacted.

NT-proBNP measurement

Blood samples for NT-proBNP measurement were collected in plastic tubes containing potassium ethylenediaminetetraacetic acid (Vacutainer; Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA). After blood collection, samples were centrifuged for five minutes at 2030 *g*. NT-proBNP was measured by a commercially available electrochemiluminescence immunoassay (Elecsys proBNP; F. Hoffman-La Roche Ltd., Basel, Switzerland) on an Elecsys 2010 analyzer. The precision, analytical sensitivity, and stability characteristics of the system have been previously described.[23] All plasma samples were stored at - 80º C.

Statistical analysis

All analyses were by the intention-to-treat principle. Mann-Whitney *U* tests were used to compare non-normal distributed continuous variables. For comparison of rates χ^2 tests were used. The effect on mean costs was assessed by means of non-parametric bootstrap analysis.[24] The Bias-corrected accelerated confidence interval for difference in mean costs was computed from 10,000 bootstrap replicates. The sample size estimates for the trial were based on achieving 80% power with an alpha of 0.05 on the endpoint of time to discharge. Mueller et al found a difference of three days in time to discharge between the study and control group with a pooled standard deviation of 10.3. These estimates led to a number of at least 376 patients to be included.[20] For statistical analysis we used SPSS 12.0.1 (SPSS Inc., Chicago, Illinois) and R 2.4.0 (R Foundation for Statistical Computing, Vienna, Austria). Glomerular filtration rate was calculated using the modified Modification of Diet in Renal Disease formula.[25]

RESULTS

For a period of 14 months more than 29,000 patients were seen at the emergency department of the Erasmus MC. Of the 785 patients visiting the emergency department with dyspnea, 477 patients were randomized (Figure 1). In 5 patients of the study group NT-proBNP measurements were falsely not performed and in 5 patients of the control group NT-proBNP measurements were performed during the hospital stay. Twenty patients were transferred from the emergency department of the Erasmus MC to another hospital due to logistic reasons. The demographic and clinical characteristics of the 236 participants in the NT-proBNP group and 241 participants in the control group were well balanced (Table 1). Mean age was 58.6 years and numbers of males and females in both groups were almost equal. About two-thirds of all patients had ever smoked. Cardiac and pulmonary disease was highly prevalent with 20% of the patients having a history of cardiac disease, 35% a history of pulmonary disease and 24% a history of both cardiac and pulmonary disease. Two patients of the NT-proBNP group and one patient of the control group had a serum creatinine concentration higher than 250 µmol/l. Almost two thirds of the patients was seen by their general practitioner prior to their emergency department visit. Primary evaluation at the

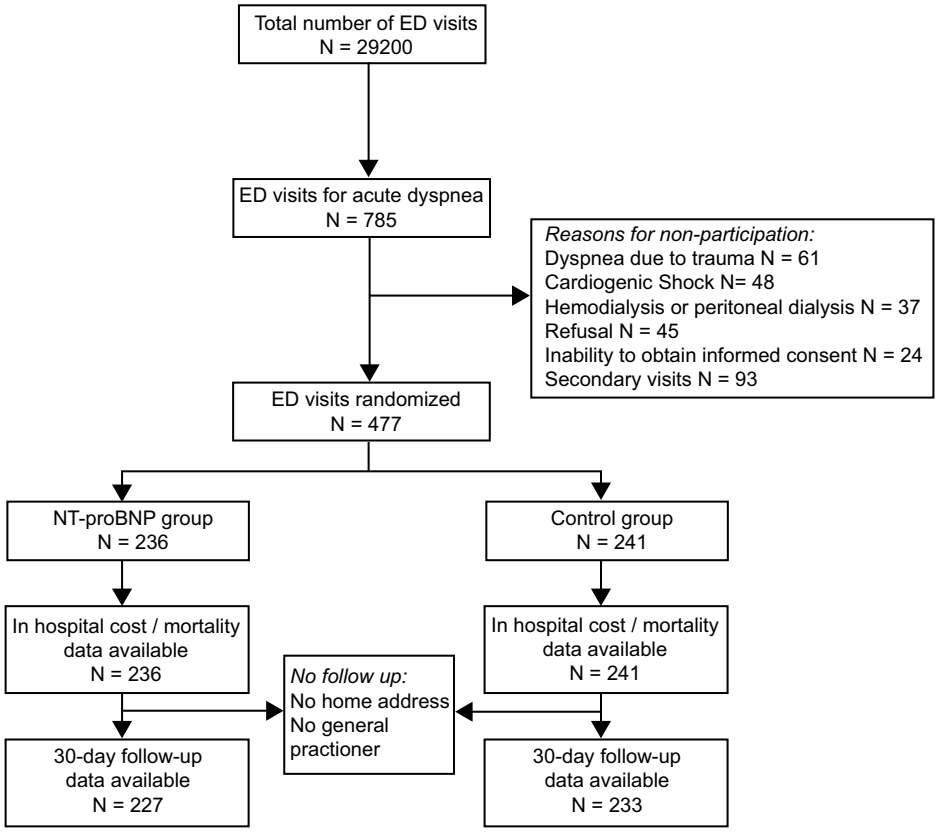

Figure 1. Flow chart of the study protocol.
Abbreviations: ED, emergency department.

emergency department was done by a resident in pulmonary medicine in 151 cases (32%), internal medicine in 115 cases (24%), emergency medicine in 113 cases (24%), cardiology in 84 cases (18%) and other specialties in 14 cases (3%). Of all patients 86% was evaluated by a senior staff member.

The median time from emergency department admission to hospital discharge was 3.9 days (interquartile range 0.16 to 11.0) in the control group and 1.9 days (interquartile range 0.12 to 8.4) in the NT-proBNP group ($p = 0.04$). The hospitalization rate between the NT-proBNP group and the control group (62% versus 67%) and the time to discharge from the emergency department between the NT-proBNP and control group did not differ (Table 2). The average costs per patient related to hospital admission, cardiopulmonary diagnostic investigations and NT-proBNP testing were 3990 euro in the NT-proBNP and 5086 euro in the control group, resulting in a mean

Table 1. Baseline characteristics of the patients of the NT-proBNP group versus the control group

Characteristic	***NT-proBNP group n = 236***	***Control Group n = 241***
Mean age (SD) - year	*58.2 (17.8)*	*59.0 (17.8)*
Male subjects (%)	*126 (53)*	*131 (54)*
Smoking Status (%)		
Never smoked	*80 (34)*	*87 (36)*
Current smoker	*80 (34)*	*90 (37)*
Previous smoker	*74 (31)*	*61 (25)*
Medical history (%)		
Heart failure	*43 (18)*	*42 (17)*
Coronary artery disease	*47 (20)*	*53 (22)*
Chronic obstructive pulmonary disease	*68 (29)*	*58 (24)*
Asthma	*29 (12)*	*35 (15)*
Arterial hypertension	*53 (23)*	*67 (28)*
Diabetes Mellitus	*35 (15)*	*43 (18)*
Chronic kidney disease	*19 (8)*	*22 (9)*
Symptoms (%)		
Dyspnea, NYHA II	*86 (36)*	*74 (31)*
NYHA III	*58 (25)*	*88 (37)*
NYHA IV	*82 (35)*	*71 (30)*
Orthopnoea	*69 (29)*	*78 (32)*
Chest pain	*52 (22)*	*70 (29)*
Coughing	*140 (59)*	*135 (56)*
Signs (%)		
Tachypnea (> 20 breaths/min)	*103 (44)*	*105 (44)*
Elevated jugular venous pressure	*29 (12)*	*39 (16)*
S3 gallop	*4 (2)*	*5 (2)*
Rales	*83 (35)*	*95 (39)*
Wheezing	*62 (26)*	*53 (22)*
Lower-extremity edema	*46 (20)*	*50 (21)*
Mean systolic blood pressure (SD) - mmHg	*148 (33)*	*148 (33)*
Mean heart rate (SD) – beats/min	*97 (23)*	*98 (25)*
Mean temperature (SD) – ºC	*37.4 (0.9)*	*37.4 (1.0)*
Kidney function		
Mean glomerular filtration rate (SD) – ml/min/1.73 m²	*91 (41)*	*87 (39)*

Abbreviations: NYHA, New York Heart Association Class; SD, standard deviation.

difference of costs of 1096 euro (bias corrected accelerated 95% confidence interval [CI] –197 to 2574 euro). There was no difference in in-hospital

mortality rate between both groups with 14 patients dying in the NT-proBNP group and 15 patients in the control group (p = 0.52). Thirty-day follow-up data was available for 460 patients. For nine in the NT-proBNP group and eight patients in the control group follow-up data could not be obtained due to logistic reasons. Thirty-day mortality rates and thirty-day readmission rates between both groups did not differ (Table 2).

Table 2. Effect of NT-proBNP testing on duration of stay at the emergency department, overall time to discharge (time from admission to emergency department to discharge from the hospital), hospitalization rate, intensive care unit admission rate, duration of hospitalization (time from admission to a ward or intensive care unit to final discharge from the hospital), mortality and re-admission rate

End Points	***NT-proBNP group n = 236***	***Control group n = 241***	***p***
Emergency department admission *– min*			
Median	*170*	*172*	*0.12**
Interquartile range	*120 - 220*	*130 - 235*	
Overall time to discharge *– days*			
Median	*1.9*	*3.9*	*0.04**
Interquartile range	*0.12 – 8.4*	*0.16 – 11.0*	
Hospitalization			
No. of patients (%)	*147 (62)*	*162 (67)*	*0.26¶*
Duration of hospitalization			
Median	*7.8*	*8.1*	*0.48¶*
Interquartile range	*4.8 – 13.9*	*4.4 – 15.6*	
Admission to intensive care			
No. of patients (%)	*38 (16)*	*38 (16)*	*0.92¶*
In-hospital mortality			
No. of patients (%)	*14 (6)*	*15 (6)*	*0.89¶*
30-day mortality			
No. of patients (%)	*15 (6)*	*18 (8)*	*0.26¶*
30-day readmission			
No. of patients (%)	*7 (3)*	*12 (5)*	*0.18¶*

*Mann-Whitney *U* test, ¶ χ^2 test

NT-proBNP testing had a favorable effect on costs without an adverse effect on 30-day mortality. By plotting the results of the bootstrap analyses in a cost-effectiveness plane, the relation between costs and effect is visualized in Figure 2. The dots below the x-axis represent the proportion of the results of the bootstrap in which costs were lower in the NT-proBNP group compared to the control group. The dots on the right side of the y-axis represent the proportion in which mortality was lower. Although the costs are not statistically lower in the NT-proBNP group versus the control group it can be appreciated from Figure 2 that introduction of NT-proBNP testing is associated with a high chance of cost-reduction and low risk for a cost-increase. Post-hoc subgroup analyses indicate that the effect on costs is largest in patients with cardiac dyspnea (mean reduction in costs 2103 euro [95% CI −1206 to 5407 euro]) compared to patient with non-cardiac dyspnea (mean reduction in costs 120 euro [95% CI −1110 to 1302 euro]).

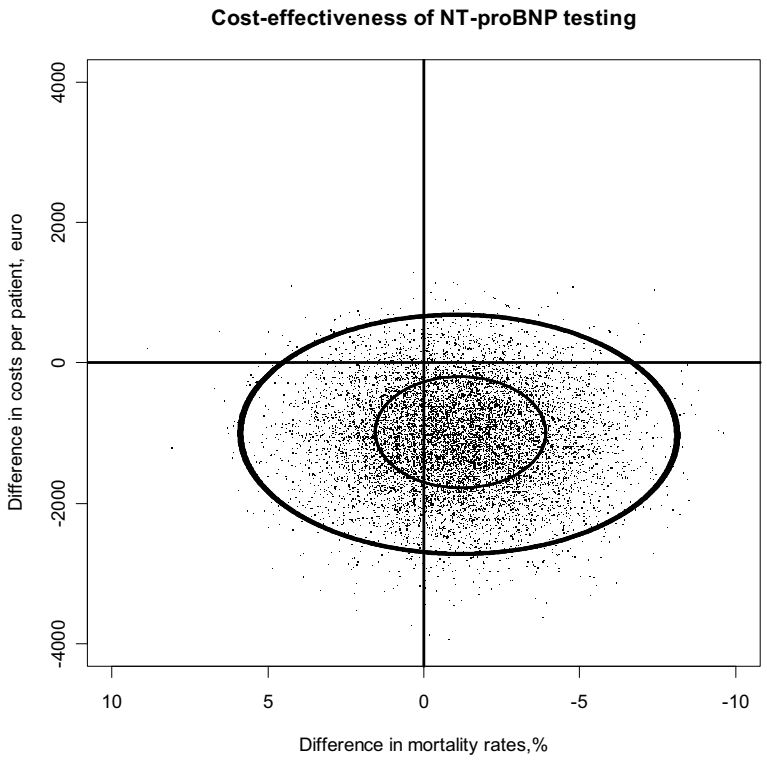

Figure 2. Results of the bootstrap analysis for the cost-efficacy of introduction of NT-proBNP testing. Each individual bootstrap sample represents a dot in the plot. The right lower quadrant of the graph represents the proportion of bootstrap samples in which a reduction in costs and mortality coincides. The ellipses show the 50% and 95% confidence intervals of the estimate.

According to the scores of the visual analogue scale filled in by the attending emergency department physicians heart failure was considered unlikely in 282 patients (59%), highly likely in 70 patients (15%), and indefinite in 105 patients (21%). In the 282 patients in whom heart failure was considered unlikely, NT-proBNP levels were above the upper cutoff value of 120 pmol/l in 61 patients (21%) (Table 3). In 29 of these 61 patients, the diagnosis was changed to a cardiac disorder after further evaluation. Fourteen out of these 61 patients had a history of cardiac disease but were admitted for other reasons. Of the remaining patients without a cardiac diagnosis but an increased NT-proBNP, 10 were diagnosed with pneumonia, 6 were critically ill due to other causes, 1 patient suffered from a pulmonary embolus, and 1 had a history of pulmonary hypertension.

Table 3. Three by three table comparing the likelihood of heart failure scored by the attending emergency department physician and the results of NT-proBNP testing

Physician's score:	***Likelihood of heart failure based on NT-proBNP level***		
	Low *< 11 pmol/l (men) < 17 pmol/l (women)*	***No definite diagnosis***	***High*** *> 120 pmol/l*
No heart failure VAS score 0 – 25% *No. patients (%)*	*128 (45)*	*93 (33)*	*61 (22)*
No definite diagnosis VAS score 26 – 75% *No. patients (%)*	*22 (20)*	*24 (23)*	*59 (56)*
Heart failure VAS score 76 – 100% *No. patients (%)*	*2 (3)*	*8 (11)*	*60 (86)*

Abbreviations: VAS, visual analogue scale.

In the 70 patients in whom heart failure was considered highly likely, NT-proBNP proved otherwise in only two. In the patients with diagnostic uncertainty heart failure could be confirmed in 59 and excluded in 22 patients by NT-proBNP testing. In 24 patients heart failure could not be excluded or

diagnosed with 100% certainty by combined clinical review and NT-proBNP testing. The agreement of the likelihood of heart failure based on the visual analogue scale and NT-proBNP testing was low (κ statistic 0.201).

DISCUSSION

Our study shows that introduction of NT-proBNP testing for heart failure in the emergency department setting results in a reduction of time to discharge and is associated with a trend towards cost reduction. Importantly, this trend to cost reduction was not associated with negative effects on 30-day mortality or readmission rates. The favorable effects on costs are most likely explained by the difference in hospitalization rate. 62% of the patients of the NT-proBNP group versus 67% of the patients of the control group were hospitalized, i.e. a difference of 9.2%. This difference in hospitalization rate also explains the overall shorter discharge time in the NT-proBNP group.

Introduction of BNP testing in patients with acute dyspnea presenting to the emergency department has been associated with an estimated reduction in mean costs of 1484 euro as reported by the investigators of the B-Type Natriuretic Peptide for Acute Shortness of Breath Evaluation (BASEL) study.[20, 21] A post-hoc cost-effectiveness analysis by decision modeling showed that NT-proBNP testing might result in a reduction in costs of 380 euro.[19] In the Improved Management of Patients with Congestive Heart Failure (IMPROVE-CHF) study a reduction of 760 euro in median costs at 60 days of follow-up, including emergency department visits, hospitalizations, and outpatient services could be demonstrated, but there was no significant difference in the median costs of the initial emergency department visit and initial hospitalization between the NT-proBNP and usual care group[22]. For cost-effectiveness evaluations, however, the mean costs per patient instead of the median costs per group is the preferred parameter.[26] Health care policy makers need to have information on the impact of NT-proBNP testing on the total budget, which is best reflected by the mean costs per patient. Therefore, we used a bootstrap technique to investigate the mean reduction in costs per patient. In our study, the costs per patient were on average 1096 euro lower for the group of patients allocated to NT-proBNP compared to the control group, but this difference did not reach statistical significance. It should be noted that in randomized clinical trials assessing the cost-effectiveness of a new

diagnostic test the difference in costs relies on only a small proportion of patients in whom the diagnosis has changed as a result of the new test, making these trials prone to a type II error.

In comparison with the other two studies our cohort was about 10 years younger, the proportion of patients with a history of coronary heart disease was lower, whereas the proportion of patients with history of chronic obstructive lung disease was higher.[20, 22] Furthermore, over 60% of our patients was first reviewed by their general practitioner prior to admittance to our emergency department, which is common in the Dutch health care system. Prehospital diagnostic investigations and treatment by a general practitioner provide important information on the possible diagnosis, thereby limiting the additional value of NT-proBNP testing in the diagnostic process. The findings of our study and of the three cited studies provide strong evidence that introduction of rapid BNP or NT-proBNP testing in the emergency department in patients presenting with acute dyspnea is cost-effective.[19, 20, 22]

In the BASEL study, BNP testing was associated with a significant reduction in hospitalizations (75% versus 85%) and a reduction in the median time to discharge (8 versus 11 days).[20] In the IMPROVE-CHF study introduction of NT-proBNP had no significant effects on the number of initial hospitalizations (57% versus 58%), the length of hospital stay (6 versus 7 days) or the proportion of patients admitted to the intensive care unit. In this regard the findings of our study are more comparable with the IMPROVE-CHF study than the BASEL study. In contrast to the BASEL study, introduction of NT-proBNP testing in our study had no effect on the proportion of patients admitted to the intensive care unit.[20] This is not unexpected as the need for intensive care treatment depends primarily on the severity of the condition and not on the diagnosis itself. Also in the IMPROVE-CHF study, no significant effect of NT-proBNP testing on the intensive care admission rate could be demonstrated.[22] We found no effect of NT-proBNP testing on the duration of stay in the emergency department in contrast to the BASEL study. This is probably due to differences in the delivery of hospital care and a difference in the incidence of heart failure between the 2 studies.

One may wonder whether the differences in outcome for the mentioned studies depend on the choice of the selected biomarker, BNP versus NT-proBNP. In head to head comparisons no superiority of either biomarker for the diagnostic value of heart failure in the emergency department could be demonstrated.[13, 16, 23] We judge it highly unlikely that the difference in

outcome across the studies is explained by the difference in the used biomarkers.

Our study has some limitations. The diagnostic value of NT-proBNP testing depends on the selected cutoff values. Our cutoff levels were based on the results of studies published before December 2004 and data provided by the manufacturer.[5, 13, 27] Recently, the results of various studies on the diagnostic value of NT-proBNP were pooled and three different cutoff values for either exclusion or diagnosis of heart failure have been proposed.[11] If the cutoff values as proposed by Januzzi et al had been used, the number of patients in whom a diagnosis of heart failure could either not be confirmed or excluded, would have been reduced from 125 to 67 patients.[11] Accordingly if we would have been able to use these cutoff values the benefit of NT-proBNP testing would probably have been greater.

Second, blinding of diagnostic strategy allocation is impossible in randomized studies assessing cost-effectiveness of diagnostic tests because the physicians are provided with the additional diagnostic information only in the study group. Since the discharge of a patient is determined by his or her clinical condition we consider it very unlikely that unblinding of study group allocation per se was of any influence on the time to discharge in our trial. Third, 62% of our patients was first reviewed by a general practitioner. This may be different for other countries and hence may influence the generalizability of our findings.

CONCLUSION

The results of our study indicate that rapid NT-proBNP testing in the emergency department for patients presenting with acute dyspnea is highly likely associated with cost savings due to an overall shorter stay in the hospital. Importantly this beneficial effect is not counterbalanced by adverse effects on in-hospital and 30-day mortality. Based on all available evidence, widespread use of natriuretic peptide testing for the management of dyspneic patients presenting to the emergency department is recommended.

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4

Associations between plasma natriuretic peptides and echocardiographic abnormalities in geriatric outpatients

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ABSTRACT

Background Identification of patients with cardiac dysfunction can be difficult in the geriatric population. Recently, different subtypes of the natriuretic peptide family have been advocated as biomarker for the diagnosis of heart failure in the emergency department setting. The study was performed to investigate the correlation between increased natriuretic peptide plasma levels and echocardiographic abnormalities in geriatric outpatients.

Methods We performed two-dimensional transthoracic echocardiography in 209 community-dwelling subjects visiting the geriatric outpatient clinic of our university hospital. Subjects were 65 years or older and had no markedly impaired cognitive function.

Results Mean ANP and BNP plasma levels were respectively 11.0 and 10.8 pmol/l. BNP correlated with left ventricular dysfunction and left ventricular mass in contrast to ANP. A natriuretic peptide level in the highest tertile was associated with a higher risk for echocardiographic abnormalities, BNP odds ratio 7.15 (95% CI 2.15 to 23.71), ANP odds ratio 3.07 (95% CI 1.15 – 8.16). At a cutoff of BNP of 6.85 pmol/l, sensitivity was 92%, specificity 31%, negative predictive value 97% and positive predictive value 16%.

Conclusions Increased natriuretic peptide levels are indicative of echocardiographic abnormalities in geriatric outpatients. If after careful history taking and physical examination still uncertainty remains regarding presence of heart disease in a geriatric outpatient BNP testing may help to exclude severe cardiac abnormalities.

INTRODUCTION

Due to an ageing population and improved therapy for acute coronary syndromes the number of elderly patients with symptoms of cardiac dysfunction is increasing.[1] For instance, in the population over 75 years the reported prevalence of heart failure is 5.5% compared to 1% in the general population.[2-4] Because medical intervention has shown to reduce morbidity and improves survival in patients with cardiac dysfunction and heart failure, identification of patients who could benefit from treatment is important.[5]

The identification of elderly subjects with cardiac dysfunction can be difficult, as they often present with atypical symptoms.[6, 7] Without the classic clinical picture, clinical suspicion of cardiac abnormalities may be low, precluding performance of echocardiography and initiation of appropriate treatment. Natriuretic peptides are potential candidates to identify patients with cardiac abnormalities. In the general population, the positive predictive value of elevated plasma levels of natriuretic peptides to diagnose cardiac dysfunction is disappointedly low.[8-10] It could be that the higher prevalence of cardiac dysfunction in the geriatric population leads to a higher positive predictive value, although it has to be taken into account that the more frequent occurrence of co-morbid conditions, like renal insufficiency, as well as older age may affect this predictive value adversely.[11-13]

Of the natriuretic peptides, BNP primarily reflects left ventricular load, whereas ANP is released predominantly upon an increase in atrial wall distension.[14] An elevated plasma BNP concentration has shown to be a biomarker for heart failure in the Emergency Department setting in several studies.[15-17] Compared to BNP, ANP may especially be useful for diagnosing increased left atrial dimension, a condition known to predict cardiovascular events in the middle-aged and elderly.[18-20]

We conducted the present study in order to investigate associations between the natriuretic peptide plasma levels and various echocardiographic abnormalities in an unselected group of geriatric patients, visiting our outpatient clinic.

METHODS

Study population

Community-dwelling patients consecutively referred to the outpatient clinic or the Diagnostic Day Center of the section of Geriatric Medicine at the Erasmus MC were invited to participate in the study. Patients were eligible if they were 65 years or older and had no markedly impaired cognitive function as defined by a Mini-Mental State Examination score of 21 points or higher.[21] The study was approved by the Medical Ethics Committee of the Erasmus MC, all patients gave written informed consent. All patient underwent a complete geriatric assessment including medical history, interview, and physical examination, including cardiac auscultation by a geriatrician.[22] Supine blood pressure was measured in duplicate after five minutes of rest.

Echocardiographic measurements

Two-dimensional transthoracic echocardiography was performed with an ultrasound scanner (Sonos 5500, Philips, Best, the Netherlands). Patients were examined in left lateral decubital position using a standard broad band S4 transducer (2-4 MHz). One and the same investigator (N.V.) performed echocardiography. All echocardiographic measurements were reviewed by a second echocardiographer (W.V.). Both researchers were blinded to the natriuretic peptide plasma levels.

Left atrial and left ventricular diameters (end diastolic diameter and end systolic diameter) as well as posterior wall and septal thickness were measured in agreement with the guidelines of the American Society of Echocardiography.[23] Left ventricular mass was calculated from the M-mode measurements according to the formula described by Devereux et al.[24] To correct for differences in body size, left ventricular mass was indexed to body surface area. Systolic left ventricular function was scored visually as good, fair, moderate or poor. Left ventricular ejection fraction was calculated using 2 dimension-guided M-mode measurements of left ventricular end-diastolic and end-systolic internal dimensions.[25] Doppler recordings of mitral and pulmonary venous flow velocities and pattern were used to assess diastolic function. Impaired relaxation was defined as an E/A ratio < 1, atrial reversal flow > 0.4 msec and a pulmonary venous flow pattern with systolic flow velocity larger than diastolic flow velocity. A restrictive pattern was defined as an E/A ratio > 1.5, a shortened deceleration time (< 160 msec) and a

pulmonary venous pattern with systolic flow velocity smaller than diastolic flow velocity.

Valvular lesions were assessed using both Doppler and color Doppler. Valvular regurgitation was assessed as mild, moderate, moderately severe or severe on basis of the jet extension (no regurgitation or trivial; 1: mild; 2: moderate; 3: moderately severe, with a long jet; and 4: severe, with a regurgitant jet along the length of either the left ventricle or the right ventricle).[26] The degree of valvular stenosis was quantified by continuous wave Doppler velocity measurements using the modified Bernoulli equation.[27] Aortic valve stenosis was defined as a flow higher than 2.2 m/sec.

Laboratory investigations

Blood samples were taken after 30 minutes rest in supine position from an intravenous catheter in one of the forearm veins. For natriuretic peptide measurements blood was collected in chilled plastic tubes containing ethylenediaminetetraacetic acid and apoprotin. All samples were immediately centrifuged at 4°C for 10 minutes at 3000 *g*, and plasma was stored at –80°C until assayed. We measured plasma ANP and BNP levels by two commercially available immunoradiometric assays (Shionoria; Osaka, Japan). Besides sampling for natriuretic peptides blood was also collected for routine laboratory investigations of serum electrolytes, urea and creatinine. The Cockcroft and Gault formula was used to estimate the glomerular filtration rate.[28]

Statistical analysis

Data are presented as mean values and standard deviations, or median values and ranges if indicated. Comparisons between groups were made using analysis of variance for independent samples or Student's two-tailed *t* test as appropriate. Geometric means are given for natriuretic peptide levels, comparisons of natriuretic peptide levels between sexes are made by Student's two-tailed *t* tests after logarithmic transformation of ANP and BNP values to normalize the positively skewed distributions. Associations between different variables were evaluated by use of Pearson's correlations. The relative risk of patients with high plasma levels of natriuretic peptides for different echocardiographic abnormalities was estimated by calculation of the odds ratio using binary logistic regression, corrected for age, sex, weight and estimated glomerular filtration rate. Left ventricular function and valvu-

lar function were categorized as either normal or abnormal (left ventricular dysfunction, visual score of fair to poor; left ventricular diastolic dysfunction, relaxation abnormalities to restrictive pattern; valvular lesion, valvular insufficiency or stenosis of moderate to severe). Sex was not corrected for if the cutoff for abnormality was sex-specific, i.e. left ventricular hypertrophy, men > 134 g/m^2, women > 110 g/m^2, left atrial diameter enlargement men > 42 mm, women > 38 mm. A *p* value less than 0.05 was considered to indicate statistical significance. Statistical analysis was performed using SPSS (version 12.0, SPSS Inc; Chicago, Illinois, USA).

RESULTS

Patient characteristics

Two hundred and nine patients (65% female, mean age 77.5 years) were consecutively included. The baseline characteristics are presented in Table 1. Ischemic heart disease was the most prevalent cardiac disease with 12% of patients having anginal complaints and 8% of patients having a history of myocardial infarction. Atrial fibrillation (continuous or paroxysmal) was present in 10% of patients. 5% of patients had a history of heart failure. Physical examination revealed cardiac murmurs in 35% of patients (Table 1).

In almost all patients full echocardiographic data were available for analysis. In 10 patients diastolic function could not be assessed due to anatomic abnormalities, obesity or chronic obstructive pulmonary disease. Left ventricular hypertrophy was twice as prevalent in women as in men (Table 2). Almost all patients had a good to fair left ventricular ejection fraction assessed by visual inspection, but left ventricular diastolic function was normal in only 19% of patients. In six patients the inferior caval vein collapse upon inspiration was less than 50%, indicating an increase in right atrial pressure. Tricuspid regurgitation, of which more than 20% mild regurgitation, was the most prevalent valvular lesion (Table 2).

Determinants of natriuretic peptides

The BNP plasma levels differed between sexes, 8.4 pmol/l for males and 12.6 pmol/l for females (p = 0.008). Also ANP plasma levels differed between males and females, 8.6 and 12.1 pmol/l, respectively (p = 0.001). Log-BNP was positively correlated with age (r = 0.406). Male sex, weight and

Table 1. Baseline characteristics of all patients (n = 209), if not indicated number of patients and percentages are given

Characteristic	
Age, years (mean, SD)	*77.5 (5.9)*
Males (%)	*73 (35)*
Current smoker (%)	*29 (14)*
Former smoker (%)	*82 (39)*
Known diabetes mellitus (%)	*26 (12)*
Prior myocardial infarction (%)	*17 (8)*
Prior or current angina pectoris (%)	*26 (12)*
Prior or current heart failure (%)	*11 (5)*
Prior or current arrhythmia (%)	*36 (17)*
Systolic blood pressure, mm Hg (mean, SD)	*150 (26)*
Diastolic blood pressure, mm Hg (mean, SD)	*79 (12)*
Cardiac murmur (%)	*73 (35)*
Leg edema (%)	*47 (22)*
Increased central venous pressure (%)	*6 (3)*
Body mass index, kg/m^2 (mean, SD)	*26.6 (4.2)*
Glomerular filtration rate, ml/min/1.73 m^2 (mean, SD)	*63.6 (17.2)*
Atrial natriuretic peptide, pmol/l, (median, range)	*11.0 (1.9-140.0)*
B-type natriuretic peptide, pmol/l, (median, range)	*10.8 (0.7-126.7)*

Abbreviations: SD, standard deviation.

glomerular filtration rate were negatively correlated with log-BNP. The mean plasma level of BNP did increase with an increasing number of echocardiographic abnormalities (Figure 1). Both left ventricular dysfunction and left ventricular mass correlated with log-BNP (Table 3). Left ventricular systolic function as assessed by visual scoring correlated with log-BNP. In contrast no relation could be demonstrated between left ventricular ejection fraction and natriuretic peptide plasma levels (Table 3). Correlations between single echocardiographic abnormalities were mainly found for log-BNP.

Table 2. Echocardiographic systolic and diastolic parameters

Echocardiographic measurement	
Left atrium diameter, mm, (mean, SD)	*42.8 (8.0)*
left ventricular mass index, g/m² (mean, SD)	*89.5 (31.0)*
Men	*94.3 (36.5)*
Women	*86.9 (43.0)*
left ventricular function, (n, %)	
Good	*178 (84)*
Fair	*29 (14)*
Moderate	*3 (1)*
Poor	*1 (1)*
Ejection fraction, %, (mean, SD)	*59.8 (13.1)*
Diastolic dysfunction, (n, %)	
No diastolic dysfunction	*39 (19)*
Relaxation abnormalities	*157 (78)*
Pseudonormalization	*4 (2)*
Restrictive pattern	*1 (<1)*
<50% collaps inferior caval vein, (n, %)	*6 (3)*
*Aortic stenosis **	*7 (3)*
*Aortic regurgitation **	*20 (10)*
*Mitral regurgitation **	*19 (9)*
*Pulmonary regurgitation **	*27 (13)*
*Tricupid regurgitation **	*42 (20)*

Definition left ventricular hypertrophy (LVH): men, normal 71g/m², LVH > 134 g/m²; women, normal 62 g/m², LVH > 110 g/m². * Moderate to severe stenosis or regurgitation.

Risk for echocardiographic abnormalities with increased natriuretic peptide levels

To determine the clinical relevance of a high plasma level of either BNP or ANP for echocardiographic abnormalities, patients with a natriuretic plasma level in the highest tertile (BNP 17.2 – 126.7 pmol/l, ANP 13.6 – 140.0 pmol/l) versus the lowest tertile (BNP 0.7 - 7.6 pmol/l, ANP 1.9 – 8.3 pmol/l) were compared. Adjusted for known confounders a high BNP was associated with a higher risk for moderate to severe valvular lesions (odds ratio 3.79 [1.67 to 8.56], p = 0.001) or left ventricular dysfunction (odds ratio 2.88 [95% CI 1.04 to 7.94], p = 0.04) at echocardiography (Figure 2). A trend towards an increased risk for having left atrial enlargement was noted (2.14 [95% CI 0.94 to 4.88], p = 0.07). A high ANP was associated with a higher risk for valvular lesions (odds ratio 2.63 [95% CI 1.20 to 5.73], p = 0.015)

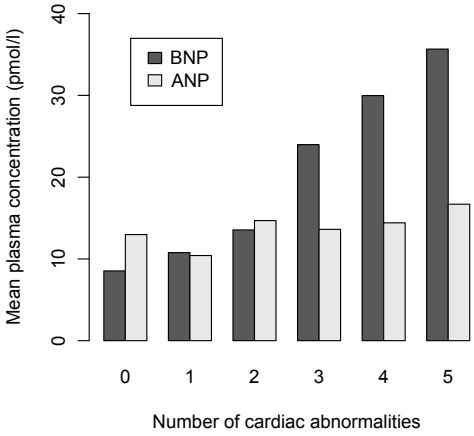

Figure 1. Mean plasma BNP and ANP plasma concentrations for patients with no echocardiographic abnormalities up to five echocardiographic abnormalities. An echocardiographic abnormality was defined by one of the following conditions: left atrial enlargement, left ventricular hypertrophy, left ventricular systolic dysfunction, left ventricular diastolic dysfunction, valvular lesion more severe than mild.

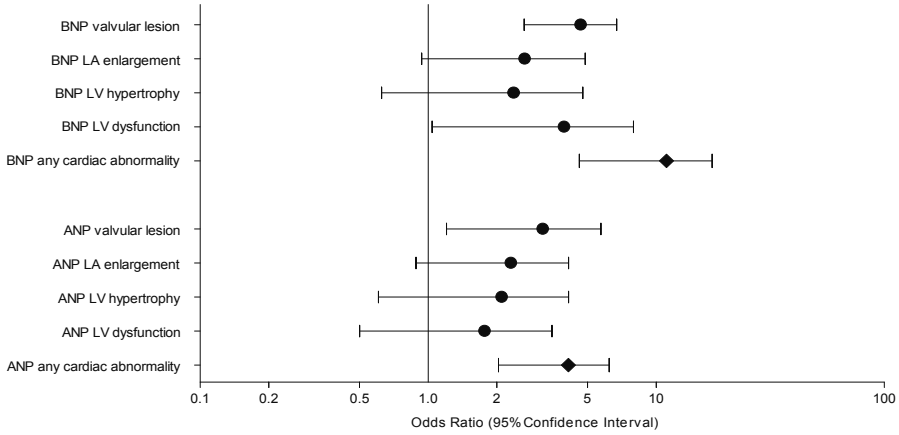

Figure 2. Odds ratio plot for echocardiographic abnormalities, respective odds ratios are given for having a moderate to severe valvular lesion, left atrial enlargement, left ventricular hypertrophy, left ventricular systolic dysfunction or any of these abnormalities for patients in the highest BNP or ANP tertile compared to patients in the lowest tertile.
Abbreviations: LA, left atrial; LV, left ventricular.

(Figure 2). For the echocardiographic abnormalities combined, odds ratios for BNP and ANP were respectively 7.15 (95% CI 2.15 to 23.71) and 3.07 (95% CI 1.15 to 8.16) (Figure 2).

Table 3. Unadjusted linear regression analysis of BNP and ANP (logarithmically transformed), Pearson's correlation coefficients are given

	BNP		***ANP***	
	r	*p*	*r*	*p*
Age	**0.406**	*0.000*	**0.276**	*0.000*
Sex ¶	**-0.186**	*0.007*	**-0.246**	*0.000*
Weight	**-0.248**	*0.000*	**-0.173**	*0.012*
Glomerular filtration rate	**-0.305**	*0.000*	**-0.221**	*0.001*
Systolic blood pressure	*0.016*	*0.832*	*0.044*	*0.550*
left ventricular systolic dysfunction ±	**0.224**	*0.001*	*0.081*	*0.248*
Ejection fraction	*0.049*	*0.482*	*0.07*	*0.925*
left ventricular diastolic dysfunction ¥	*0.004*	*0.951*	*0.082*	*0.252*
left ventricular mass	**0.160**	*0.022*	*0.045*	*0.521*
Left atrial diameter	**0.270**	*0.000*	**0.188**	*0.007*
Valvular lesion *	**0.329**	*0.000*	**0.185**	*0.008*

¶ Male = 1; ± Left ventricular function at visual score < good = 1; ¥ Left ventricular diastolic function relaxation abnormalities, pseudonormalization or restrictive pattern = 1; * Moderate to severe valvular stenosis or regurgitation = 1.

DISCUSSION

In the present study we assessed the associations between natriuretic plasma levels and echocardiographic abnormalities in an unselected group of geriatric outpatients. The plasma level of BNP but not of ANP progressively increased with the number of echocardiographic abnormalities Patients with high BNP levels had the highest risk for valvular dysfunction compared to the risk of other cardiac abnormalities.

Patient characteristics such as age, weight, kidney function and gender are known to be of influence on the natriuretic peptide plasma levels. In our study particularly age was an important determinant of natriuretic peptide levels, even in this group of elderly patients (mean age 77, range 65-89).

Clerico et al reported mean reference plasma values of ANP and BNP of 5.8 and 2.8 pmol/l in subjects with a mean age of 43 years.[11] In comparison to these values, mean plasma ANP (13.4 pmol/l) and BNP (18.1 pmol/l) in our geriatric outpatients were considerably higher. The combination of

higher age, higher prevalence of cardiac abnormalities and age-dependent decrease of glomerular filtration rate likely explains this difference in plasma natriuretic peptide values. In our patients without echocardiographic abnormalities, plasma ANP and BNP were respectively 13.0 and 8.5 pmol/l. These values are still higher than those reported by Clerico et al, stressing the importance of consideration of the patient's age (and renal function) in interpretation of plasma natriuretic peptide concentrations.

For all echocardiographic parameters evaluated the correlation coefficients were stronger for BNP than ANP, suggesting that measurement of plasma ANP in conjunct with BNP in this category of patients has no additional value. This agrees well with studies showing that under pathological conditions BNP rather than ANP is synthesized and released from cardiac myocytes.[29] Left atrial dimension in our population was more strongly correlated with plasma BNP than ANP. This is probably so as left atrial enlargement is associated with left ventricular remodeling and BNP is a maker of left ventricular disease.[30]

A remarkable finding was the relatively strong association between moderate to severe valvular lesions with plasma BNP. In our cohort, BNP was more closely related to these valvular lesions than to left ventricular systolic dysfunction. Unexpectedly, and contrary to previous reports, ejection fraction and plasma BNP did not correlate.[29] A possible explanation for this finding is that calculation of the ejection fraction was based on 2 dimension-guided M-mode measurements of left ventricular end-diastolic and end-systolic internal dimensions. These measurements are less accurate than visual scoring of left ventricular dysfunction.[31, 32] Indeed plasma BNP in our study correlated with left ventricular dysfunction as assessed by visual scoring.

Left ventricular diastolic dysfunction is a strongly age-dependent cardiac abnormality.[33] In our cohort four out of five patients had relaxation abnormalities. This high prevalence of diastolic dysfunction likely explains why identification of patients with diastolic dysfunction by measurement of plasma BNP was not possible. It might be argued that the echocardiographic definition of relaxation abnormalities as used in the present study is not specific enough for a geriatric population. Other parameters (such as tissue Doppler imaging of the mitral annular motion) should probably be taken into account to characterize diastolic function.[34] In a study in which the diagnosis of diastolic dysfunction was based on standard echocardiographic criteria as used in our study it was not possible to establish a relation with NT-proBNP,

whereas it was in another study in which left ventricular was assessed by tissue Doppler.[34, 35]

A potential limitation of our study could be generalizibility of our finding, as subjects were referred by their general practitioner to the outpatient clinic and the Diagnostic Day Center of the section of Geriatric Medicine. Probably the characteristics of our cohort differ from the general population and cardiac abnormalities could be more prevalent in our cohort. Nevertheless, the total percentages of patients with systolic or diastolic dysfunction in our study correspond with the prevalences in the general population older than 75 years of age, although severity of cardiac dysfunction seems less in our population.[4] Therefore cardiac disease was probably not more prevalent in our cohort than in the middle aged to elderly population. A potential second limitation is the scoring of left ventricular function by visual assessment (eyeballing), which results in an ordinal scale. However, assessment of left ventricular ejection fraction by visual scoring is accurate and correlates well with quantitative methods.[31, 32]

CONCLUSION

In comparison to ANP, BNP correlates closer with echocardiographic abnormalities in geriatric outpatients. Even in this elderly population age remains an important determinant of ANP and BNP plasma levels. As compared to plasma BNP, plasma ANP has no additional value for the detection of echocardiographic abnormalities including left atrial enlargement. Because of the close relationship between plasma BNP and the number of echocardiographic abnormalities, we expect that measurement of this marker is helpful for diagnostic decision making if after careful history taking and physical examination still uncertainty remains regarding presence of heart disease.

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5

Arterial stiffness as determinant of increased amino-terminal pro-B-type natriuretic peptide levels in individuals with and without cardiovascular disease - the Rotterdam study

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ABSTRACT

Background Large artery stiffening has adverse effects on cardiac structure and function and, therefore, may be associated with elevated circulating levels of NT-proBNP.

Methods In a large community-dwelling older population (n = 6211, mean age 69.2 years) serum NT-proBNP, brachial pulse pressure and carotid-femoral pulse wave velocity were measured. Linear regression analyses were adjusted for age, weight, height, mean arterial pressure, heart rate, smoking, diabetes, estimated glomerular filtration rate, total and high density lipoprotein cholesterol and use of lipid lowering and antihypertensive medication.

Results In individuals without cardiovascular disease, median NT-proBNP was 6.7 pmol/l in men (n = 2073) and 10.1 pmol/l in women (n = 3085) ($p < 0.001$). In these individuals, indices of arterial stiffness correlated with NT-proBNP with beta coefficients for brachial pulse pressure and carotid-femoral pulse wave velocity of 0.315 and 0.255 in men and 0.233 and 0.232 in women (all $p < 0.001$). After multivariable adjustment these associations remained significant for brachial pulse pressure and carotid-femoral pulse wave velocity in men and for brachial pulse pressure in women. In multivariable adjusted models, brachial pulse pressure explained 20.3% and carotid-femoral pulse wave velocity 10.7% of the variation of NT-proBNP in men and respectively 10.8% and 9.4% in women. In patients with prevalent cardiovascular disease indices of arterial stiffness and NT-proBNP were unrelated in multivariable adjusted models.

Conclusions Our findings show that arterial stiffness is independently associated with elevated NT-proBNP levels in subjects without prevalent cardiovascular disease. The association between vascular stiffness and NT-proBNP is stronger in men than in women and absent in individuals with prevalent cardiovascular disease.

INTRODUCTION

Observational studies have shown that arterial stiffening is an independent predictor of coronary heart disease, heart failure, atrial fibrillation and stroke, both in the general population and in populations with hypertension, diabetes mellitus or renal disease.[1-6] When the aorta and large elastic arteries become stiffer, their cushioning function decreases resulting in adverse hemodynamic effects, including an increase in systolic blood pressure and pulse pressure with increased systolic cardiac load and decreased myocardial perfusion pressure.[7, 8]

NT-proBNP is produced and secreted by cardiomyocytes.[9] Increased left ventricular wall stretch and myocardial ischemia are both important stimuli for the release of these peptides.[9] In the general population elevated NT-proBNP and BNP plasma levels have been associated with an increased risk for heart failure as well as coronary heart disease and stroke and in clinical medicine NT-proBNP and its C-terminal counterpart BNP are established biomarkers for the diagnosis and management of heart failure.[10-12] Because of the adverse effects of arterial stiffening on cardiac load and coronary perfusion we hypothesized that arterial stiffness is a determinant of circulating levels of NT-proBNP.

In the Framingham population a positive association was found between BNP and carotid-femoral pulse wave velocity in men, but remarkably an inverse association was observed in women.[13] This inverse association is difficult to explain. It might be related to the fact that measurement of BNP and carotid-femoral pulse wave velocity were not performed at the same examination cycle and that circulating BNP in stead of NT-proBNP levels were measured. Evidence indicates that NT-proBNP measurements have an advantage over BNP in the detection of mild or asymptomatic structural heart disease not yet detectable by echocardiography.[14, 15] An explanation for this advantage could be the longer half-life of NT-proBNP, resulting in more stable circulating levels.[16] In light of the potential differences between BNP and NT-proBNP as marker of subclinical cardiac disorders our study was aimed to obtain insight in the associations between indices of arterial stiffness and NT-proBNP levels in a large population-based cohort. In our studies we especially focused on sex-specific effects as well as on the influence of prevalent cardiovascular disease on the associations between arterial stiffness and NT-proBNP levels.

METHODS

Study Population

The Rotterdam Study is a prospective cohort study of subjects, aged 55 years or older, living in Rotterdam, the Netherlands. Its aim is to investigate the incidence of and risk factors for chronic disabling diseases.[17] Of the 7983 participants recruited in 1990-1993, blood was sampled in 3930 participants at the third examination cycle during 1997-1999. Furthermore we included 2568 out of 3011 participants, additionally recruited and examined in 2000-2001, fulfilling the same inclusion criteria as the original cohort. Of the total cohort (n = 6498), 5158 subjects had no history of coronary heart disease, heart failure, stroke and NT-proBNP levels below the age-specific cutoff for the diagnosis of heart failure as proposed by Januzzi et al (50-75 years, 108 pmol/l; older than 75 years, 216 pmol/l)[10] and 1053 subjects had a history of cardiovascular disease or high NT-proBNP levels. Of 287 subjects complete follow-up for cardiovascular disease was not available. The numbers of subjects for multivariate analysis were: subjects without cardiovascular disease (pulse pressure, n = 4826; carotid-femoral pulse wave velocity, n = 4340), subjects with cardiovascular disease (pulse pressure, n = 919; carotid-femoral pulse wave velocity, n = 809). A more detailed description of the Rotterdam Study and the collection of data can be found elsewhere.[3, 17-19] The Medical Ethics Committee of Erasmus Medical Center, Rotterdam, approved the study.

Cardiovascular risk factors

A trained interviewer using a computerized questionnaire collected information on current health status, medical history, drugs use and smoking behavior from the participants of the original cohort (n = 3930) at the third examination round (1997-1999). From the participants added to the cohort (n = 2568), information was collected at their first examination round (2000-2001). In addition to the interview the cardiovascular risk factors and measures of arterial stiffness were assessed during 2 visits at the research center. Serum glucose, serum total cholesterol and high-density lipoprotein cholesterol were measured using standard laboratory techniques. Diabetes mellitus was considered to be present when fasting blood glucose exceeded 7.0 mmol/l, non-fasting glucose exceeded 11.0 mmol/l and/or anti-diabetic medication was used or all. Creatinine clearance was estimated according to the simplified Modification of Diet in Renal Disease formula.[20]

NT-proBNP Measurement

Blood samples for NT-proBNP measurement were collected in glass tubes containing clot activator and gel for serum separation. After blood collection, the samples were left to clot for 30 minutes and then centrifuged for 20 minutes at 3000 rotations per minute at 4º C. Subsequently, the serum was stored at −80°C. All tubes were stored on ice before and after blood sampling. NT-proBNP was measured using a commercially available electrochemiluminescence immunoassay (Elecsys proBNP, F. Hoffman-La Roche Ltd., Basel, Switzerland) on an Elecsys 2010 analyzer. The precision, analytical sensitivity, and stability characteristics of the system have previously been described.[21]

Measures of Arterial Stiffness

a. Brachial pulse pressure

Systolic (first Korotkoff phase) and diastolic (fifth Korotkoff phase) blood pressures were measured twice on the right arm with a random-zero sphygmomanometer, after the participant had been seated for at least 5 minutes. The mean of the 2 blood pressure values was used in the analyses. Brachial pulse pressure was defined as the difference between systolic and diastolic blood pressure.

b. Carotid-femoral pulse wave velocity

Carotid-femoral pulse wave velocity, a measure of aortic stiffness, was calculated with the subjects in supine position. Carotid-femoral pulse wave velocity was assessed with an automatic device (Complior; Artech Medical, Pantin, France) that measures the time delay between the rapid upstroke of the feet of simultaneously recorded pulse waves in the carotid and the femoral arteries.[22] The distance between the recording sites in the carotid and the femoral arteries (the carotid artery and the groin) was measured. Carotid-femoral pulse wave velocity was calculated as the ratio between the distance and the foot-to-foot time delay and expressed in meters per second. In a reproducibility study the intra-class correlation coefficient was 0.80.[23]

Statistical analysis

Parametric data are presented as mean value ± standard deviation. Comparisons of baseline characteristics between both sexes were made using

Student's *t* test for normally distributed variables and χ^2 tests for categorical variables. The distribution of NT-proBNP values was positively skewed, therefore, calculations were performed on logarithmically transformed data and NT-proBNP values are presented as median with interquartile range. The associations between log NT-proBNP and measures of arterial stiffness and covariates were investigated using linear regression models. To investigate whether NT-proBNP was independently related to arterial stiffness, the models were adjusted for age, weight, height, mean arterial pressure, heart rate, smoking, diabetes, estimated glomerular filtration rate, total and high density lipoprotein cholesterol, use of lipid lowering and antihypertensive medication. To adjust for a possible cohort effect between the subjects originally included in 1996-1999 and the subjects additionally recruited in 2000-2001 an indicator variable was used in all adjusted models. The strength of the associations is indicated by standardized beta-coefficients and corresponding 95% confidence intervals. The relative contribution of the covariates to the totally explained variation was calculated by averaging sequential sums of squares over orderings of regressors.[24] We performed separate analyses for men and for women with or without a history of cardiovascular disease. For subjects without a history of cardiovascular disease and low NT-proBNP levels data was missing for the following covariates cholesterol 0.5%, diabetes mellitus 1.0%, high-density lipoprotein cholesterol 1.3%, height 0.9%, medication use 3.9%, smoking 0.9%, weight 0.9%. For the subjects with a history of cardiovascular disease or high NT-proBNP levels data was missing for the following covariates: cholesterol 2.8%, diabetes mellitus 2.4%, high-density lipoprotein cholesterol 4.0%, height 3.7%, medication use 5.7%, smoking 2.4%, weight 3.5%. A *p* value less than 0.05 was considered to indicate statistical significance. Statistical analyses were performed using SPSS (version 16.0, SPSS Inc, Chicago, Illinois, USA) and R 2.7.2 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

The baseline characteristics for individuals with or without a history of cardiovascular disease are given in Table 1. As anticipated, patients with a history of cardiovascular disease were older and had a higher pulse pressure, carotid-femoral pulse wave velocity and NT-proBNP levels than subjects

without cardiovascular disease. In both groups brachial pulse pressure was lower in men than in women, whereas carotid-femoral pulse wave velocity was slightly and NT-proBNP markedly higher in women than in men (Table 1).

Table 1. Baseline characteristics of the subjects without or with a history of cardiovascular disease

	Subjects without cardiovascular disease		**Subjects with cardiovascular disease**		**Difference***	
	Men	*Women*	*Men*	*Women*	*Men*	*Women*
Characteristics					*p*	*p*
N	*2073*	*3085*	*625*	*428*	-	-
Age, years	*67.4 (7.6)*	*68.8 (8.3)*	*72.6 (7.8)*	*75.8 (8.7)*	*< 0.001*	*< 0.001*
Mean blood pressure, mm Hg	*101 (13)*	*98 (13)*	*98 (13)*	*98 (14)*	*< 0.001*	*0.925*
Total cholesterol, mmol/l	*5.6 (0.9)*	*6.0 (0.9)*	*5.3 (1.0)*	*5.8 (1.1)*	*< 0.001*	*< 0.001*
HDL-C, mmol/l	*1.2 (0.3)*	*1.5 (0.4)*	*1.2 (0.3)*	*1.4 (0.4)*	*< 0.001*	*< 0.001*
Diabetes mellitus, %	*10.3*	*8.5*	*18.2*	*15.1*	*< 0.001*	*< 0.001*
Current smokers, %	*18.5*	*17.1*	*17.8*	*12.9*	*0.385*	*0.016*
Use of antihypertensives, %	*19.2*	*24.3*	*32.0*	*36.8*	*< 0.001*	*< 0.001*
Use of lipid-lowering agents, %	*8.5*	*11.1*	*33.3*	*23.1*	*< 0.001*	*< 0.001*
Body mass index, kg/m²	*26.6 (3.2)*	*27.3 (4.4)*	*26.6 (3.3)*	*27.7 (4.4)*	*0.832*	*0.053*
eGFR, ml/kg/1,73m²	*83.1 (16.6)*	*79.3 (15.7)*	*74.8 (19.7)*	*72.0 (18.2)*	*< 0.001*	*< 0.001*
Brachial PP, mm Hg	*65 (17)*	*66 (18)*	*67 (17)*	*73 (19)*	*0.001*	*< 0.001*
cfPWV, m/s†	*13.4 (3.1)*	*12.5 (2.9)*	*14.5 (3.3)*	*14.2 (3.3)*	*< 0.001*	*< 0.001*
NT-proBNP, median (IQR), pmol/l	*6.7 (3.7 – 13.5)*	*10.1 (5.8 – 17.9)*	*23.0 (10.6 – 71.7)*	*23.2 (12.4 – 62.9)*	*< 0.001*	*< 0.001*

* *P* values for difference between subjects without and with cardiovascular disease, separately for men and women. † Carotid-femoral pulse wave velocity was measured in 1768 men and 2530 women. Abbreviations: HDL-C, high density lipoprotein cholesterol; eGFR, estimated glomerular filtration rate; PP, pulse pressure; cfPWV, carotid-femoral pulse wave velocity; NT-proBNP, amino-terminal pro-B-type natriuretic peptide; IQR, interquartile range.

NT-proBNP levels correlated with brachial pulse pressure and carotid-femoral pulse wave velocity levels in subjects without cardiovascular disease (Table 2, Figure 1). In patients with cardiovascular disease associations

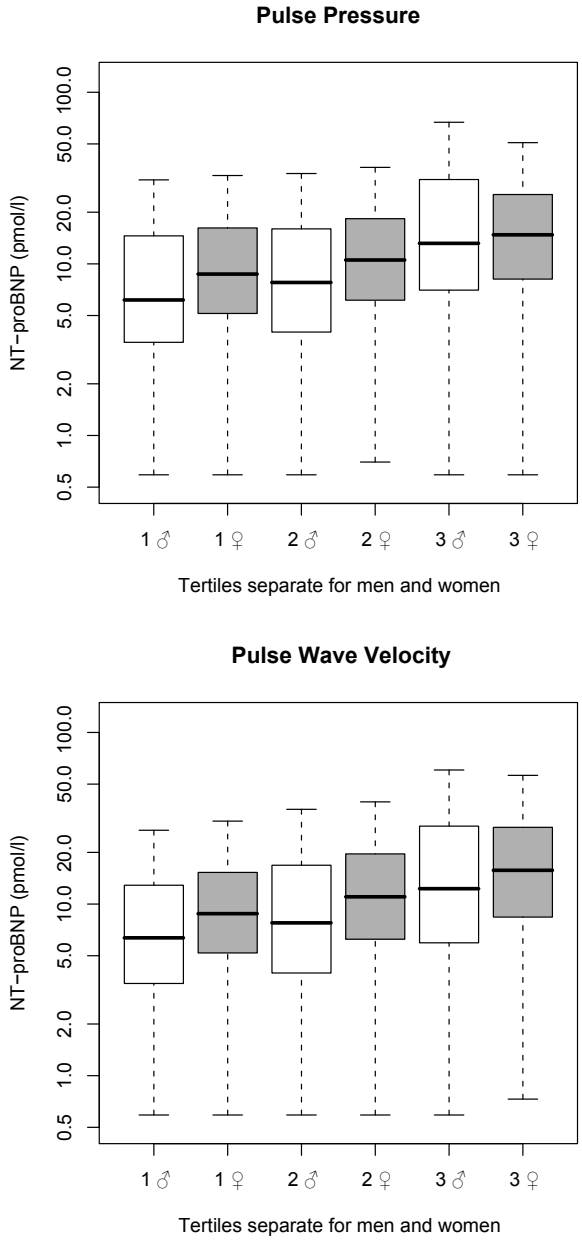

Figure 1. Box plots showing an increase in serum NT-proBNP levels with increasing tertiles of pulse pressure (left panel) and pulse wave velocity (right panel) in men (white bars) and women (gray bars), p for trend < 0.001 (both figures).

between NT-proBNP and brachial pulse pressure or carotid-femoral pulse wave velocity were considerably weaker and only significant for carotid-femoral pulse wave velocity. In individuals without cardiovascular disease,

NT-proBNP remained associated with brachial pulse pressure in men and in women, and with pulse wave velocity in men in the multivariable adjusted model (Table 2). In individuals without cardiovascular disease (mean age 67 years) an increase in brachial pulse pressure from 70 to 80 mmHg associated with an increase in NT-proBNP levels from 9.5 to 10.6 pmol/l in men (n = 1943) and from 15.2 to 15.8 pmol/l in women (n = 2835) in the fully adjusted model. In the individuals without cardiovascular disease an increase in carotid-femoral pulse wave velocity from 10 m/s to 20 m/s resulted in an increase in NT-proBNP from 8.1 to 9.7 pmol/l in men (n = 1769), while no increase was seen in women.

Table 2. Univariate and multivariate adjusted standardized beta-coefficients for the association between measures of vascular stiffness and NT-proBNP

		Univariate standardized β coefficient			***Multivariate standardized β coefficient****		
		β	*95% CI*	*p*	*β*	*95% CI*	*p*
Pulse Pressure							
Subjects without cardiovascular disease§	*Men*	*0.315*	*0.274, 0.356*	*< 0.001*	*0.172*	*0.123, 0.221*	*< 0.001*
	Women	*0.233*	*0.198, 0.267*	*< 0.001*	*0.056*	*0.014, 0.097*	*0.009*
Subjects with cardiovascular disease§	*Men*	*0.073*	*-0.005, 0.152*	*0.067*	*0.034*	*-0.051, 0.118*	*0.438*
	Women	*0.050*	*-0.046, 0.146*	*0.306*	*-0.072*	*-0.185, 0.041*	*0.208*
Carotid-Femoral Pulse Wave Velocity							
Subjects without cardiovascular disease§	*Men*	*0.255*	*0.211, 0.299*	*< 0.001*	*0.060*	*0.012, 0.108*	*0.015*
	Women	*0.232*	*0.196, 0.268*	*< 0.001*	*0.010*	*-0.036, 0.057*	*0.658*
Subjects with cardiovascular disease§	*Men*	*0.149*	*0.065, 0.234*	*< 0.001*	*-0.071*	*-0.158, 0.016*	*0.111*
	Women	*0.163*	*0.062, 0.264*	*0.002*	*-0.058*	*-0.168, 0.052*	*0.299*

* Adjusted for age, weight, height, mean arterial pressure, heart rate, smoking, diabetes, estimated glomerular filtration rate, total and high density lipoprotein cholesterol, use of lipid lowering and antihypertensive medication, cohort effect. § Cardiovascular disease was defined as a history of coronary heart disease, stroke, heart failure, including a NT-proBNP above the cutoff level for heart failure.

The unadjusted and multivariable adjusted associations of cardiovascular risk factors, including height and weight and estimated glomerular filtration rate, with NT-proBNP are provided in Table 3 for men and in Table 4 for women. In men without cardiovascular disease the fully adjusted model explained 28.2% of the variation in NT-proBNP. Of the explained variation, age was the most important covariate (49.6%) followed by brachial pulse pressure (20.3%) and estimated glomerular filtration rate (6.5%), the other covariates explained each less than 3%. The model using carotid-femoral pulse wave velocity instead of brachial pulse pressure as covariate explained 27.0% of the variation in NT-proBNP, with carotid-femoral pulse wave velocity being the second most influential covariate (12.1%) after age (54.5%). In women without cardiovascular disease, the full model explained 23.7% of the variability in NT-proBNP, of which 56.2% by age, 10.8% by brachial pulse pressure and 6.9% by estimated glomerular filtration rate. Comparable findings were observed for carotid-femoral pulse wave velocity (Table 4).

Table 3. Explained variation of NT-proBNP in men without a history of cardiovascular disease, top five covariates of a model including brachial pulse pressure (left) and a model including carotid-femoral pulse wave velocity (right)

Model including brachial PP		***Model including cfPWV***	
n = 1943		***n = 1769***	
R^2 full model: 28.2%		***R^2 full model: 26.9%***	
Parameter	*% of R^2*	*Parameter*	*% of R^2*
Age	*49.6*	*Age*	*56.3*
Brachial PP	*20.3*	*cfPWV*	*10.8*
eGFR	*6.5*	*eGFR*	*6.0*
Total cholesterol	*2.6*	*Mean arterial pressure*	*3.4*
Antihypertensives	*2.1*	*Antihypertensives*	*2.5*
Other covariates	*< 2.0*	*Other covariates*	*< 2.0*

Abbreviations as in Table 1. Covariates for each model: age, weight, height, mean arterial pressure, heart rate, smoking, diabetes, estimated glomerular filtration rate, total and high density lipoprotein cholesterol, use of lipid lowering and antihypertensive medication, cohort effect.

Table 4. Explained variation of NT-proBNP in women without a history of cardiovascular disease, top five covariates of a model including brachial pulse pressure (left) and a model including carotid-femoral pulse wave velocity (right)

Model including brachial PP		***Model including cfPWV***	
n = 2835		***n = 2530***	
R2 full model:23.7%		***R2 full model: 23.5%***	
Parameter	*% of R^2*	*Parameter*	*% of R^2*
Age	*56.2*	*Age*	*53.1*
Brachial PP	*10.8*	*cfPWV*	*9.4*
eGFR	*6.9*	*eGFR*	*7.1*
Antihypertensives	*4.7*	*Heart rate*	*5.3*
Heart rate	*4.4*	*Antihypertensives*	*5.0*
Other covariates	*< 3.0*	*Other covariates*	*< 5.0*

Abbreviations as in Table 1. Covariates for each model: age, weight, height, mean arterial pressure, heart rate, smoking, diabetes, estimated glomerular filtration rate, total and high density lipoprotein cholesterol, use of lipid lowering and antihypertensive medication, cohort effect.

DISCUSSION

The main findings of this community-based population study are that serum NT-proBNP levels are independently associated with carotid-femoral pulse wave velocity and brachial pulse pressure, that the associations are stronger in men than in women and that the associations are stronger in individuals without than in patients with a history of cardiovascular disease. In patients with a history of cardiovascular disease, associations between carotid-femoral pulse wave velocity or brachial pulse pressure and NT-proBNP were no longer significant after multivariable adjustment. Our analysis further revealed that brachial pulse pressure after age is the strongest determinant of NT-proBNP levels in subjects without a history of cardiovascular disease.

Studies investigating the association between natriuretic peptide levels and measures of arterial stiffness are scarce. In a sample of the Framingham cohort (mean age 61 years) the associations between plasma BNP levels and carotid-femoral pulse wave velocity or carotid pulse pressure were positive in men with correlation coefficients in multivariable-adjusted models of 0.115 and 0.129.[13] In contrast, in women no association between plasma BNP and

carotid pulse pressure was present, whereas, unexpectedly, the association between BNP and carotid-femoral pulse wave velocity was negative.[13]

Recently, in the same Framingham cohort sample, the associations between five vascular function measures and seven different biomarkers, including BNP, amino-terminal pro-atrial natriuretic peptide (NT-proANP), aldosterone, renin, aldosterone-renin ratio, C-reactive protein, plasminogen activator inhibitor, fibrinogen and homocysteine were reported.[25] In this analysis, which was not performed separately for men and women, a positive association between carotid pulse pressure and BNP, but not between carotid-femoral pulse wave velocity and BNP, persisted after adjustment for the mentioned biomarkers and cardiovascular risk factors.

The association between pulse wave velocity and BNP has also been assessed in healthy Japanese men.[26] In this study brachial-ankle pulse wave velocity was measured using a plethysmographic method. Although carotid-femoral pulse wave velocity and brachial-ankle pulse wave velocity were correlated, the reported squared correlation coefficient between these two measurements was not higher than 0.60, making comparison with the findings of the present study and of those obtained in the Framingham population difficult.[27]

In the aforementioned studies BNP was measured, whereas NT-proBNP was measured in our study. Compared to BNP the half-life of NT-proBNP is considerably longer (120 versus 20 minutes), in addition NT-proBNP is much more stable *in vitro* at room temperature.[16] Due to these properties NT-proBNP may provide a better estimate of chronic cardiovascular load than BNP.[14, 15] This may explain why contrary to the findings in the Framingham cohort a positive association between brachial pulse pressure and NT-proBNP was found in women in our study.

The strength of the association between measures of arterial stiffness and NT-proBNP levels became considerably weaker after multivariable adjustment. Evaluation of the individual components of the multivariable-adjusted model showed that age was by far the most important covariate (Tables 3 and 4), which accords well with the knowledge that NT-proBNP, pulse pressure and carotid-femoral pulse wave velocity are all closely related to age.[8, 28, 29] Our subjects aged 67 years as compared to 61 years in the Framingham cohort. The correlation between carotid-femoral pulse wave velocity and BNP found in the Framingham cohort was larger than the correlation between carotid-femoral pulse wave velocity and NT-proBNP in the Rotterdam cohort,

which suggests that the influence of vascular stiffness on natriuretic peptides is greater in younger subjects. Since no subjects below 55 years of age were included in the Rotterdam study our results can only be extrapolated to middle-aged and elderly individuals.

Impaired renal function, the level of blood pressure, diabetes, cardiovascular disease and heart failure may affect the association between NT-proBNP levels and arterial stiffness because of their concordant effects on both parameters.[3, 4, 9-12, 29-31] To account for these confounding effects, adjustments were made for the mentioned variables and separate analyses were performed for subjects with and without a history of cardiovascular disease. In subjects with prevalent cardiovascular disease, NT-proBNP levels were markedly and carotid-femoral pulse wave velocity and pulse pressure slightly higher than in subjects without cardiovascular disease, whereas the associations between NT-ProBNP and pulse pressure or carotid-femoral pulse wave velocity were considerably weaker and no longer existent after multivariable adjustment. This observation indicates that in subjects with prevalent cardiovascular disease the rise in NT-proBNP is dissociated from the rise in pulse pressure or carotid-femoral pulse wave velocity. This may especially be the case in subjects with heart failure, where the association between NT-proBNP and pulse pressure can become inverse due to the opposing effects of heart failure on these two parameters.[32]

Compared with men, the association between NT-proBNP and carotid-femoral pulse wave velocity was less pronounced and no longer significant after multivariable adjustment in women. As reported in previous studies NT-proBNP levels were almost 50% higher in women than in men.[33] Because the variation in NT-proBNP levels in men and women were comparable, we don't think that these higher NT-proBNP levels *per se* accounted for this weaker association in women. It is possible that the effect of vascular stiffness on NT-proBNP release is less in women than in men. This accords with previous observations, showing that for the same level of left ventricular dysfunction or left ventricular hypertrophy the rise in natriuretic peptides is less in women than in men.[34] Our findings are in line with these observations, supporting the presence of gender-specific differences in NT-proBNP responses to ventricular stress.

The observation that in multivariable adjusted models, changes in brachial pulse pressure or carotid-femoral pulse wave velocity only explained a small proportion of the variation in NT-proBNP seems at variance with the

idea that increased vascular stiffness has adverse effects on cardiac function and structure.[13, 25, 26] It has been demonstrated that infusion of atrial natriuretic peptide, which acts on the same receptor as BNP, results in a decline in venous return due to intravascular volume contraction and venoconstriction thereby reducing cardiac preload and hence circulating NT-proBNP levels.[35, 36] These beneficial effects of natriuretic peptides on cardiac preload and large artery function potentially have weakened the strength of the associations between indices of large artery stiffness and circulating NT-proBNP levels. Furthermore the fully adjusted models only explained 24% to 28% of the variation in NT-proBNP. These findings indicates that other, yet undetermined factors are important as well.

The strength of our study is that data on circulating NT-proBNP levels and indices of large arterial stiffness were collected in an extensive cohort of well-characterized subjects, making reliable analyses possible for men and for women and for subjects with and without a history of cardiovascular disease. Nevertheless, our study has limitations. First, the cross-sectional nature of our study does not allow to draw definite conclusions about a possible causal relation between arterial stiffness and NT-proBNP levels. This can only be established if follow-up of both parameters is available. Second, most of the subjects included in the Rotterdam study are white and of older age. Our findings can therefore not be extrapolated to other ethnic groups or to a younger population. Third, brachial pulse pressure is only a surrogate measure of aortic pulse pressure; the possibility of a different outcome if aortic pulse pressure as a more appropriate marker of ventricular wall stress had been used, can therefore not be excluded. As peripheral amplification of blood pressure decreases with age, the difference between peripheral and central blood pressure in our older population was probably not very large; nevertheless the intra-individual difference could still be substantial.

CONCLUSION

Our findings are compatible with the hypothesis that arterial stiffening is a determinant of circulating NT-proBNP levels in older subjects free of cardiovascular disease. This effect appears to be gender-specific as it was less pronounced in women than in men. Our findings also demonstrate that the association between arterial stiffness and NT-proBNP is confounded by

several factors of which age is most important. In this regard it would be worthwhile to investigate whether the association between arterial stiffness and NT-proBNP is stronger in a younger population. Finally, our data were obtained under resting conditions. It is possible that the adverse effects of large artery stiffening on the heart and hence on NT-proBNP levels are more evident during exercise and studies exploring this possibility should be performed.

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6

B-type natriuretic peptide and amino-terminal pro-atrial natriuretic peptide predict survival in peritoneal dialysis

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ABSTRACT

Background Different subtypes of the natriuretic peptide family have emerged as diagnostic markers for heart failure and have been identified as predictors of mortality. In this study we determined the predictive power of NT-proANP and BNP for mortality in peritoneal dialysis.

Methods From the Netherlands Co-operative Study Adequacy of Dialysis (NECOSAD) cohort, a large, prospective multi-center study (n = 1464), we selected a random sample of 68 peritoneal dialysis patients without clinically overt heart failure. Patients were included at the time of initiation of dialysis, and followed up at regular intervals. Patient characteristics were recorded at baseline. Six months after the start of dialysis blood was collected for measurement of NT-proANP and BNP.

Results Patients were followed for maximal follow-up of 4.5 years, mortality rate 15%. Median NT-proANP and median BNP levels were 1112 pmol/l and 7.5 pmol/l. An NT-proANP and a BNP level above the median was associated with an increased mortality risk (NT-proANP: hazard ratio 11.3 [95% CI 1.4 to 91.9]; BNP: hazard ratio 11.3 [95% CI 1.4 to 91.4]). This association remained significant for BNP after adjustment for age, co-morbidity and residual glomerular filtration rate (hazard ratio 8.5 [95% CI 1.0 to 73.8]).

Conclusion BNP is a predictor of survival in peritoneal dialysis patients even after adjustment for age, co-morbidity and residual glomerular filtration.

INTRODUCTION

Mortality in patients with end stage renal disease is largely determined by cardiovascular disease.[1, 2] Congestive heart failure is especially highly prevalent in the hemodialysis population. In comparison with the general population, congestive heart failure is 12 - 36 times more common in patients with end stage renal disease.[2] After a diagnosis of heart failure the probability of survival is diminished by almost 50%.[3] Recently, different subtypes of natriuretic peptides have been introduced as biochemical markers for the clinical diagnosis of heart failure and as prognostic markers for mortality.[4-6] Two biologically active forms of natriuretic peptides produced by the myocardium can be distinguished: ANP and BNP. BNP is predominantly secreted by ventricular cardiomyocytes. NT-proANP is mainly formed by atrial cardiomyocytes after splicing of ANP, the biologically active substance, from pro-ANP.[7] Compared to ANP, NT-proANP has a longer half-live and consequently its plasma concentration is several times higher than the concentration of ANP.

Plasma concentrations of natriuretic peptides are frequently increased to extremely high levels in patients with end stage renal disease, which may be caused by the combined effects of different factors, such as volume overload, concomitant cardiac disease and decreased renal clearance.[8] Importantly, raised levels of BNP in patients with end stage renal disease, who require renal replacement therapy with hemodialysis, have been shown to be associated with increased mortality.[5, 6] Probably, natriuretic peptides are also predictive for mortality in patients on peritoneal dialysis; however, this has not been reported before. In the present study we explored the prognostic value of BNP and NT-proANP for overall mortality in patients on peritoneal dialysis.

METHODS

The Netherlands Cooperative Study on the Adequacy of Dialysis (NECOSAD) is a multi-center, prospective, cohort study in which end stage renal disease patients are consecutively included at the time of initiation of dialysis and followed at 6-month intervals until transplantation, end of follow-up or death. Eligibility criteria are age of 18 years or older, and dialysis as first renal replacement therapy. All patients gave their informed consent before

inclusion. The local medical ethics committees of the different centers approved the study.The inclusion period started January 1, 1997. Until January 1, 2001 1464 patients were included, of which 527 patients (36%) started peritoneal dialysis. The dialysis modality of a patient was defined as the dialysis modality at the follow-up visit six months after inclusion. We took a random sample of 75 peritoneal dialysis patients; 7 patients were excluded because of clinically overt heart failure at baseline, leaving 68 peritoneal dialysis patients. The nephrologists of each study center decided whether heart failure was present or not based upon review of patient files, clinical history and physical examination. All patients were followed until the 1st of July 2002. If no event (death or transplantation) occurred, patients had a minimal follow-up of 1.5 years. At the start of dialysis data were collected consisting of demographics, etiology of renal disease and co-morbidity. Body mass index, blood pressure and renal function were assessed six months after the start of dialysis in combination with blood sampling for natriuretic peptides and other laboratory investigations.

Blood for NT-proANP and BNP was sampled from an antecubital vein into a polystyrene tube containing heparin or ethylenediaminetetraacetic acid . Samples were stored at a temperature of at least -20° C. We measured plasma NT-proANP levels and plasma BNP levels by two commercially available kits, namely NT-proANP radioimmunoassay (Biotop OY, Oulu, Finland) and Shionoria-BNP (Shionoria, Osaka, Japan) respectively. Coefficients of variation of between-assay and within-assay measurements were 11.2% and 5.3% for NT-proANP and 2.3% and 2.2% for BNP, respectively.

Data are expressed as mean and standard deviation or as median and range. To assess the risk for all-cause mortality the group was dichotomized based upon median NT-proANP or BNP levels. Survival was assessed by Kaplan-Meier analysis. The difference between survival rates was investigated by using log-rank tests. Unadjusted hazard ratios were calculated with Cox proportional hazards analysis. Subsequently, the hazard ratios were adjusted for age, comorbidity, and residual glomerular filtration rate. Patients were censored at transplantation or at the end of the follow-up period. All statistical analyses were performed using SAS version 9 (SAS Institute, Cary, North Carolina, USA).

RESULTS

Table 1 shows the main demographic and clinical characteristics of all patients included in the study. By the end of follow up 10 out of 68 (15%) patients had died. The median NT-proANP level was significantly higher

Table 1. Characteristics of patients on peritoneal dialysis at six months after start of dialysis treatment

Characteristics	
N	*68*
Age (years)	*49.9 (14.4)*
Gender (% male)	*59*
Primary kidney disease (%)	
Diabetes mellitus	*18*
Glomerulonephritis	*21*
Renal Vascular Disease	*4*
Other	*57*
Comorbidity (%)	
Acute coronary syndromes	*7*
Diabetes Mellitus	*19*
Stroke	*1*
Pulmonary disease	*3*
Cancer	*4*
GFR (ml/min/1.73m2)	*3.8 (3.9)*
BMI (kg/m2)	*24.7 (3.5)*
Serum albumin (g/dl)	*3.5 (0.5)*
Systolic BP (mmHg)	*138 (20)*
NT-proANP (pmol/l) (median, range)	*1112 (15 – 5523)*
BNP (pmol/l) (median, range)	*7.5 (1 – 116)*

Unless stated otherwise, mean values and standard deviations are given for continuous variables. Abbreviations: GFR, glomerular filtration rate; BMI, body mass index; BP, blood pressure; NT-proANP, amino-terminal pro-atrial natriuretic peptide; BNP, B-type natriuretic peptide.

than BNP level, 1112 pmol/l versus 7.5 pmol/l. There were no statistically significant differences in baseline characteristics between the peritoneal dialysis sample and the remainder of the peritoneal dialysis population without congestive heart failure of the NECOSAD (n = 488) cohort (data not shown). The maximal follow-up was 4.5 years. As shown in Figure 1, peritoneal dialysis patients with NP levels above the median had a significantly increased mortality compared to peritoneal dialysis patients with NP levels below the median. (log rank $test_{median\ NT\text{-}proANP}$, $p < 0.01$, log rank $test_{median\ BNP}$, $p < 0.01$). The distribution of the deceased patients over the groups above or below the median level was identical for NT-proANP and BNP, moreover the time of censoring of the survived patients over the both groups was largely similar, resulting in similar survival curves.

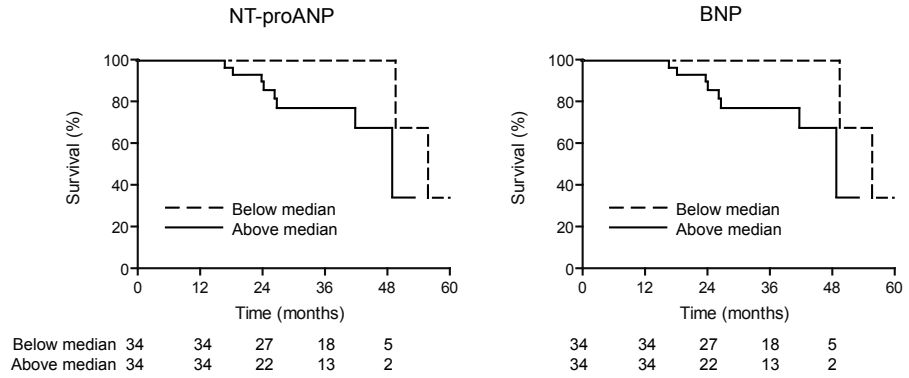

Figure 1. Survival curves of patients on peritoneal dialysis below and above median NT-proANP 1112 pmol/l (left panel) and median BNP 7.5 pmol/l (right panel).

Cox proportional hazard analysis for prediction of all-cause mortality in peritoneal dialysis patients resulted in a hazard ratio of 11.3 (95% CI 1.4 to 91.9) for patients with NT-proANP levels above median. Patients with a BNP level above median had a significantly increased mortality risk as well (hazard ratio 11.3 (95% CI 1.4 to 91.4). After adjustment for age, comorbidity and residual glomerular filtration rate, the hazard ratios were 7.9 (95% CI 0.9 to 72.1) and 8.5 (95% CI 1.0 to 73.8), respectively for NT-proANP and BNP (Table 2).

Table 2. Mortality hazard ratio's for peritoneal dialysis patients with NT-proANP or BNP above the median value

	Unadjusted HR	***95% CI***	***p***	***Adjusted HR***	***95% CI***	***p***
NT-proANP < median	*1.0*	*na*		*1.0*	*na*	
NT-proANP > median	*11.3*	*1.4 - 91.9*	*0.02*	*7.9**	*0.9 - 72.1*	*0.07*
BNP < median	*1.0*	*na*		*1.0*	*na*	
BNP > median	*11.3*	*1.4 - 91.4*	*0.02*	*8.5**	*1.0 - 73.8*	*0.05*

* Adjusted for age, comorbidity and residual GFR. Abbreviations: HR, hazard ratio; NT-proANP, amino-terminal pro-atrial natriuretic peptide; BNP, B-type natriuretic peptide; CI, confidence interval.

DISCUSSION

In the present study we assessed the prognostic value of NT-proANP and BNP in patients on peritoneal dialysis. An increased BNP level was associated with a significantly increased mortality risk, whereas for plasma NT-proANP levels a trend towards an increased mortality risk was found. Our results concerning the prognostic value of BNP obtained in peritoneal dialysis patients are in accordance with reports in other end stage renal disease populations. Zoccali et al found BNP and ANP to be the strongest independent predictors of outcome after age in 246 patients on renal replacement therapy.[9] Several other authors have confirmed these findings within the hemodialysis population establishing the prognostic role of natriuretic peptides for either all-cause mortality or cardiovascular events.[10, 11] Also, in patients with chronic renal failure, but not yet on renal replacement therapy, BNP and NT-proBNP have been shown to be prognostic markers for mortality.[12, 13] Moreover, it has been suggested that high levels of NT-proBNP reflect myocardial damage due to ischemia.[14]

Limitations of our study include the relative small sample size of both groups, although samples were randomly selected from a large group of patients collected in several centers. Also, because we aimed to determine the predictive value of NT-proANP and BNP as could be collected during clinical practice, the differences in blood sampling and handling techniques between different study centers may have introduced variation. Still, data collected in this manner, will represent daily clinical practice. Patients with heart failure were excluded from the analysis. The diagnosis of heart failure was based upon clinical information. This practice may have led to inclusion

of patients with subclinical heart failure. Finally, we used all-cause mortality to determine the predictive value of natriuretic peptides, whereas natriuretic peptides probably predict the risk for cardiovascular mortality better. The use of all-cause mortality will most likely underestimate the true predictive value of an increased NT-proANP and BNP on mortality.

CONCLUSION

BNP is a predictor of survival in peritoneal dialysis patients and NT-proANP tends to predict survival. While confirming the findings of other authors on the predictive potential of natriuretic peptides in the end stage renal disease population, this study points toward a predictive value of natriuretic peptides for mortality in the peritoneal dialysis population.

NECOSAD STUDY GROUP

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Amino-terminal pro-B-type natriuretic peptide improves cardiovascular and cerebrovascular risk prediction in the population - the Rotterdam study

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ABSTRACT

Background Increased circulating NT-proBNP levels are a marker of cardiac dysfunction but also associate with coronary heart disease and stroke. We aimed to investigate whether increased circulating NT-proBNP levels have additive prognostic value for first cardio- and cerebrovascular events beyond classical risk factors.

Methods In a community-based cohort of 5063 participants free of cardiovascular disease, aged 55 years or older, circulating NT-proBNP levels and cardiovascular risk factors were measured. Participants were followed for the occurrence of first major fatal or non-fatal cardiovascular event.

Results 420 Participants developed a first cardiovascular event (108 fatal). After adjustment for classical risk factors the hazard ratio for cardiovascular events was 2.32 (95% CI 1.55 to 2.70) in men, and 3.08 (1.91 to 3.74) in women for participants with NT-proBNP in the upper compared to the lowest tertile. Corresponding hazard ratio's with 95% confidence intervals for coronary heart disease, heart failure and ischemic stroke were 2.01 (1.14 to 2.59), 2.90 (1.33 to 4.34) and 2.06 (0.91 to 3.18) for men and 2.95 (1.30 to 4.55), 5.93 (2.04 to 11.2) and 2.07 (1.00 to 2.97) for women. Incorporation of NT-proBNP in the classical risk model significantly improved the C statistic both in men and women and resulted in a net reclassification improvement of 9.2% (95% CI 3.5% to 14.9%, $p = 0.001$) in men and 13.3% (95% CI 5.9% to 20.8%, $p < 0.001$) in women.

Conclusion In an asymptomatic older population NT-proBNP improves risk prediction not only of heart failure, but of cardiovascular disease in general beyond classical risk factors, resulting in a substantial reclassification of participants to a lower or higher risk category.

INTRODUCTION

The decision to start treatment in individuals without prevalent cardiac or vascular disease is based on the estimated risk for cardiovascular disease using established prediction models, like the Framingham Heart or Systematic Coronary Risk Evaluation (SCORE) risk function models.[1, 2] A disadvantage of these risk models is that a considerable proportion of individuals without prevalent cardiovascular disease is classified in an intermediate cardiovascular risk category. In these individuals the decision to initiate or withhold treatment is not straightforward. To overcome this shortcoming several biomarkers have been investigated for their additive predictive value for cardiovascular events in the general population beyond the traditional risk models, either by assessing the improvement in C statistic[3] or the reclassification of subjects in different risk categories[4], however with disappointing results.[5-7]

In the present study we explored whether addition of NT-proBNP to a risk model based on classical risk factors improves the accuracy of 10-year cardiovascular risk prediction in an older population and what proportion of individuals at an intermediate risk category could be reclassified in either a lower or higher risk category. NT-proBNP is a cardiac hormone, secreted by cardiomyocytes in response to an increase in ventricular wall stretch.[8] In clinical practice, measurement of NT-proBNP or BNP plasma levels is routinely applied for the diagnosis and management of subjects with (suspected) heart failure.[9, 10] Moreover, in community-based populations increased NT-proBNP or BNP plasma levels have been associated with an increased risk for cardiac and non-cardiac vascular disesase.[6, 8, 11-15] Because of these properties and the knowledge that the risk prediction of cardiovascular disease by classical risk factors is diminished at advancing age,[16] we hypothesized that NT-proBNP may be a useful candidate to improve the accuracy of cardiovascular disease prediction.

METHODS

Setting and participants

The Rotterdam Study is a prospective cohort study of subjects, aged ≥ 55 years, living in Rotterdam. Its aim is to investigate the incidence of and

risk factors for chronic disabling diseases. Of the 7983 participants recruited in 1990-1993, 3930 participants had blood sampled at the third examination cycle during 1997-1999. Furthermore, we included 2568 out of 3011 participants, additionally recruited and examined in 2000-2001, fulfilling the same inclusion criteria as the original cohort. After excluding participants with prevalent coronary heart disease, heart failure and/or stroke 5211 participants remained. Additionally 53 participants with a NT-proBNP level above the age-specific cutoff value for the diagnosis of heart failure as proposed by Januzzi et al (50-75 years, 108 pmol/l; > 75 years, 216 pmol/l) were excluded[9]. From these 5158 participants follow-up was available for all endpoints in 5063 participants (2032 men, 3031 women). A more detailed description of the Rotterdam Study and the collection of data can be found elsewhere.[17-20] The Medical Ethics Committee of Erasmus MC approved the study.

Risk Factors for Cardiovascular Disease

A trained interviewer using a computerized questionnaire collected information on current health status, medical history and smoking behavior from the subjects of the original cohort (n = 3930) at the third examination round (1997-1999). From the subjects added to the cohort (n = 2568), information was collected at their first examination round (2000-2001). In addition to the interview, the classical cardiovascular risk factors were measured during 2 visits at the research center. Two blood pressure measurements were taken with a random-0 sphygmomanometer after 5 minutes of rest with the subject in a sitting position. Serum glucose, serum total cholesterol and high-density lipoprotein cholesterol were measured using standard laboratory techniques. Diabetes mellitus was considered to be present when fasting blood glucose exceeded 7.0 mmol/l, non-fasting glucose exceeded 11.0 mmol/l and/or anti-diabetic medication was used. Hypertension was defined as systolic or diastolic blood pressure of ≥ 140/90 mmHg or use of antihypertensive medication. Glomerular filtration rate was estimated using the simplified Modification of Diet in Renal Disease equation.[21]

NT-proBNP Measurement

Blood samples for NT-proBNP measurement were collected in glass tubes containing clot activator and gel for serum separation. The serum was stored at −80°C. NT-proBNP was measured using a commercially available elec-

trochemiluminescence immunoassay (Elecsys proBNP, F. Hoffman-La Roche Ltd., Basel, Switzerland) on an Elecsys 2010 analyzer. The precision, analytical sensitivity, and stability characteristics of the system have previously been described.[22]

Cardiovascular Disease Monitoring

A history of coronary event was considered present in case of a self-report of coronary event confirmed by electrocardiogram or additional clinical information. A validated score for the diagnosis of heart failure, similar to the criteria of the European Society of Cardiology, was used during the interview.[23] A history of stroke was determined by direct interview, and subsequent review of general practioner`s records of the subjects with a positive history of stroke.

Subjects participating in the Rotterdam Study are monitored continuously for the occurrence of cardiovascular events through automated linkage with the files from general practitioners in the study district. For the diagnosis of cardiac events, 2 research physicians independently coded all reported events. In case of disagreement, a decision was made by a medical expert in the field. In case of stroke, events were coded by 2 research physicians and an experienced neurologist. All events were coded according to the *International Classification of Diseases, 10th edition*.[24] Coronary heart disease was defined as the occurrence of a nonfatal myocardial infarction (International Classification of Diseases, 10th edition code I21), a percutaneous transluminal coronary angioplasty, a coronary artery bypass graft, other forms of acute (I24) or chronic ischemic heart disease (I25) and mortality due to coronary artery disease (I20, I21, I24, I25, I46, and R96). Heart failure was defined as incident fatal and non-fatal heart failure (I50). Ischemic stroke was defined as incident fatal and non-fatal ischemic stroke (I63). The composite endpoint cardiovascular disease included all first events due to coronary heart disease, heart failure or ischemic stroke. Information concerning the vital status of the participants was obtained from the municipal health service of Rotterdam and was available for all participants up to January 2007.

Statistical Analysis

Baseline characteristics between men and women were compared using student's *t* tests for continuous variables. Categorical data were compared using χ^2 tests. Calculations on NT-proBNP values were made after logarithmic transformation to correct for its skewed distribution.[11-13] The additional

predictive value of NT-proBNP on top of the classical risk factors age, systolic blood pressure, cholesterol, high-density lipoprotein, smoking (categories: never, former, current) and diabetes (categories: yes, no) was assessed in three steps. The effect of adjustment for Modification of Diet in Renal Disease equation, body mass index and use of antihypertensives on top of the classical risk factors for the predictive value of NT-proBNP for cardiovascular disease was also investigated. The results are reported for men and women separately to allow for sex-specific effects.

First, we calculated the hazard ratios for cardiovascular events related to a one standard deviation increase in logarithmically transformed NT-proBNP. Hazard ratios were adjusted for the aforementioned classical risk factors. The hazard ratios were also calculated for the 2 upper, age-corrected tertiles of NT-proBNP, with the first tertile as reference. In the analysis for incident cardiovascular events subjects were censored at end of follow-up or non-cardiovascular death. In the sub-analyses for coronary heart disease, heart failure and stroke, subjects were also censored if a cardiovascular event occurred that was not the endpoint of that specific analysis. Using likelihood ratio tests we assessed the improvement of global model fit.

Second, we analyzed the influence of NT-proBNP on the discrimination of the model, that is, the ability to distinguish subjects who will develop disease from subjects who will be free of disease during follow-up, by assessing the change in the C statistic after adding NT-proBNP to a model on the basis of the aforementioned risk factors. All of the reported C statistics were corrected for optimism by bootstrap analysis.[25] The 95% confidence interval around the change in optimism-corrected C statistic was also calculated using bootstrap analysis (1000 bootstrap replicates).[26]

Third, as treatment decisions are based using cutoff levels of 10-year risk, reclassification tables were constructed to investigate the number of patients, classified in a higher or lower category of 10-year risk for cardiovascular disease. Reclassification tables were constructed using three different risk categories, that is, 0 to 10%, > 10 to 20% and > 20%.[3] We used Weibull proportional hazard models to calculate 10-year risk predictions. To evaluate the net percentage of reclassified subjects we calculated the net reclassification improvement (NRI) on the entire sample and we evaluated the relative integrative discrimination improvement (RIDI)[4, 27]. In additional analyses we tested the predictive value of NT-proBNP over a model including the classical risk factors and C-reactive protein in the 2726 subjects in whom

C-reactive protein was measured. Also, we investigated whether NT-proBNP resulted in an improvement of cardiovascular risk prediction in the subjects < 75 years of age. Finally, we investigated whether NT-proBNP predicted the combined endpoint of coronary heart disease and ischemic stroke.

Missing data, < 2% for all covariates, were handled using multiple imputation (R-library: Hmisc, function: aregImpute). Calibration of the model was assessed using the Hosmer-Lemeshow statistic[28]. Values are presented as mean ± standard deviation or as median with interquartile range. A *p* value less than 0.05 was considered to indicate statistical significance. We used SPSS 15.0 (SPSS Inc, Chicago, Illinois, USA) and R 2.7.2 (R Foundation for Statistical Computing, Vienna, Austria) for the statistical analysis.

RESULTS

Baseline characteristics of cohort are shown in Table 1. The mean age was 67 ± 8 years in men and 69 ± 8 years in women. There were small differences between sexes, with a lower systolic blood pressure and a smaller proportion of smokers and diabetics in women (Table 1). The median NT-proBNP level was higher in women than in men (10.2 versus 6.8 pmol/l; p < 0.001). Follow-up was 9291 years for men and 14810 years for women. During follow-up 224 (59 fatal) men and 196 (49 fatal) women experienced a cardiovascular event. Coronary heart disease (n = 107) was the most frequent cardiovascular event in men followed by heart failure (n = 60) and ischemic stroke (n = 54). Incidences of coronary heart disease (n = 67), heart failure (n = 59) and ischemic stroke (n = 70) in women were almost identical.

An increase in the risk for cardiovascular disease was seen from the lowest to the highest tertile of NT-proBNP after adjustment for the classical risk factors (Figure 1 and Table 2). A one standard deviation increase in log-NT-proBNP was related to an increase in the future risk for the composite endpoint cardiovascular events (men: hazard ratio 1.47 [95% CI 1.28 to 1.68]; women: hazard ratio 1.69 [95% CI 1.46 to 1.95]). The hazard ratios for the covariates of the classical model and the NT-proBNP model are given in Table 3. The hazard ratios for the separate endpoints related to a one standard deviation increase in log-NT-proBNP were: coronary heart disease (men: 1.31 [95% CI 1.07 to 1.59]; women: 1.63 [95% CI 1.27 to 2.11]), heart failure (men: 1.70 [95% CI 1.08 to 4.40]; women: 2.17 [95% CI 1.68

Table 1. Baseline characteristics of included participants of the Rotterdam study

Characteristics	***Total group***	***Men***	***Women***	***p****
No. of participants	*5063*	*2032*	*3031*	
Age, years	*68 (8)*	*67 (8)*	*69 (8)*	*< 0.001*
Systolic blood pressure, mmHg	*143 (21)*	*144 (21)*	*142 (21)*	*0.009*
Hypertension, No. (%)	*3040 (60.0)*	*1223 (60.2)*	*1817 (59.9)*	*0.764*
Total cholesterol, mmol/l	*5.9 (1.0)*	*5.6 (0.9)*	*6.0 (0.9)*	*< 0.001*
High-density lipoprotein cholesterol, mmol/l	*1.4 (0.4)*	*1.3 (0.3)*	*1.5 (0.4)*	*< 0.001*
Diabetes mellitus, No. (%)	*462 (9.1)*	*207 (10.2)*	*255 (8.4)*	*0.035*
Smoking, No. (%) *Never* *Past* *Current*	 *1708 (33.7)* *2416 (47.7)* *891 (17.6)*	 *329 (16.2)* *1312 (64.6)* *376 (18.5)*	 *1379 (45.5)* *1104 (36.4)* *515 (17.0)*	*< 0.001*
eGFR, ml/kg/1,73m^3	*81 (16)*	*83 (16)*	*79 (16)*	*< 0.001*
Body mass index, kg/m^2	*27 (4)*	*27 (3)*	*27 (4)*	*< 0.001*
NT-proBNP, median (IQR), pmol/l	*8.7 (4.8 -16.4)*	*6.8 (3.7 -13.6)*	*10.2 (5.8 - 18.0)*	*< 0.001*

If not indicated mean values and standard deviations are given. *p value for difference between genders. Abbreviations: eGFR, estimated glomerular filtration rate; IQR, interquartile range.

Table 2. Hazard ratios for cardiovascular events related to the upper tertiles of NT-proBNP (first tertile as reference) adjusted for the classical risk factors*

Outcome	***Men***		***Women***	
	HR	*95% CI*	*HR*	*95% CI*
Cardiovascular events				
2nd tertile	*1.34*	*0.88 – 1.57*	*1.82*	*1.11 – 2.23*
3rd tertile	*2.32*	*1.55 – 2.70*	*3.08*	*1.91 – 3.74*
Coronary heart disease				
2nd tertile	*1.67*	*0.96 – 2.12*	*2.63*	*1.17 – 4.02*
3rd tertile	*2.01*	*1.14 – 2.59*	*2.95*	*1.30 – 4.55*
Heart failure				
2nd tertile	*0.84*	*0.34 – 1.40*	*1.90*	*0.60 – 3.84*
3rd tertile	*2.90*	*1.33 – 4.34*	*5.93*	*2.04 - 11.2*
Ischemic stroke				
2nd tertile	*1.07*	*0.45 – 1.71*	*1.30*	*0.61 – 1.91*
3rd tertile	*2.06*	*0.91 – 3.18*	*2.07*	*1.00 – 2.97*

* Adjusted for: age, systolic blood pressure, cholesterol, high-density lipoprotein, smoking (categories: never, former, current) and diabetes (categories: yes, no). Abbreviations: HR, hazard ratio; CI, confidence interval.

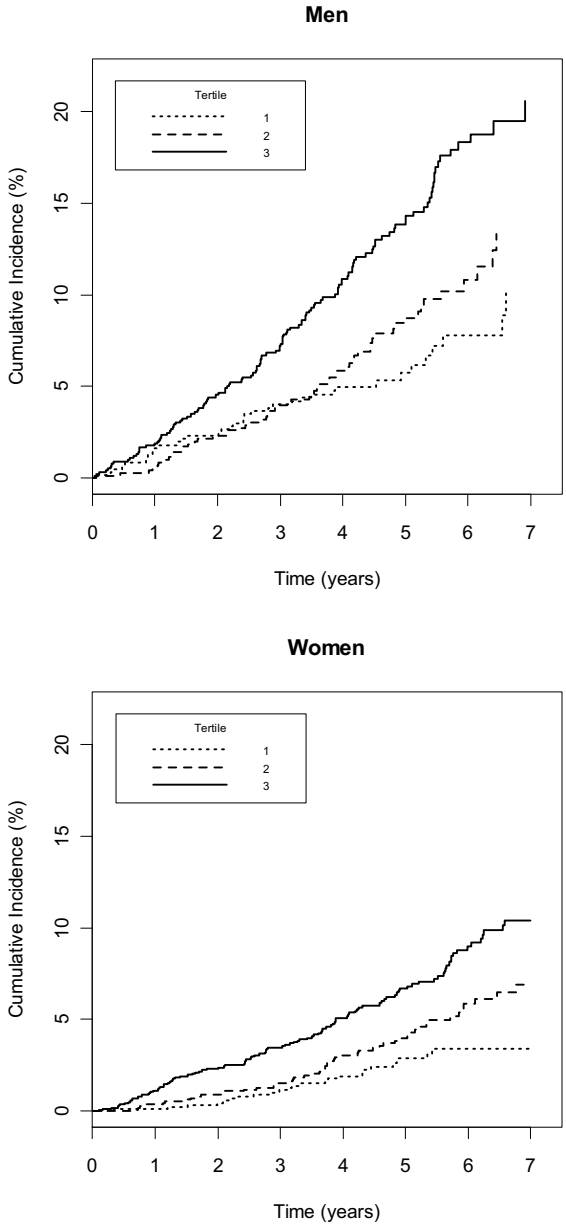

Figure 1. Cumulative incidence of cardiovascular disease in men (left panel) and women (right panel) for each tertile of NT-proBNP. Median NT-proBNP values for tertiles of NT-proBNP for men (2.8; 6.8 and 18.3 pmol/l) and women (4.6; 10.2 and 23.3 pmol/l). See Table 3 for hazard ratios with 95% confidence intervals.

to 2.81]) and ischemic stroke (men: 1.49 [95% CI 1.13 to 1.69]; women: 1.39 [95% CI 1.08 to 1.79]). Global model fit of the prediction model for cardiovascular events improved substantially after adding NT-proBNP to the

classical risk factors (men: likelihood ratio 29, p < 0.001; women: likelihood ratio 46, p < 0.001). The calibration of the predictive models was adequate (Hosmer-Lemeshow tests not significant). Additional adjustment for Modification of Diet in Renal Disease equation, body mass index and use of antihypertensives did not influence the predictive value of NT-proBNP (data not shown).

The discrimination of cases and non-cases improved by using NT-proBNP from 0.661 to 0.694 in men (change in C statistic: 0.033 [95% CI 0.012 to 0.052]) and from 0.729 to 0.761 in women (change in C statistic: 0.032 [95% CI 0.016 to 0.047]). Of the separate cardiovascular endpoints the largest increase in C statistic was seen in the prediction of heart failure (men: change in C statistic 0.075 [95% CI 0.030 to 0.113]; women: change in C statistic 0.043 [95% CI 0.016 to 0.068]; Table 4). The improvement of C statistic related to NT-proBNP did not change after including Modification of Diet in Renal Disease equation, body mass index and use of antihypertensives.

Table 4. C statistics of Cox regression models predicting cardiovascular events

Outcome	***Classical model****		***NT-proBNP model**†**	
	C statistic	*95% confidence interval*	*C statistic*	*95% confidence interval*
Cardiovascular events				
Men	*0.661*	*0.630 - 0.691*	*0.694*	*0.665 - 0.724*
Women	*0.729*	*0.702 - 0.756*	*0.761*	*0.736 - 0.787*
Coronary heart disease				
Men	*0.676*	*0.634 - 0.716*	*0.691*	*0.651 - 0.732*
Women	*0.726*	*0.683 - 0.770*	*0.761*	*0.722 - 0.801*
Heart failure				
Men	*0.702*	*0.651 - 0.756*	*0.777*	*0.729 - 0.830*
Women	*0.768*	*0.723 - 0.815*	*0.811*	*0.768 - 0.858*
Ischemic stroke				
Men	*0.699*	*0.650 - 0.750*	*0.714*	*0.660 - 0.767*
Women	*0.721*	*0.678 - 0.765*	*0.734*	*0.692 - 0.777*

*Classical model include age (continuous), systolic blood pressure (continuous), total cholesterol (continuous), high density lipoprotein (continuous), smoking (never, past, current), diabetes mellitus (yes, no). †NT-proBNP model include the covariates of the classical model and NT-proBNP (continuous). Abbreviation: NT-proBNP, amino-terminal pro-B-type natriuretic peptide.

Of the 54 men who experienced an event during follow-up and classified initially in the intermediate risk category 17 (31.5%) were reclassified to the higher and 4 (7.4%) to the lower risk category using NT-proBNP as risk predictor (Table 5). Of the 783 men who did not experience an event and were initially classified in the intermediate risk category, 104 (13.3%) were reclassified to the lower and 113 (14.4%) to the higher risk category (Table 5). Of the 65 women who experienced an event initially classified as having an intermediate risk, 21 (32.2%) were reclassified to the higher and 11 (16.9%) to the lower risk category (Table 6). Of the 867 women without an event in the intermediate risk category 298 (34.3%) were reclassified to a lower and 136 (15.7%) to a higher risk category (Table 6). In total 238 out of 837 men (28.4%) and 466 out of 932 women (50.0%) were reclassified. The NRI of the total sample was 9.2% (95% CI 3.5% to 14.9%, p = 0.001) in men and 13.3% (95% CI 5.9% to 20.8%, p < 0.001) in women. The RIDI improved significantly both in men 9.1% (95% CI 5.0% to 12.9%) and women 16.8% (95% CI 10.9 to 23.2%).

Table 5. Change in risk stratification by NT-proBNP for first cardiovascular events in men

Classical model*		***NT-proBNP model**†*		
Predicted 10-Year Risk	***≤ 10%***	***> 10% - ≤ 20%***	***> 20%***	*Total*
Participants who experience a cardiovascular disease event				
≤ 10%	*3 (50.0)*	*3 (50.0)*	*0*	*6*
> 10% - ≤ 20%	*4 (7.4)*	*33 (61.1)*	*17 (31.5)*	*54*
> 10% - ≤ 20%	*0 (0.0)*	*11 (6.7)*	*153 (93.3)*	*164*
Total	*7*	*47*	*170*	*224*
Participants who do not experience a cardiovascular disease event				
≤ 10%	*118 (72.0)*	*46 (28.1)*	*0*	*164*
> 10% - ≤ 20%	*104 (13.3)*	*566 (72.3)*	*113 (14.4)*	*783*
> 10% - ≤ 20%	*0 (0.0)*	*181 (21.0)*	*680 (79.0)*	*861*
Total	*222*	*793*	*793*	*1808*

Reclassification tables separated for cases (upper panel) and non-cases (lower panel) with rows indicating the risk categories based upon the classical model without NT-proBNP and columns indicating the new risk stratification after addition of NT-proBNP to the model. The cells give the number and percentage of subjects reclassified using Weibull proportional hazards models to estimate ten year cardiovascular disease risk. *For covariates of classical model see Table 4. † NT-proBNP model include the covariates of the classical model and NT-proBNP (continuous).

Table 6. Change in risk stratification by NT-proBNP for first cardiovascular events in women

Classical model*		***NT-proBNP model**†*		
Predicted 10-Year Risk	***≤ 10%***	***> 10% - ≤ 20%***	***> 20%***	*Total*
Participants who experience a cardiovascular disease event				
≤ 10%	*19 (59.4)*	*11 (34.4)*	*2 (6.3)*	*32*
> 10% - ≤ 20%	*11 (16.9)*	*33 (50.8)*	*21 (32.3)*	*65*
> 10% - ≤ 20%	*0 (0.0)*	*7 (7.1)*	*92 (92.9)*	*99*
Total	*30*	*51*	*115*	*196*
Participants who do not experience a cardiovascular disease event				
≤ 10%	*1179 (86.6)*	*172 (12.6)*	*10 (0.7)*	*1361*
> 10% - ≤ 20%	*298 (34.4)*	*433 (49.9)*	*136 (15.7)*	*867*
> 10% - ≤ 20%	*9 (1.5)*	*158 (26.0)*	*440 (72.5)*	*607*
Total	*1486*	*763*	*586*	*2835*

For explanation see legend of Table 5.

In sub-analysis, including the participants in whom C-reactive protein was measured (n = 2726), C-reactive protein did not improve prediction of the classical model, whereas addition of NT-proBNP to a model including the classical risk factors and C-reactive protein improved the C statistic from 0.669 to 0.695 in men (change in C statistic 0.034 [95% CI 0.013 to 0.052]) and from 0.735 to 0.761 in women (change in C statistic 0.033 [95% CI 0.017 to 0.048]). When restricting the analysis to men and women < 75 years of age (n = 3939, mean age 65.0 yrs), the hazard ratio of cardiovascular events related to a one standard deviation increase in NT-proBNP increased in men (1.63 [95% CI 1.40 to 1.91]) and remained the same in women (1.59 [95% CI 1.36 to 1.85]). Incorporation of NT-proBNP in the model improved the C statistic both in men (0.632 to 0.684; change in C statistic: 0.052 [95% CI 0.021 to 0.079]) and women (0.681 to 0.725; change in C statistic: 0.044 [95% CI 0.016 to 0.069]). In men below 75 yrs of age there was a significant reclassification (NRI 15.4% [95% CI 6.3% to 24.5%]; RIDI 20.6% [95% CI 13.7% to 27.6%]). In women below 75 yrs of age a trend towards an improvement in reclassification was seen (NRI 10.2% [95% CI –3.0% to 23.5%]) and the RIDI improved (19.9% [95% CI 11.5% to 28.5%]). NT-proBNP predicted in men and women the combined endpoint coronary heart disease and ischemic stroke with a improvement in C statistic of 0.022 (95% CI 0.001 to 0.038) in men and 0.026 (95% CI 0.008 to 0.042)

in women. The RIDI improved both in men 6.1% (95% CI 2.0% to 10.0%) and women 8.6% (95% CI 2.4% to 15.1%). Due to the lower rate of events after exclusion of heart failure the NRI decreased to 3.3% (95% CI -5.5% to 12.2%) in women, and 7.5% (95% CI 0.0% to 14.7%) in men.

DISCUSSION

In this population-based cohort study, we found that NT-proBNP has independent prognostic value for first cardiovascular events beyond the classical risk factors. An increase in the risk not only for heart failure but also for coronary heart disease and ischemic stroke was seen, resulting in a doubling of the risk for first cardiovascular events in subjects with a NT-proBNP above the 80th percentile. Furthermore, NT-proBNP improved the prediction of 10-year cardiovascular risk, reflected by an improvement in C statistic. The addition of NT-proBNP to a model including the classical risk factors improved risk classification, with a net reclassification improvement of 9% in men and 13% in women. Our findings indicate that addition of NT-proBNP to a risk model based on the classical risk factors improves the risk prediction of first fatal and non-fatal cardiovascular disease in healthy older individuals.

In our study a one standard deviation increase in log-NT-proBNP was associated with 47% increase in first fatal and non-fatal cardiovascular events in men and 69% in women after adjustment for classical risk factors. This increase in risk is comparable to what has been found in other population-based studies adding BNP or NT-proBNP to established risk models. In the Framingham population (mean age: men: 59 years, women: 58 years) a one standard deviation increase in log-BNP was associated with a 35% increase in the risk for first cardiovascular events after adjustment for classical risk factors.[29] In a population-based study (mean age: 68 years) from Copenhagen, Denmark, a one standard deviation increase in log-NT-proBNP was associated with a 92% increase in the risk for first major cardiovascular events and in a population-based study (mean age 71 years) from Uppsala, Sweden a one standard deviation increase in NT-proBNP was associated with 116% increase in risk for cardiovascular death.[11, 15] More recently, Melander et al showed in a Swedish population (mean age: 58 years) that a one standard deviation increase in NT-proBNP related to an increased risk for cardiovascular disease of 22%.[7]

The association of BNP and NT-proBNP with cardiovascular events or overall mortality together with biomarkers of other pathophysiological pathways such as C-reactive protein, troponin I and urinary albumin-to-creatinine ratio has also been evaluated in population-based studies.[6-8, 11, 13, 15] Of the 10 biomarkers investigated by Wang et al, BNP had the strongest association with major cardiovascular events.[6] Kistorp et al, evaluating NT-proBNP, C-reactive protein and urinary albumin-creatinine ratio, found that NT-proBNP was the biomarker with the highest risk ratio for first major cardiovascular events[11], and Zethelius et al, evaluating NT-proBNP, troponin I, C-reactive protein and cystatin C, also found that NT-proBNP had the highest risk ratio for death from cardiovascular causes.[15] In the recent study of Melander et al NT-proBNP was related to the highest hazard ratio for cardiovascular events in comparison to C-reactive protein, cystatin C, lipoprotein-associated phospholipase 2, midregional proadrenomedullin, midregional proatrial natriuretic peptide.[7] These findings indicate that among the biomarkers tested head-to-head, B-type natriuretic peptides have the strongest association with cardiovascular disease.

In our study, increased NT-proBNP levels improved the risk stratification for first cardiovascular events, as evidenced by a significant increase in the C statistic. In most of the previously reported studies no change in C statistic for primary cardiovascular events was demonstrated using NT-proBNP as biomarker. In a study in elderly men with a high prevalence of cardiovascular disease an improvement was seen using NT-proBNP in combination with three other biomarkers.[15] Also in the study of Melander et al no improvement in C statistic or reclassification was found for NT-proBNP for first cardiovascular events and only a borderline significant improvement of IDI was found for the prediction of first coronary events.[7]

Several factors may account for these discrepant results between our study and previous studies. First, it is well known that the role of the classical risk factors in cardiovascular risk prediction diminishes with advancing age.[16,28] As a consequence, the contribution of other factors to risk prediction, like NT-proBNP, may increase. The mean age of the participants in our study was 68 years compared to 59 years of the participants in the Framingham population[6], this possibly explains why NT-proBNP improved the C statistic in our study. Second, in the Framingham population, BNP instead of NT-proBNP was tested. NT-proBNP and BNP are released in the circulation in equimolar amounts, but the half-life of NT-proBNP is longer than that of

BNP, resulting in more stable circulating levels. The information about the "chronic" cardiovascular condition provided by NT-proBNP may therefore be more accurate than that provided by BNP.

In clinical practice, BNP or NT-proBNP are principally used as biomarkers for the diagnosis and management of subjects with (suspected) heart failure.[9, 10] In our population-based study we found that modestly elevated NT-proBNP levels not only were associated with heart failure, but remarkably also with coronary artery disease and ischemic stroke. Apparently, mild elevations of these peptides are present in subjects with an increased risk for heart failure as well as in subjects with an increased risk for cardiovascular disease in general. One possible explanation for this intriguing observation is that an increase in B-type natriuretic peptides not only occurs in response to myocardial stretch, as in heart failure, but also in response to myocardial ischemia.[30] Especially at older age myocardial ischemia is mainly due to coronary artery disease, which in turn is closely linked to cerebrovascular disease.[31]

In primary cardiovascular disease prevention, decisions regarding medical intervention are guided by the predicted cardiovascular risk. Our findings show that addition of NT-proBNP resulted in a reclassification of a substantial number of subjects. This still remained after additional adjustment for body mass index and use of blood pressure lowering agents. From a clinical point of view accurate risk classification is especially relevant for individuals with an intermediate cardiovascular risk, because the necessity to start or withhold treatment in these subjects is uncertain. Of the 1769 subjects in the intermediate risk category 28% of men and 50% of women were reclassified, with both in men and women an up-classification of subjects who experienced an event during follow-up. Our findings support the incorporation of biomarkers that reflect subclinical cardiovascular disease, like NT-proBNP, in risk models based on the classical etiological risk factors in order to improve risk prediction.

The strength of our study is the availability of NT-proBNP levels in a large number of subjects of a well-characterized cohort with a substantial number of endpoints, but several limitations have to be mentioned. First, we extrapolated the data to compute 10-year predictions. Second, we performed our analyses in a cohort of subjects older than 55 years; it is not justified therefore to extrapolate our findings to younger subjects. As NT-proBNP is a marker of (subclinical) disease it may predict cardiovascular

disease better in older subjects. Third, C-reactive protein was only measured in a subcohort, we found no signification improvement of cardiovascular risk prediction after incorporation of C-reactive protein in the classical model. This could be due to a lack of statistical power or due to the decreasing predictive value of C-reactive protein in an elderly population. Fourth, we investigated a white population; whether our results can be extrapolated to other ethnic groups requires further investigation. Finally, NT-proBNP was measured only once, and we have no information on the effect of increases or decreases in NT-proBNP levels. Possibly, the change of NT-proBNP level over time is a better predictor of someone's future cardiovascular risk than a single measurement.

CONCLUSION

Our study shows that incorporation of NT-proBNP in a traditional risk model improves cardiovascular risk prediction and classification in asymptomatic older subjects. Our study therefore lends support for the idea of introducing biomarkers into classical risk models in order to improve risk prediction of individual subjects. It should be remarked that our findings were obtained in an older population. Especially in older populations the use of biomarkers for risk prediction is attractive owing to the fact that cardiovascular disease risk prediction by classical risk factors is diminished with advancing age. Introduction of NT-proBNP testing for risk prediction is relatively easy because of the wide-spread availability of this measurement in clinical centers, its low costs, and the lack of requirement for special training of health care providers.

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8

Multimarker approach for the prediction of primary cardiovascular events - the Rotterdam study

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ABSTRACT

Introduction With advancing age the predictive value of traditional risk factors decreases and markers of vascular disease, inflammation, and end organ damage may provide additive prognostic value.

Methods 5063 subjects from a community-based cohort study, aged 55 years or older, living in Rotterdam, The Netherlands, free of cardiovascular disease and heart failure were followed for a median duration of 4.7 years for first major non-fatal or fatal cardiovascular event (coronary artery disease, stenting and by-pass grafting, heart failure and ischemic stroke). The predictive value for cardiovascular disease of each separate risk marker was assessed by calculating the hazard ratio related to a one standard deviation increase in each marker, the improvement in C statistic and the net reclassification index.

Results After adjustment for age, blood pressure, total cholesterol, high-density lipoprotein cholesterol, diabetes mellitus and smoking, a one standard deviation increase in NT-proBNP, hazard ratio 1.47 (95% CI 1.28 to 1.69), and intima media thickness, hazard ratio 1.15 (95% CI 1.03 to 1.29) was independently related to an increased risk for cardiovascular disease in men. In women, NT-proBNP, hazard ratio 1.69 (95% CI 1.46 to 1.95), was independently associated with cardiovascular disease. Addition of NT-proBNP to the model based on traditional risk factors resulted in an increase in C statistic of 0.034 (95% 0.012 to 0.052) in men and a net reclassification index of 9.2% (95% CI 3.5% to 14.9%). In women NT-proBNP improved the C statistic by 0.034 (0.017 to 0.048), with a net reclassification index of 13.3% (95% CI 5.9% to 20.8%).

Conclusion NT-proBNP improves the prediction of first fatal and nonfatal cardiovascular events, but carotid-femoral pulse wave velocity, ankle-brachial index, creatinine clearance, intima media thickness and C-reactive protein have no additional predictive value above the traditional risk factors in older subjects free of cardiovascular disease.

INTRODUCTION

Interventions to prevent cardiovascular disease, such as treatment of dyslipidemia or hypertension, are especially effective in individuals with a high cardiovascular risk. To estimate the 10-year risk of cardiovascular disease various risk models have been developed like the Framingham score[1], the Adult Treatment Panel III risk assessment tool[2] or the Systematic Coronary Risk Evaluation (SCORE) risk function model.[3] The risk prediction of these models is based on the traditional risk factors for cardiovascular disease, such as age, sex, blood pressure, lipid profile, smoking and diabetes. The estimated cardiovascular disease risk explained by the traditional risk factors diminishes with advancing age.[4] For example, the relative risk for ischemic heart disease related to a 1 mmol/l increase in total cholesterol decreases from 2.2 for subjects aged 40-49 years to 1.2 for subjects aged 70-89 years.[5]

Exposure to cardiovascular risk factors may lead to vascular damage in different organs. Therefore, measurement of markers reflecting subclinical cardiovascular disease has been applied in an attempt to further improve risk stratification.[6-9] Both markers of vascular disease such as aortic pulse wave velocity[6, 10, 11], ankle brachial index[7], and intima media thickness[12], as markers of end-organ damage such as creatinine clearance[8, 13-15] and natriuretic peptide plasma levels[16-19] have been reported to be associated with cardiovascular disease beyond the traditional risk factors. Also elevated levels of C-reactive protein, reflecting inflammation and atherosclerosis, have shown to be an independent cardiovascular risk predictor in middle-aged women.[20]

Risk prediction is especially suitable in individuals without a history of cardiovascular disease. In these individuals, as opposed to those with a history of cardiovascular disease, the decision to initiate medical treatment is determined by the estimated cardiovascular disease risk based on risk models.[21, 22] Since the number of subjects to be screened for cardiovascular disease risk in the general population is large, measurement of an extensive number of markers will not likely be cost-effective; selection of the most appropriate tests is therefore mandatory. Moreover, to be implemented in daily clinical practice, the cardiovascular risk marker should be easy to measure at a relatively low cost, and its incorporation in a traditional risk model must improve the risk prediction of individual subjects.

The objective of the present study was to compare the clinical usefulness of six risk markers for cardiovascular disease prediction in a large community-

based cohort of older subjects. In this cohort, we measured carotid-femoral pulse wave velocity, ankle-brachial index, intima media thickness, C-reactive protein, creatinine clearance and NT-proBNP at baseline and assessed their predictive value for the occurrence of fatal and nonfatal coronary heart disease, heart failure and ischemic stroke beyond the traditional risk factors.

METHODS

Study Population

The Rotterdam Study is a prospective cohort study of subjects, aged 55 years or older, living in Rotterdam, the Netherlands. Its aim is to investigate the incidence of and risk factors for chronic disabling diseases. Of the 7983 participants recruited in 1990-1993, in 3930 participants blood was sampled at the third examination cycle during 1997-1999. Furthermore we included 2568 out of 3011 participants, additionally recruited and examined in 2000-2001, fulfilling the same inclusion criteria as the original cohort. After excluding participants with prevalent coronary heart disease, heart failure, stroke or a NT-proBNP level above the age-specific cutoff for the diagnosis of heart failure as proposed by Januzzi et al (50-75 years, 108 pmol/l; > 75 years, 216 pmol/l) 5158 out of 6498 participants remained.[23] Follow-up for all endpoints was available in 5063 participants (2032 men, 3031 women). A more detailed description of the Rotterdam Study and the collection of data can be found elsewhere.[6, 24-27] The Medical Ethics Committee of the Erasmus MC, Rotterdam, approved the study.

Risk Factors for Cardiovascular Disease

A trained interviewer using a computerized questionnaire collected information on current health status, medical history, drugs use, smoking behavior, and family history of cardiovascular disease from the subjects of the original cohort (n = 3930) at the third examination round (1997-1999). From the subjects added to the cohort (n = 2568), information was collected at their first examination round (2000-2001). In addition to the interview the classical cardiovascular risk factors were measured during 2 visits at the research center. Two blood pressure measurements were taken with a random-0 sphygmomanometer after 5 minutes of rest with the subject in a sitting position. Serum glucose, serum total cholesterol and high-density lipoprotein

cholesterol were measured using standard laboratory techniques. Diabetes mellitus was considered to be present when fasting blood glucose exceeded 7.0 mmol/l, non-fasting glucose exceeded 11.0 mmol/l and/or anti-diabetic medication was used.

Markers of subclinical cardiovascular organ damage

a. NT-proBNP

NT-proBNP was measured using a commercially available electrochemiluminescence immunoassay (Elecsys proBNP, F. Hoffman-La Roche Ltd., Basel, Switzerland) on an Elecsys 2010 analyzer. The precision, analytical sensitivity, and stability characteristics of the system have previously been described.[28] NT-proBNP measurements were available in all participants (n = 5063).

b. Carotid-femoral pulse wave velocity

Carotid-femoral pulse wave velocity, a measure of aortic stiffness, was assessed with the subjects in supine position using an automatic device (Complior; Artech Medical, Pantin, France) that measures the time delay between the rapid upstroke of the feet of simultaneously recorded pulse waves in the carotid and the femoral arteries. The distance between the recording sites in the carotid and femoral arteries (the carotid artery and the groin) was measured with a tape over the surface of the body. Carotid-femoral pulse wave velocity was calculated as the ratio between the distance and the foot-to-foot time delay and was expressed in meters per second. Carotid-femoral pulse wave velocity measurements were available in 4405 participants.

c. Ankle-brachial arm index

Ankle brachial index, a measurement of lower-extremity atherosclerosis, was calculated as the ratio of the systolic blood pressure at the ankle to the systolic blood pressure at the arm. The lowest value of two legs was used in the analysis. Values of ankle brachial index above 1.50 were considered invalid as increased vascular stiffness may lead to spurious high ankle brachial index. Ankle brachial index measurements were available in 4691 participants.

d. Creatinine clearance

Serum creatinine was measured using an enzymatic assay (Roche Diagnostics GmBH, Mannheim, Germany), which was calibrated by isotope dilution mass spectrometry. Creatinine clearance was estimated using the Cockcroft and Gault formula[29] and was adjusted for 1.73 m^2 body surface area, calculated according to the DuBois and Dubois formula: body surface area = 0.007184*(weightˆ0.425)*(heightˆ0.725), and expressed in ml/min/1.73 m^2. Creatinine clearance was assessed in 5015 participants.

e. C-reactive protein

High-sensitivity C-reactive protein was determined in serum, which was stored at -20°C. C-reactive protein was measured with the Rate Near Infrared Particle Immunoassay (Image Immunochemistry System, Beckman Coulter, Fullerton, California). This system measures concentrations from 0.2 to 1440 mg/l, with a within-run precision < 5.0%, a total precision < 7.5% and a reliability coefficient of 0.995. C-reactive protein measurements were available in 2726 participants. Participants with a log C-reactive protein of 3 standard deviations above the geometric mean (n = 42) were excluded.

f. Intima media thickness

The maximum common carotid intima media thickness was determined as the average of the maximum intima media thickness of near- and far-wall measurements over a length of 1 cm, and the average of left and right maximum common carotid intima media thickness was computed. Intima media thickness was measured in 3675 subjects.

Cardiovascular Endpoints

A history of a coronary event was considered present in case of a self-report of coronary event confirmed by electrocardiogram or additional clinical information. A validated score for the diagnosis of heart failure, similar to the criteria of the European Society of Cardiology, was used during the interview.[30] A history of stroke was determined by direct interview, and subsequent review of general practioner's records of the subjects with a positive history of stroke.

Subjects participating in the Rotterdam Study are monitored continuously for the occurrence of cardiovascular events through automated linkage with the files from general practitioners in the study district. When a cardiovascular event was reported, the research assistants collected additional information

from medical records of the general practitioner and, in addition, obtained information from the hospital discharge records or nursing home records including letters from medical specialists. For the diagnosis of cardiac events, 2 research physicians independently coded all reported events. In case of disagreement, a decision was made by a medical expert in the field. In case of stroke, events were coded by 2 research physicians and an experienced neurologist. All events were coded according to the *International Classification of Diseases*, 10th edition.[31] Coronary heart disease was defined as the occurrence of a nonfatal myocardial infarction (*International Classification of Diseases*, 10th edition code I21), a percutaneous transluminal coronary angioplasty, a coronary artery bypass graft, other forms of acute (I24) or chronic ischemic heart disease (I25) and mortality due to coronary artery disease (I20, I21, I24, I25, I46, and R96). Heart failure was defined as incident fatal and non-fatal heart failure (I50). Ischemic stroke was defined as incident fatal and non-fatal ischemic stroke (I63). The composite endpoint cardiovascular disease included all first events due to coronary heart disease, heart failure or ischemic stroke. Information concerning the vital status of the participants was obtained from the municipal health service of Rotterdam and was available for all participants up to January 2007.

Statistical Analysis

Baseline characteristics between men and women were compared using student's *t* tests for continuous variables. Categorical data were compared using χ^2 tests. Calculations on NT-proBNP and C-reactive protein values were made after logarithmic transformation to correct for their skewed distributions.[17-19] As cardiovascular risk profiles differ between sexes we analyzed the data separately for men and women. In the analysis for incident cardiovascular events subjects were censored at end of follow-up or non-cardiovascular death. NT-proBNP, carotid-femoral pulse wave velocity, ankle brachial index and creatinine clearance were measured in both the original cohort and the participants subsequently added to the cohort. Intima media thickness and C-reactive protein were only measured in the original cohort. Therefore the predictive value of intima media thickness and C-reactive protein was investigated in separate analyses of the 2857 subjects of the original cohort.

To investigate the association of each risk marker with cardiovascular disease, we first calculated the hazard ratios for cardiovascular events related to a one standard deviation increase in each risk marker adjusting only

for age. Second, we adjusted for systolic blood pressure, total cholesterol, high-density lipoprotein cholesterol, diabetes mellitus and smoking. For illustrative purposes we also calculated the risk related to the four upper quintiles of each risk markers using the first quintile as reference. Third, we investigated the hazard ratio's for cardiovascular disease, coronary heart disease, heart failure and ischemic stroke associated with a one standard deviation increase in risk markers.

The influence of the six risk markers on the ability of the prediction model to distinguish subjects who will develop disease from subjects who will not during follow-up was investigated by assessing the change in C statistic. The C statistic is the equivalent of the area under the curve of the receiver operator characteristic's curve in the analyses of survival data. All reported C statistics were corrected for optimism by bootstrap analysis.[32] The 95% confidence interval around the change in optimism-corrected C statistic was also calculated using bootstrap analysis (1,000 bootstrap replicates).[33]

Lastly, as treatment decisions are based on cutoff levels of 10-year risk, reclassification tables were constructed to investigate the percentage of patients, classified in a higher or lower category of 10-year risk for cardiovascular disease. Reclassification tables were constructed using three different risk categories, i.e. 0 – 10%, > 10 – 20%, > 20%.[34] We used Weibull proportional hazard models to make predictions of 10-year risk. The net reclassification index (NRI) was calculated using the estimated number of cases from the Weibull models. The NRI is the percentage of participants that moves correctly from one risk category to another - upwards for events and downwards for non-events.[35]

Values are presented as mean ± standard deviation or as median with interquartile range. A *p* value less than 0.05 was considered to indicate statistical significance. We used SPSS 15.0 (SPSS Inc, Chicago, Illinois, USA) and R 2.7.1 (R Foundation for Statistical Computing, Vienna, Austria) for the statistical analysis. Missing values were handled by multiple imputation (R-library: Hmisc, function: aregImpute). Missing values were imputed in the following covariates: systolic blood pressure (n = 20), total cholesterol (n = 28), high density lipoprotein cholesterol (n = 68), diabetes mellitus (n = 50), smoking status (n = 48), carotid-femoral pulse wave velocity (n = 658), ankle brachial index (n = 372) and creatinine clearance (n = 48). In the analysis of the original cohort we imputed intima media thickness (n = 246) and C-reactive protein (n = 131).

RESULTS

The baseline characteristics of the study sample are shown in Table 1. The mean age of participants at time of measurement of biomarkers was 67.5 ± 7.6 years in men and 68.9 ± 8.2 years in women. Men had on average a higher blood pressure, a higher total cholesterol to high density lipoprotein cholesterol ratio and a greater likelihood of having diabetes mellitus than women. Levels of cardiovascular risk markers are shown in Table 1. Men had on average a lower NT-proBNP, a higher carotid-femoral pulse wave velocity, a lower creatinine clearance, a higher ankle brachial index and a higher intima media thickness than women, whereas C-reactive protein levels did not differ. In comparison with the participants included in 2000-2001, the participants from the original cohort were older (71.7 versus 64.0 years) and

Table 1. Baseline characteristics of included participants of the Rotterdam study

Characteristic	***Men***	***Women***	***p***
No. of participants	*2032*	*3031*	
Age, years	*67.5 (7.6)*	*68.9 (8.2)*	*< 0.001*
Systolic blood pressure, mmHg	*144 (21)*	*142 (21)*	*0.011*
Total cholesterol, mmol/l	*5.61 (.95)*	*6.04 (0.93)*	*< 0.001*
HDL-C, mmol/l	*1.25 (0.32)*	*1.51 (0.40)*	*< 0.001*
Diabetes mellitus, No. (%)	*207 (10.2)*	*255 (8.4)*	*0.039*
Smoking, No. (%)			*< 0.001*
Never	*329 (16.2)*	*1379 (45.5)*	
Past	*1312 (64.6)*	*1104 (36.4)*	
Current	*376 (18.5)*	*515 (17.0)*	
Body mass index, kg/m^2	*26.5 (3.2)*	*27.3 (4.4)*	*< 0.001*
NT-proBNP, median (IQR), pmol/l	*6.8 (3.7 – 13.6)*	*10.2 (5.8 – 18.0)*	*< 0.001*
PWV, m/s	*13.4 (3.1)*	*12.6 (2.9)*	*< 0.001*
ABI	*1.06 (0.18)*	*1.04 (0.17)*	*< 0.001*
CrC, ml/kg/1,73m^3	*74.7 (16.5)*	*76.4 (17.9)*	*0.001*
IMT, mm	*0.89 (0.16)*	*0.84 (0.14)*	*< 0.001*
CRP, mg/l	*2.24 (1.15 - 4.34)*	*2.30 (1.15 - 4.34)*	*0.984*

Abbreviations: HDL-C, high density lipoprotein cholesterol; cfPWV, carotid-femoral pulse wave velocity; ABI, ankle brachial index, CrC, creatinine clearance; IMT, intima media thickness; CRP, C-reactive protein.

had the percentage of smokers (15.4% versus 20.4%) and diabetics (8.2% versus 10.4%) was lower (Table 2).

The total follow-up time was 9,291 years for men and 14,810 years for women. During follow-up 224 men and 196 women experienced a cardiovascular event, of these, 59 were fatal in men and 49 in women. Coronary heart disease (n = 107) was the most frequent cardiovascular event in men followed by heart failure (n = 60) and ischemic stroke (n = 54). Three men were diagnosed with heart failure and myocardial infarction at the same time. In women, incidences of coronary heart disease (n = 67), heart failure (n = 59) and ischemic stroke (n = 70) were comparable.

Table 2. Characteristics of the original cohort (recruited in 1990-1993) and the cohort extension (recruited in 2000-2001)

Characteristics	***Total Cohort***	***Original Cohort***	***Extension Cohort***	***p***
No. of participants	*5063*	*2857*	*2206*	*-*
Sex, No. males (%)	*2032 (40.1)*	*1080 (37.8)*	*952 (43.2)*	*< 0.001*
Age, years	*68.3 (8.0)*	*71.7 (6.8)*	*64.0 (7.4)*	*< 0.001*
Systolic blood pressure, mmHg	*143 (21)*	*143 (21)*	*142 (21)*	*0.692*
Total cholesterol, mmol/l	*5.9 (1.0)*	*5.9 (1.0)*	*5.8 (1.0)*	*0.208*
HDL-C, mmol/l	*1.4 (0.4)*	*1.4 (0.4)*	*1.4 (0.4)*	*0.002*
Diabetes mellitus, No. (%)	*462 (9.1)*	*233 (8.2)*	*229 (10.4)*	*0.007*
Smoking, No. (%)				*< 0.001*
Never	*1708 (33.7)*	*1021 (35.7)*	*687 (31.1)*	
Past	*2416 (47.7)*	*1356 (47.5)*	*1060 (48.1)*	
Current	*89 (17.6)*	*441 (15.4)*	*450 (20.4)*	
Body mass index, kg/m^2	*27.0 (4.0)*	*26.8 (3.9)*	*27.2 (4.1)*	*0.001*
NT-proBNP, median (IQR), pmol/l	*8.7 (4.8 - 16.4)*	*10.3 (5.9 - 18.3)*	*6.8 (3.7 - 13.3)*	*< 0.001*
PWV, m/s	*12.9 (3.0)*	*13.2 (2.9)*	*12.5 (3.0)*	*< 0.001*
ABI	*1.05 (0.18)*	*1.05 (0.17)*	*1.05 (0.18)*	*0.341*
CrC, ml/kg/1,73m^3	*75.7 (17.4)*	*71.0 (15.5)*	*81.7 (17.7)*	*< 0.001*
IMT, mm	*0.86 (0.15)*	*0.86 (0.15)*	*-*	*-*
CRP, mg/l	*2.3 (1.2 - 4.3)*	*2.3 (1.2 - 4.3)*	*-*	*-*

Abbreviations: HDL-C, high density lipoprotein cholesterol; cfPWV, carotid-femoral pulse wave velocity; ABI, ankle brachial index, CrC, creatinine clearance; IMT, intima media thickness; CRP, C-reactive protein.

In men, after adjusting for age a one standard deviation increase in carotid-femoral pulse wave velocity, creatinine clearance, NT-proBNP and intima media thickness was related to an increased, whereas a one standard deviation increase in ankle brachial index was related to a decreased hazard ratio of cardiovascular disease (Table 3). In women one standard deviation increments in NT-proBNP, ankle brachial index and intima media thickness, but not in carotid-femoral pulse wave velocity, ankle brachial index and creatinine clearance were related to future cardiovascular events (Table 3). C-reactive protein did not associate with an increased risk for cardiovascular disease, neither in men nor in women. After additional adjustment for systolic blood pressure, total cholesterol, high-density lipoprotein cholesterol, diabetes mellitus and smoking, NT-proBNP (hazard ratio 1.47 [95% CI 1.28 to 1.69]), and intima media thickness (hazard ratio 1.15 [95% CI 1.03 to 1.29]) remained independently related to an increased risk for cardiovascular disease in men. In women only NT-proBNP (hazard ratio 1.69 [95% CI 1.46 to 1.95]) remained independently associated with cardiovascular disease. There was a gradual increase in risk for cardiovascular disease from the lowest to the highest quintile in NT-proBNP both in men and women (Figure 1).

Table 3. Hazard ratios for cardiovascular events related to a one standard deviation increase in 6 different cardiovascular risk markers, adjusted for age

		Men		**Women**	
Endpoint	***Risk marker***	*Hazard Ratio*	*95% Confidence interval*	*Hazard Ratio*	*95% Confidence interval*
Cardiovascular Disease	*Pulse wave velocity*	*1.18*	*1.06 - 1.33*	*1.04*	*0.90 - 1.20*
	Ankle brachial index	*0.87*	*0.77 - 0.98*	*0.86*	*0.77 - 0.97*
	Creatinine clearance	*1.19*	*1.01 - 1.40*	*1.11*	*0.92 - 1.33*
	NT-proBNP	*1.54*	*1.35 - 1.76*	*1.71*	*1.48 - 1.98*
	Intima media index	*1.23*	*1.12 - 1.35*	*1.21*	*1.04 - 1.39*
	C-reactive protein	*1.13*	*0.98 - 1.31*	*1.16*	*1.00 - 1.35*

Analysis of the separate endpoints showed that a one standard deviation increase in NT-proBNP was related with an increased risk for coronary heart disease, heart failure and ischemic stroke both in men and women (Table 4). Of the other risk markers increases in intima media thickness and C-reactive

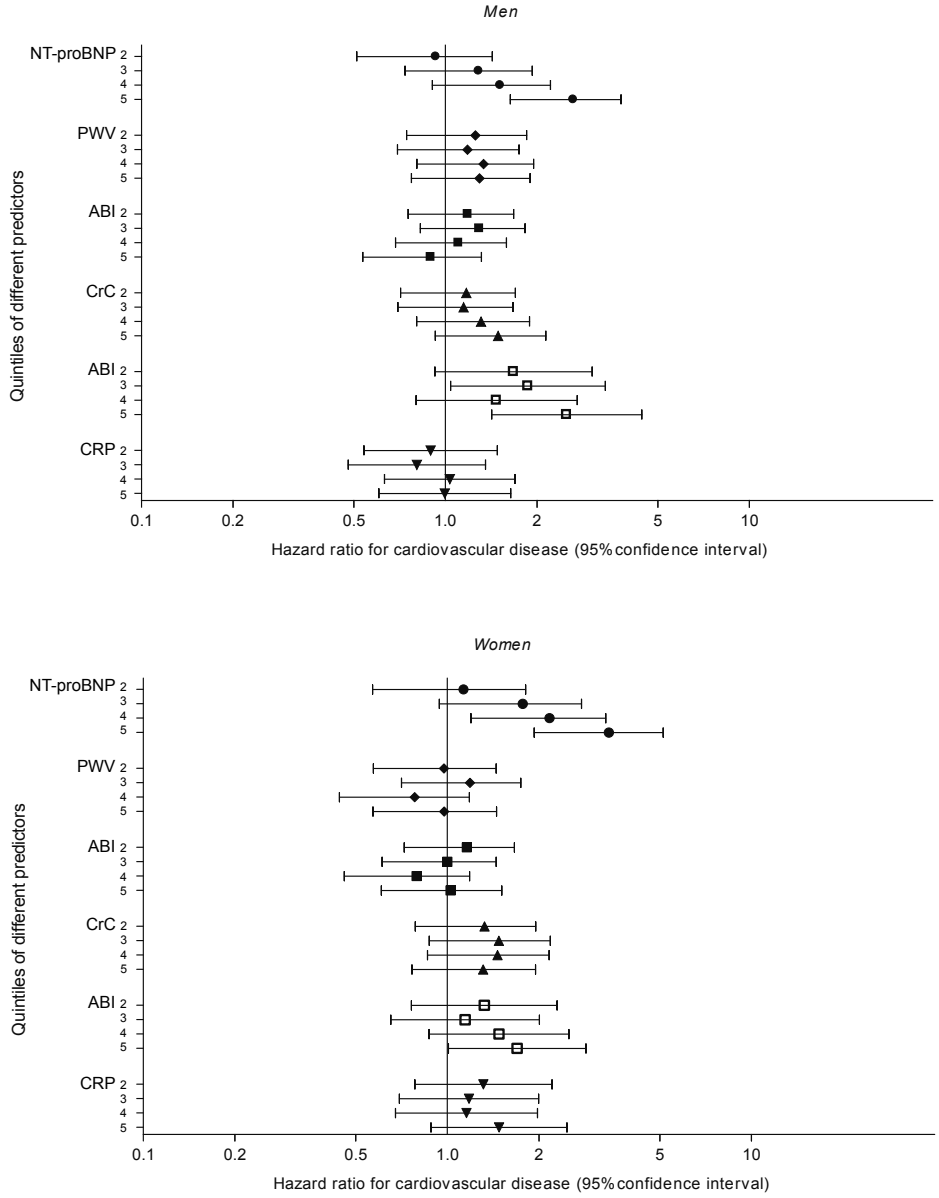

Figure 1. Adjusted hazard ratios for cardiovascular disease related to the four upper quintiles compared to the lowest quintile of amino-terminal pro-B-type natriuretic peptide, carotid-femoral pulse wave velocity, ankle-brachial index, creatinine clearance, intima media thickness and C-reactive protein. Adjusted for age, systolic blood pressure, total cholesterol, high-density lipoprotein cholesterol, smoking, diabetes mellitus. Abbreviations: PWV, pulse wave velocity; ABI, ankle brachial index; creatinine clearance, CrC; IMT, intima media thickness; CRP, C-reactive protein.

Table 4. Hazard ratios for cardiovascular disease, coronary heart disease, heart failure and ischemic stroke related to a one standard deviation increase in six different cardiovascular risk markers, adjusted for traditional risk factors

		Men		Women	
Endpoint	***Risk marker***	*HR*	*95% CI*	*HR*	*95% CI*
Cardiovascular Disease	*Pulse wave velocity*	*1.07*	*0.94 - 1.21*	*1.15*	*0.88 - 1.51*
	Ankle brachial index	*0.95*	*0.84 - 1.08*	*0.92*	*0.81 - 1.05*
	Creatinine clearance	*1.16*	*0.99 - 1.37*	*1.06*	*0.88 - 1.27*
	NT-proBNP	*1.47*	*1.28 - 1.69*	*1.69*	*1.46 - 1.95*
	Intima media index	*1.15*	*1.03 - 1.29*	*1.15*	*0.99 - 1.34*
	C-reactive protein	*1.06*	*0.90 - 1.24*	*1.10*	*0.94 - 1.29*
Coronary Heart Disease	*Pulse wave velocity*	*1.14*	*0.95 - 1.37*	*1.09*	*0.85 - 1.39*
	Ankle brachial index	*0.93*	*0.78 - 1.10*	*0.89*	*0.72 - 1.10*
	Creatinine clearance	*1.25*	*1.00 - 1.57*	*1.10*	*0.81 - 1.51*
	NT-proBNP	*1.31*	*1.07 - 1.60*	*1.64*	*1.27 - 2.11*
	Intima media index	*1.06*	*0.86 - 1.30*	*1.04*	*0.79 - 1.38*
	C-reactive protein	*0.86*	*0.67 - 1.10*	*1.16*	*0.88 - 1.52*
Heart Failure	*Pulse wave velocity*	*1.07*	*0.86 - 1.35*	*0.87*	*0.66 - 1.15*
	Ankle brachial index	*0.82*	*0.66 - 1.01*	*0.81*	*0.66 - 1.00*
	Creatinine clearance	*1.21*	*0.90 - 1.64*	*1.19*	*0.86 - 1.66*
	NT-proBNP	*1.70*	*1.32 - 2.18*	*2.18*	*1.68 - 2.82*
	Intima media index	*1.18*	*0.95 - 1.46*	*1.16*	*0.90 - 1.49*
	C-reactive protein	*1.12*	*0.84 - 1.49*	*1.12*	*0.85 - 1.47*
Ischemic Stroke	*Pulse wave velocity*	*0.89*	*0.66 - 1.19*	*0.88*	*0.68 - 1.14*
	Ankle brachial index	*1.31*	*0.96 - 1.80*	*1.08*	*0.86 - 1.36*
	Creatinine clearance	*0.96*	*0.68 - 1.35*	*0.90*	*0.65 - 1.24*
	NT-proBNP	*1.49*	*1.13 - 1.97*	*1.39*	*1.08 - 1.78*
	Intima media index	*1.26*	*1.07 - 1.50*	*1.23*	*0.96 - 1.59*
	C-reactive protein	*1.42*	*1.06 - 1.92*	*1.03*	*0.79 - 1.35*

protein were related with an increased risk for ischemic stroke in men, but not with coronary heart disease or heart failure (Table 4). Carotid-femoral pulse wave velocity, creatinine clearance, and ankle brachial index were not related with any of the separate endpoints.

To investigate the potential of the new cardiovascular risk markers to discriminate future cases from non-cases we compared the C statistic of the traditional risk prediction model with a prediction model including the traditional risk factors and the risk markers (table 5). The C statistic improved by 0.034 (95% CI 0.012 to 0.052) from 0.660 to 0.694 in men and by 0.034 (95% CI 0.017 to 0.048) from 0.728 to 0.762 in women after incorporation of NT-proBNP in the prediction model. For the other risk markers the increases in C statistic were smaller, maximum 0.008, and non-significant (Table 5). To investigate whether the C statistic of the model including the traditional risk factors and NT-proBNP could be further improved we added carotid-femoral pulse wave velocity, ankle brachial index and creatinine clearance. This did not result in a significant improvement either in men (change in C statistic 0.008 [95% CI –0.002 to 0.016] or in women (change in C statistic 0.000 [95% CI –0.007 to 0.004]).

Incorporation of NT-proBNP in the traditional prediction model resulted in a reclassification of almost 30% of participants, changing from the lowest risk category to higher risk categories or from the intermediate risk category to either the low or high risk category. The net reclassification index was 9.2% (95% CI 3.5% to 14.9%) in men and 13.3% (95% CI 5.9% to 20.8%) in women.

DISCUSSION

In the present study, we investigated the usefulness of six cardiovascular risk markers for the prediction of fatal and nonfatal cardiovascular disease in more than 5000 participants of a community-based cohort followed for a median duration of almost 5 years. We found that incorporation of NT-proBNP in the standard risk model substantially improved the cardiovascular disease risk prediction both in men and in women as reflected by an increase in C statistic. The use of NT-proBNP as risk marker resulted in a significant reclassification of both men and women to a lower or higher risk category. Conversely, the measurement of aortic stiffness, creatinine clearance, ankle

Table 5. Improvement in C statistic of the prediction model for cardiovascular disease for all different risk markers, with 95% confidence interval of the improvement in C statistic

	Men				***Women***			
	C-stat classical	*C-stat new*	*Difference C-stat*	*95% CI*	*C-stat classical*	*C-stat new*	*Difference C-stat*	*95% CI*
Pulse wave velocity	*0.660*	*0.661*	*0.001*	*-0.008, 0.003*	*0.728*	*0.727*	*-0.001*	*-0.003, 0.001*
Ankle brachial index	*0.660*	*0.659*	*0.000*	*-0.005, 0.001*	*0.728*	*0.729*	*0.001*	*-0.004, 0.003*
Creatinine clearance	*0.660*	*0.668*	*0.008*	*-0.003, 0.013*	*0.728*	*0.727*	*-0.001*	*-0.005, 0.000*
NT-proBNP	*0.660*	*0.693*	*0.034*	*0.012, 0.052*	*0.728*	*0.762*	*0.034*	*0.017, 0.048*
Intima media index	*0.655*	*0.661*	*0.006*	*-0.008, 0.016*	*0.675*	*0.678*	*0.004*	*-0.006, 0.010*
C-reactive protein	*0.654*	*0.654*	*0.000*	*-0.007, 0.002*	*0.674*	*0.673*	*-0.002*	*-0.008, 0.002*

Abbreviations: C-stat, C statistic ; classical, classical prediction model; new, new prediction model, CI, confidence interval.

brachial index, C-reactive protein and intima media thickness offered no additional value above standard risk models and no significant reclassification of the participants was seen after introduction of these five markers.

Improvement of cardiovascular risk stratification beyond models based on the traditional risk factors by using a multimarker approach is difficult[36], owing to the fact that substantial independent associations with new markers and outcome are required in order to increase the C statistic. Within the framework of the Framingham study no improvement of risk prediction was found after testing a combination of ten different biomarkers, notwithstanding that for all tested biomarkers an independent association with an increased risk for cardiovascular disease was demonstrated in previous studies.[16] In the Atherosclerosis Risk in Communities (ARIC) study, of the tested 19 biomarkers six were associated with cardiovascular disease, but only lipoprotein-associated phospholipase A2 had an effect on the C statistic (increase by 0.006).[37] In the Uppsala Longitudinal Study of Adult Men, improvement of risk stratification for cardiovascular death was demonstrated in a cohort of elderly men after the simultaneous addition of the biomarkers troponin I, NT-proBNP, cystatin C, and C-reactive protein to the traditional risk model.[36] Recently, in another Swedish cohort, the Malmö Diet and Cancer study, both NT-proBNP and C-reactive protein were retained in the prediction model for cardiovascular disease after a backward selection procedure starting with six different biomarkers.[38] However, the increase in C statistic for the prediction of cardiovascular events was minimal and no significant reclassification was achieved. The four mentioned studies investigated the predictive value of several blood biomarkers, whereas the present study assessed the predictive value of blood biomarkers as well as direct markers of vascular damage. Of the markers tested only NT-proBNP provided substantially additional value. In Uppsala Longitudinal Study of Adult Men a one standard deviation increase in NT-proBNP was associated with the highest hazard ratio for cardiovascular mortality compared to a one standard deviation increase of troponin I, cystatin and C-reactive protein. Also in the Malmö Diet and Cancer study NT-proBNP was retained in the prediction models for cardiovascular and coronary heart disease. In conjunction with the results of the studies mentioned, it appears that NT-proBNP has superior predictive value compared to other risk markers.

In our study carotid-femoral pulse wave velocity, ankle brachial index, creatinine clearance and C-reactive protein were not associated with cardio-

vascular disease after adjustment for the traditional risk factors. We excluded all participants with a history of cardiovascular disease and a NT-proBNP level above the age-specific cutoff value for the diagnosis of heart failure. The majority of participants was included in 1990-1993 and followed until the third examination round in 1997-2000. All participants that experienced a cardiovascular event in the years before the third examination round were excluded from the analysis. Related to this long run-in period the number of participants incorrectly classified as being free of cardiovascular disease was undoubtedly very low, leaving a cohort with a relatively low baseline cardiovascular disease risk. The exclusion of subjects with NT-proBNP levels above the diagnostic cutoff value of heart failure further reduced the number of participants with subclinical cardiovascular disease. The predictive value of cardiovascular risk markers may become weaker when tested in a low-risk population. Elevated pulse wave velocity has been associated with an increased risk for cardiovascular disease in populations with hypertension[39-41], diabetes mellitus[42] and renal disease.[43, 44] In a Danish community-based cohort elevated pulse wave velocity was associated with an increased risk for cardiovascular disease, but the association was not as strong as in the mentioned patient populations and disappeared after exclusion of participants on antihypertensive therapy.[11] There is also evidence that the association between creatinine clearance and cardiovascular disease is influenced by the characteristics of the studied population, such as age or the prevalence of cardiovascular disease. In patients with a myocardial infarction or hypertension[45, 46] the risk for cardiovascular disease was found increased with decreasing creatinine clearance, whereas the findings in community-based cohorts varied from study to study.[8, 9, 14, 15, 46, 47] In our study no association between creatinine clearance and cardiovascular disease was present after adjustment for the traditional risk factors and after excluding subjects with high NT-proBNP plasma levels.

In a recent meta-analysis of 16 large scale population-based studies, an ankle-brachial index below 1.11 was associated with an increased hazard ratio for total mortality, cardiovascular mortality and major cardiovascular events.[7] The participants from the original cohort of the Rotterdam study were included in this meta-analysis.[12] In our analysis, including most of the participants of the original cohort, we did not find an increased risk for cardiovascular disease with a lower ankle-brachial index. This might be explained by the exclusion of all subjects with a history of cardiovascular

disease, whereas in the studies of the meta-analysis only participants with coronary heart disease were excluded. Apparently, the clinical characteristics of the cohorts under consideration largely explain why markers such as pulse wave velocity and ankle brachial index may or may not have additional predictive value over the traditional risk factors.

The most interesting finding of the present study was that NT-proBNP had by far the strongest predictive value in comparison with the other risk markers. Our findings are in agreement with previous findings and underscore the predictive potential of NT-proBNP in populations with a relative low risk for cardiovascular disease.[17-19, 36, 48] In our study a continuous increase in risk was seen with increasing elevated NT-proBNP levels. In addition elevations of NT-proBNP were associated not only with heart failure, but also with ischemic heart disease and stroke. Our findings demonstrate that elevated NT-proBNP plasma levels, still below the diagnostic cutoff value of heart failure, have the ability to identify older subjects with generalized subclinical cardiovascular disease. Not only ventricular wall stress, but also cardiac ischemia is a stimulus for natriuretic peptide release by cardiomyocytes.[49, 50] There is evidence that myocardial scars, identified by cardiac magnetic resonance imaging, are present in substantial proportion of older subjects without recognized myocardial infarction.[51] It could be that these subjects might have modestly elevated NT-proBNP levels as well, explaining why NT-proBNP is a predictor of heart failure as well as cardiovascular disease in older subjects.

Our study has several limitations. First, we performed our analyses in a cohort of subjects older than 55 years; therefore our findings cannot be extrapolated to younger subjects. Second, all risk markers were not assessed in each participant, and missing variables were imputed. This may have led to a decreased predictive value of especially carotid-femoral pulse wave velocity for which the largest number of missing data had to be imputed. C-reactive protein and intima media thickness were only measured in the original cohort, therefore our analyses may have been underpowered to demonstrate significant improvement of prediction by using C-reactive protein and intima media thickness. Third, we investigated a Caucasian population; whether our results can be extrapolated to other ethnic groups requires further investigation. Fourth, the risk markers were measured only once, and we have no information on the changes of these markers over time. Possibly, the change

over time of a particular marker is a better predictor of someone's future cardiovascular risk than only a single measurement.

CONCLUSION

In the general population without prevalent cardiovascular disease the serum NT-proBNP level is predictive for first fatal and nonfatal cardiovascular events, including coronary heart disease, heart failure and stroke, both in men and women beyond the traditional risk factors, but carotid-femoral pulse wave velocity, ankle brachial index, creatinine clearance, intima media thickness and C-reactive protein lack additional predictive value.

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9

General discussion

COST-EFFECTIVENESS OF NATRIURETIC PEPTIDE TESTING IN THE EMERGENCY DEPARTMENT

NT-proBNP and BNP are accurate biomarkers for heart failure in the Emergency Department.[1, 2] Both peptides have been introduced in daily clinical practice to improve the diagnostic accuracy in patients presenting with acute shortness of breath. Since their introduction one question remained unanswered: does the use of point of care BNP or NT-proBNP measurements improve the care of patients with acute dyspnea? Worldwide, three randomized controlled trials, besides our study (Chapter 3), have been performed to address this question (Table 1).[3-5] In our study we could demonstrate that introduction of NT-proBNP in the emergency department results in a decrease in the time to discharge and in overall costs. A reduction in time to

Table 1. Baseline characteristics and main results of four randomized trials on the impact of introduction of BNP or NT-proBNP on the length of hospital stay and costs of patients presenting with acute dyspnea

	Mueller et al, 2004	***Moe et al, 2007***	***Rutten et al, 2008***	***Schneider et al, 2009***
No. of subjects	*452*	*500*	*477*	*612*
Country	*Switzerland*	*Canada*	*Netherlands*	*Australia*
Natriuretic peptide	*BNP*	*NT-proBNP*	*NT-proBNP*	*BNP*
Threshold to rule in heart failure	*145 pmol/l*	*53 pmol/l < 50 y* *106 pmol/l > 50 y*	*120 pmol/l*	*145 pmol/l*
Threshold to rule out heart failure	*29 pmol/l*	*35 pg/ml*	*17 pmol/l ♀* *11 pmol/l ♂*	*29 pmol/l*
Age, years	*71*	*70*	*59*	*73*
Male, %	*58*	*51*	*54*	*53*
Smoking, %	*24*	*-*	*36*	*13*
History heart failure, %	*-*	*34*	*43*	*36*
NYHA class IV, %	*27*	*52*	*32*	*-*
Time to discharge	*8.0 vs 11.0*	*1.9 vs 3.9*	*-*	*-*
Relative Risk for 30 day mortality, study vs control (95% CI)	*0.79 (0.47-1.34)*	*-*	*0.86 (0.44-1.66)*	*0.95 (0.53-1.72)*
Cost reduction per patient, €	*1484*	*760*	*1096*	*-*

hospital discharge was also found by Mueller et al, whereas a reduction in costs was demonstrated both by Mueller et al and Moe et al.[3, 4]

The study of Mueller et al was the only study to demonstrate a decrease in the number of hospital and intensive care unit admissions. Recently, Schneider et al published results of a randomized clinical trial on the effect of introduction of point of care BNP measurements in two Australian academic hospitals.[5] In this study no effect on the number of hospital admissions was found, which agrees with our findings and with those of Moe et al. This is not unexpected as the need for admission to a general ward or the intensive care unit depends more on the severity of the condition than on the underlying diagnosis.

The difference between on one hand the reductions in time to discharge, observed in our study and the study of Mueller et al, and on the other hand the similar duration of hospital stay in both the study and control group in our study can be explained as follows. The time to discharge included the total time from entering the emergency department to discharge from the hospital. The length of hospital stay is the time from admission to a ward to discharge from the hospital. The time of discharge is therefore influenced by the number of admissions. As the duration of an emergency department visit is considerably shorter than an admission to a ward, a relative small decrease in the number of admitted patients will result in a relatively large reduction in time to discharge even if the decrease in the percentage of admitted patients is not statistically significant. This reduction in time to discharge ultimately results in a reduction in overall costs.

From the results of the four different studies one could speculate for which category of patients the largest reduction in costs can be found. In a post-hoc analysis we found that the largest effect on costs was present in patients with cardiac disease, pointing towards the high sensitivity of NT-proBNP for diagnosing heart failure. Mueller et al reported that the proportion of patients with an exacerbation of chronic pulmonary obstructive disease was higher in the study than in the control group.[4] As BNP is an excellent marker to exclude cardiac disease it is plausible that in the study of Mueller the high negative predictive value of BNP resulted in a better outcome of the study group with regard to hospital admission and costs. Indeed, if the negative predictive value of BNP is the most important factor, this also explains the negative findings of Schneider et al, because in their study the proportion of patients with heart failure was higher in the study than in the control group.[5]

In addition, in the study of Schneider et al only subjects with severe dyspnea were included.[5] We also included subjects with less severe dyspnea and one could argue that especially in this group a high level of diagnostic accuracy will preferably result in out-hospital treatment instead of admission to the hospital for analysis and treatment.

A recent meta-analysis by Trinquart et al, including all four studies, concluded that the introduction of point of care measurements of BNP and NT-proBNP results in a reduction in the time to discharge and costs without negative effects on outcome (length of hospital stay, hospital admission, secondary admission, or mortality).[6] Although none of the four trials were individually powered to show a difference in mortality rates, analysis of more than 2000 subjects showed that there was no increased risk of mortality. To determine which category or categories of patients will benefit most of point of care BNP or NT-proBNP measurements would require a large multi-center trial. However, as a cost-reduction is seen with natriuretic peptide testing without an increase in mortality or hospital readmission rate, point of care BNP and NT-proBNP measurements can be advocated for all patients with acute dyspnea presenting to the emergency department.

DETECTION OF ECHOCARDIOGRAPHIC ABNORMALITIES

Detection of echocardiographic abnormalities using natriuretic peptides as biomarker has been tested in the population, primary care and hospital-based settings. The regulation and production of proANP and proBNP derived peptides are different, hence it was expected that the diagnostic characteristics for detection of echocardiographic abnormalities would be different as well. The largest part of BNP and NT-proBNP secretion is from the left ventricle, being the largest and most muscular heart chamber. In healthy subjects the main source of ANP are the atrial cardiomyocytes.[7] In patients with left ventricular hypertrophy ANP is also produced by ventricular cardiomyocytes.[8] In our study (chapter 4) left ventricular mass correlated with BNP, but not with ANP. Similar findings were reported by Yamamoto et al, showing that BNP is a more superior biomarker to detect cardiac abnormalities than ANP.[9] BNP and NT-proBNP have also been shown to correlate with the volume, dimensions and ejection fraction of the left ventricle.[10]

In our study only a weak association with varying degrees of diastolic heart failure and natriuretic peptides was found. Possibly, this was due to the higher mean age of our cohort and to the fact that the majority of our subjects was asymptomatic. The stronger associations between diastolic heart failure and BNP reported by other investigators were demonstrated in less aged subjects with chronic heart failure[11] and in subjects with symptomatic diastolic heart failure.[12, 13] The difference in test characteristics of BNP for diastolic cardiac dysfunction between subjects with and without symptoms has been recently highlighted in an asymptomatic cohort in which BNP discriminated poorly between subjects with and without diastolic heart disease (area under the receiving operator characteristics curve 0.71).[14]

Aortic or mitral valvular lesions may result in a pressure and/or volume overload of the left ventricle. This may explain why we found a strong association between BNP and valvular lesions. In subjects with aortic stenosis the increase in BNP has been reported to be related to the increase in left ventricular mass.[15] Especially pressure overload, causing an increase in left ventricular wall stress, is related to increased BNP levels. In a study in subjects with mitral regurgitation BNP was not related to left ventricular volume, while a strong association was found with pulmonary artery pressure.[16] Elevated BNP levels predicted the presence of pulmonary hypertension and left atrial enlargement even when left ventricular systolic function at rest or during dobutamine stress was normal.[16]

In our study on the association between natriuretic peptides and echocardiographic abnormalities (chapter 4) we concluded that normal reference BNP levels should predominantly be used to exclude echocardiographic abnormalities. This corresponds with the recent guidelines of the European Society of Cardiology for the diagnosis and treatment of heart failure, in which the use of BNP and NT-proBNP as rule-out test for heart failure in primary care patients is recommended.[17] Although normal reference values of BNP and NT-proBNP vary with age, renal disease and other co-morbid conditions, the guidelines recommended one common lower cutoff level of either BNP or NT-proBNP for exclusion of heart failure. Recently, Hildebrandt et al pooled the data of 11 studies (total n = 5508) to investigate the effect of age on the diagnostic value of NT-proBNP for systolic heart failure, defined as a left ventricular ejection fraction of 40% or lower.[18] General diagnostic performance was best in the youngest subjects and the area under the receiving operator characteristics curve gradually declined from 0.95 in subjects

< 50 years, to 0.90 in subjects 50-75 years and to 0.82 in subjects > 75 years. When instead age-specific cutoff levels of NT-proBNP were used, the negative predictive value (NPV) was fairly good in all three age categories (< 50 years, NT-proBNP 5.9 pmol/l, NPV 99.7%; 50-75 years, NT-proBNP 8.8 pmol/l, NPV 96.8%; > 75 years, NT-proBNP 29.5 pmol/l, NPV 92.3%). The same seems to be true for BNP, but less data on BNP is available and the number of included elderly subjects in all BNP studies is relatively small.[19-21] In conclusion, to exclude echocardiographic abnormalities relatively low serum levels of NT-proBNP or BNP have to be used.

ASSOCIATION OF NT-PROBNP AND MARKERS OF VASCULAR STIFFNESS

When the aorta and large elastic arteries become stiffer as normally occurs with increasing age, their cushioning function decreases. This leads to adverse hemodynamic effects, including an increase in systolic blood pressure and pulse pressure with increased systolic cardiac load and decreased myocardial perfusion pressure.[22, 23] We hypothesized that this increase in cardiac load and decreased cardiac perfusion would result in higher NT-proBNP plasma levels in subjects with increased vascular stiffness. In chapter 5 it is shown that after age, vascular stiffness, as estimated by pulse pressure and carotid femoral pulse wave velocity, is the covariate with the largest influence on the baseline levels of NT-proBNP in men and women free of cardiovascular disease. Both vascular stiffness and NT-proBNP increase with age, but an independent effect of vascular stiffness on NT-proBNP levels was found in multivariate linear regression models.

In the on average younger subjects of the Framingham cohort (mean 61 versus 68 years in our cohort) a comparable association between BNP and measures of vascular stiffness as in our study was found in men, whereas in women no association was present.[24] Recently, the results of two studies were published, both reporting a positive relation between BNP and measures of vascular stiffness, however compared to the Framingham study and our study the number of participants was too small to draw definite conclusions.[25, 26] Based on the studies on this subject, it can be concluded that an independent positive association between natriuretic peptides and measures of vascular stiffness likely exists, but that the magnitude of the association is

relatively weak. Although being the second most influential covariate in our study, only 5.7% of the variation in NT-proBNP in men and 2.6% in women could be explained by vascular stiffness.[24, 27]

The relation between age, arterial stiffness and natriuretic peptides cannot be ascertained completely unequivocally in a cohort study. In humans the degree of vascular stiffness is mainly determined by structural changes in elastin and collagen content of the vessel wall due to physiologic aging of the vessels. This is especially true in individuals without cardiovascular disease at older age, because in this group premature vascular aging due to hypertension, smoking and other cardiovascular risk factors is less pronounced. Probably in a younger population (age < 40 years) with a relatively large variation in vascular stiffness the effect on natriuretic peptides is more pronounced. In addition the negative effect of vascular stiffness on the left ventricle and hence on B-type natriuretic peptides may be more evident during exercise than under resting conditions. Therefore, it might be hypothesized that the association between vascular stiffness and BNP or NT-proBNP becomes stronger when the two biomarkers are measured after exercise.

PREDICTING OUTCOME USING NATRIURETIC PEPTIDES

Cardiovascular disease can be prevented by various lifestyle interventions and treatment of risk factors. Obviously the number of life years gained and decrease in morbidity in response to treatment will be largest in individuals with the highest risk for cardiovascular disease. Researchers have therefore investigated large groups of subjects to pinpoint risk factors and risk markers for cardiovascular disease for more than thirty years. Risk factors can be modifiable conditions or habits that are related to cardiovascular disease, such as hypertension and smoking, but also conditions that cannot be modified, such as age and male sex. Various cardiovascular risk models, like the Framingham Risk Score and Systematic Coronary Risk Evaluation (SCORE) are used in daily clinical practice. Because in patients with a history of cardiovascular disease treatment of risk factors is always required, risk models are especially useful to assess the risk of subjects without a history of cardiovascular disease. There is a continuing search for new risk factors and risk markers to further improve the accuracy of the prediction of cardiovascular disease and treatment in a subject.

In a preliminary study we showed that increased natriuretic peptide levels (ANP and BNP) in subjects with end-stage renal disease undergoing peritoneal dialysis were associated with an increased risk for mortality (Chapter 6). Although the number of subjects was fairly small, the mortality risk was clearly increased in subjects with relatively high natriuretic peptide levels. Our results have been confirmed by others. Roberts et al found an increased risk for mortality in subjects undergoing long-term dialysis when their BNP level was increased.[28] Furthermore, in 965 subjects on peritoneal dialysis NT-proBNP was independently associated with cardiovascular disease and mortality after correction for age, diabetes, presence of peripheral vascular disease, baseline albumin, normalized protein nitrogen appearance, urine volume, peritoneal ultrafiltration, and measures of chronic inflammatory status (interleukin 6, high-sensitivity C-reactive protein), which all are factors known to influence outcome in patients on peritoneal dialysis.[29]

We (Chapter 7) and others have shown that an increase in NT-proBNP is related to an increase in the risk for cardiovascular disease.[30-32] Although a risk factor may be significantly related to cardiovascular events, this does not necessarily mean that addition of such a risk factor to an existing prediction model has additive value for the risk prediction of individuals. For instance, the risk related to a risk factor or marker may be significant but very small or a risk marker may only improve the risk prediction in individuals with a high risk, whereas the main purpose of the risk model is to differentiate between subjects with a low and intermediate risk. The C statistic of a prediction model is a measure of the probability that the predicted risk is higher for a case than for a non-case. The C statistic has been used to assess the discrimination of a model, i.e. the power of a model to discriminate between subjects that will develop an event and or not.[33] The risk ratio related to a marker needs to be substantial in order to have a significant impact on the C statistic.[33]

In chapter 7 we report that incorporation of NT-proBNP in a risk model based on the classical risk factors age, systolic blood pressure, cholesterol, high-density lipoprotein, smoking and diabetes significantly improves the C statistic for the prediction of cardiovascular events in subjects aged 55 years or older without a history of cardiovascular disease. Recently, deFilippi et al demonstrated that the prediction of cardiovascular events could be further improved by serial measurements of NT-proBNP.[34] As clinical decisions to start or withhold preventive cardiovascular drugs are based on cutoff levels

of 10-year risk, we also investigated the reclassification of subjects after adding NT-proBNP to the classical risk model. We could establish that the classification improved with cases classified in higher risk categories and non-cases classified in lower risk categories.

NT-proBNP improves the risk prediction of subjects above 55 years of age without a history of cardiovascular disease. However, it is unclear whether NT-proBNP also predicts cardiovascular events in subjects without hypertension, diabetes mellitus, dyslipidemia. In the subgroup of the cohort of Olmsted (Rochester Epidemiology Project, follow-up of 8.9 years) including only subjects without hypertension, smoking, diabetes mellitus and echocardiographic abnormalities (n = 703) NT-proBNP did not predict cardiovascular disease.[35] Probably the number of subjects was too small to assess the predictive value of any new biomarker. Second, the incidence of cardiovascular events was considerably lower than in our study (10% versus 16% in women and 25% in men) diminishing the power to detect an incremental value of NT-proBNP. Third, all subjects in the subgroup were screened by echocardiography and excluded if abnormalities were found.

In chapter 8 we compare the predictive value of NT-proBNP with pulse wave velocity, ankle-brachial index, creatinine clearance, intima media thickness and C-reactive protein and found that the other risk markers have no additional predictive value above the traditional risk factors in older subjects free of CVD. In three other studies NT-proBNP also appeared to be the biomarker with the highest additive value. In the Uppsala Longitudinal Study of Adult Men (ULSAM), improvement of risk stratification for cardiovascular death was demonstrated in a cohort of elderly men after the simultaneous addition of the biomarkers troponin I, NT-proBNP, cystatin C, and CRP to the traditional risk model, but NT-proBNP had the highest predictive value.[32] In another Swedish cohort, the Malmö Diet and Cancer study (MDC), only NT-proBNP and CRP were retained in the prediction model for CVD after a backward selection procedure starting with six different biomarkers.[36] In the most recent and largest study reported by Blankerberg et al (n = 7915, confirmatory cohort n = 2551) comparing 30 different biomarkers, only NT-proBNP, C-reactive protein and troponin I were retained in the final model.[37]

In conclusion, NT-proBNP improves the risk prediction of individuals above 55 years of age without a history of cardiovascular disease. The predictive value can probably be improved by adding one or two other biomarkers such as C-reactive protein and troponin I, but of all the biomarkers tested

head-to-head in various cohorts NT-proBNP has the highest predictive value. The important question whether natriuretic peptide testing in general should be included in cardiovascular prediction models in order to improve their predictive value (incremental predictive values) cannot be answered at this moment and requires further investigation. At this moment the first steps are undertaken to combine the findings of the various studies, including our study published in Hypertension, that have explored the additive value of NT-proBNP testing in cardiovascular risk prediction under the name of Natriuretic Peptides Studies Collaboration (NPSC). This study is coordinated by Professor John Danesh from the Department of Public Health & Primary Care, University of Cambridge, UK.

FUTURE RESEARCH

After introduction of BNP measurements in daily clinical practice it was observed that BNP plasma levels were reduced in obese compared to lean subjects. For two reasons this is a counterintuitive finding. First, an increase in body mass index is associated with an increase in extracellular volume and cardiac output and second the presence of adiposity or obesity increases cardiovascular risk. Obviously, these two factors would increase rather than decrease cardiac BNP production. The decrease in BNP seen with an increase in body mass index seemed at first easily explained by the increase in natriuretic peptide clearance receptors that are abundantly present on adipocytes. However, with NT-proBNP, a peptide that is not cleared by natriuretic peptide clearance receptors also an inverse association between body mass index and NT-proBNP plasma levels was found. Consequently, a decreased release of natriuretic peptides from the heart, rather than an increased clearance may be responsible for the association between higher body mass index and lower natriuretic peptide levels.

After bariatric surgery patients lose fat mass, but their NT-proBNP plasma levels increase, supporting the presence of an effect of adipose tissue on natriuretic peptide levels.[38] In another study it was shown that the inverse association between body mass index and NT-proBNP was mediated by total body lean mass rather than by total body fat mass, from which the researchers postulated that a substance produced in the lean mass may suppress cardiac synthesis of natriuretic peptides.[39] Alternatively, this effect could be

mediated by sex steroid hormones that coordinately influence natriuretic peptide synthesis as well as body position. For example androgens, which promote the development of lean mass, may suppress the natriuretic peptide release, whereas estrogens, which are associated with lower lean mass, increase natriuretic peptide levels. Because in obese subjects testosterone levels are lower due to peripheral aromatization, it has been hypothesized that this is the mechanism responsible for the lower natriuretic peptide levels in obese individuals.

Another possible explanation for the decrease in natriuretic peptide levels in obese subjects might be the increase in insulin levels. In the Rotterdam study we found a negative association between insulin and NT-proBNP. In the near future we will explore the effect of insulin on NT-proBNP, as insulin may affect the cardiac synthesis and/or release of NT-proBNP and BNP. This will be explored both in clinical studies (for instance in diabetic patients in whom insulin therapy is initiated) and in experimental studies using isolated neonatal rat and human cardiomyocytes.

The interplay between increase body mass index, natriuretic peptides, insulin and sex hormones might explain the difference in natriuretic plasma levels found between men and women and between obese and lean subjects. Men have lower natriuretic peptide levels than women, but have a higher risk for cardiovascular disease at a younger age. This underlines that the positive association found between NT-proBNP and cardiovascular disease is influenced by a number of factors. NT-proBNP and BNP reflect cardiac load, but are influenced by various cardiovascular risk factors such as hypertension, body mass index, gender and renal function. Experimental and epidemiological studies on the effects of various cardiovascular risk factors on NT-proBNP may not only provide insight in their direct effect on natriuretic peptides levels, but may also provide information on how the different risk factors contribute to the cardiovascular risk of individuals. The fact that of more than 30 new cardiovascular risk markers evaluated NT-proBNP remains the strongest predictor of cardiovascular disease underlines the potential role of natriuretic peptides in cardiovascular risk assessment. New cardiovascular risk markers such as genetic profiling will have to be compared with NT-proBNP on their incremental predictive value of cardiovascular disease.

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10

Summary

CHAPTER 1 AND 2: INTRODUCTION AND AIM OF THESIS

The natriuretic peptide family consists of three different types of peptides: atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP) and C-type natriuretic peptide (CNP). All three peptides have a central intramolecular ring structure of 17 amino acid residues. This ring structure is also found in natriuretic peptides of other species. The biologically active peptides (ANP, BNP and CNP) are cleaved from pro-peptides (proANP, proBNP, proCNP) before release in the circulation. Both the active peptide and its amino-terminal counterpart can be measured in plasma and serum (i.e. BNP, NT-proBNP). Two types of guanylyl cyclase coupled receptors have been identified: the natriuretic peptide receptor A and B (NPR-A and NPR-B). NPR-A has the highest ligand selectivity for ANP and BNP, whereas NPR-B predominantly binds CNP. Stimulation of the NRP-A results in an increase in glomerular filtration rate, an increase in renal sodium excretion, hence their name natriuretic peptides, and inhibition of renin and aldosterone. The natriuretic peptide receptor C has a high affinity for all natriuretic peptides and removes peptides from the circulation by internalization. Natriuretic peptides are also enzymatically degraded by neutral endopeptidase. In humans the majority of BNP is formed in ventricular cardiomyocytes. The BNP gene expression is mainly influenced by mechanical strain. BNP transcription is also induced by neurohormones, pro-inflammatory proteins and thyroid hormones.

BNP and NT-proBNP are increased in patients with heart failure. Both BNP and NT-proBNP have an excellent sensitivity for detection of a decreased left ventricular systolic function. The specificity is, however, too low to recommend screening of asymptomatic populations for heart failure using natriuretic peptides. The diagnostic characteristics improve when the incidence of heart failure is higher such as in patients presenting to the general practitioner with complaints of dyspnea. Sensitivity and specificity are best in subjects presenting to the emergency department with complaints of acute dyspnea. It was expected that BNP and NT-proBNP were not only outstanding markers for the diagnosis of heart failure, but that natriuretic peptide guided treatment of patients admitted for heart failure or followed in an outpatient clinic for heart failure would improve prognosis. Recent studies have shown that BNP guided treatment has no advantage over treatment guided by clinical judgment in the older heart failure patient, but may be useful in patients below 65 years of age. Due to their clear association not only with heart

failure related morbidity and mortality but also with cardiovascular disease in general BNP and NT-proBNP may have incremental predictive value for cardiovascular disease.

The aims of the present thesis were: 1) to assess the impact of introduction of NT-proBNP measurement on clinical decision making in patients presenting to the emergency department of the Erasmus MC with acute dyspnea; 2) to investigate the correlation of natriuretic peptide plasma levels with echocardiographic abnormalities in a geriatric outpatient cohort; 3) to determine the influence of increasing large artery stiffness on natriuretic peptide levels; 4) to investigate the prognostic value of natriuretic peptides in patients with end-stage renal disease; 5) to investigate the independent predictive value of natriuretic peptides for cardiovascular morbidity and mortality in older subjects without a history of cardiovascular disease and compare its predictive value with commonly used other risk markers.

CHAPTER 3: NT-PROBNP TESTING IN THE EMERGENCY DEPARTMENT

NT-proBNP is an established biomarker for heart failure in patients presenting to the emergency department with acute dyspnea. Assessment of this biomarker may result in improved patient care and reduced costs. In a Swiss study an improvement in patient care was seen after introduction of BNP testing in the emergency department. Since the effect of introduction of natriuretic peptide testing on clinical outcome and cost savings strongly depends on the way medical care is organized and delivered, the objective of this study was to investigate whether introduction of rapid NT-proBNP testing in the emergency department of our hospital associates with improved diagnostic decision making as reflected by cost savings without compromising clinical outcome.

In a prospective clinical trial, patients presenting with acute dyspnea to the emergency department of the Erasmus MC, Rotterdam, the Netherlands were randomized for either rapid measurement or no measurement of NT-proBNP. For ruling *out* heart failure, cutoff values of 11 pmol/l in male and 17 pmol/l in female patients were used, and for ruling *in* heart failure a cutoff value of 120 pmol/l was used. Time to discharge from the hospital and costs related to hospital admission were primary end-points. Bootstrap

analysis was used for comparison of costs and 30-day mortality between the NT-proBNP and control group. A total number of 477 patients (54% male) was enrolled. The mean age of the participants was 59 years, with 44% of patients having a history of cardiac disease. Median time to discharge from the hospital was 1.9 (interquartile range 0.12 to 8.4) days in the NT-proBNP group (n = 236) compared to 3.9 (interquartile range 0.16 to 11.0) days in the control group (n = 241) (p = 0.04). Introduction of NT-proBNP testing resulted in a trend towards a reduction in costs related to hospital admission and diagnostic investigations of 1096 euro per patient (95% confidence interval: –197 to 2574 euro), whereas 30-day mortality was similar (15 patients in the NT-proBNP and 18 patients in the control group).

The results of our study indicate that rapid NT-proBNP testing in the emergency department for patients presenting with acute dyspnea is highly likely associated with cost savings due to an overall shorter stay in the hospital. Importantly, this beneficial effect is not counterbalanced by adverse effects on in-hospital and 30-day mortality.

CHAPTER 4: ANP, BNP AND ECHOCARDIOGRAPHY ABNORMALITIES

Identification of elderly subjects with cardiac dysfunction can be difficult, as geriatric patients often present with atypical symptoms such as fatigue or dyspnea. Medical intervention reduces morbidity and improves survival in patients with cardiac dysfunction and heart failure, identification of patients who could benefit from treatment is therefore important. This study was performed to investigate the correlation between increased natriuretic peptide plasma levels and echocardiographic abnormalities in geriatric outpatients.

We performed two-dimensional transthoracic echocardiography in 209 community-dwelling subjects visiting the geriatric outpatient clinic of our university hospital. Subjects were 65 years or older and had no markedly impaired cognitive function. Mean ANP and BNP plasma levels were respectively 11.0 and 10.8 pmol/l. The plasma level of BNP, but not of ANP, progressively increased with the number of echocardiographic abnormalities and correlated with left ventricular dysfunction and left ventricular mass. A natriuretic peptide level in the highest tertile was associated with a greater risk for echocardiographic abnormalities, BNP odds ratio 7.15 (95% CI 2.15

to 23.71), ANP odds ratio 3.07 (95% CI 1.15 to 8.16). At a cutoff of BNP of 6.85 pmol/l, sensitivity was 92%, specificity 31%, negative predictive value 97% and positive predictive value 16%. We conclude that increased natriuretic peptide levels are indicative of echocardiographic abnormalities in geriatric outpatients. Compared with ANP, BNP correlates better with echocardiographic abnormalities in geriatric outpatients. If after careful history taking and physical examination uncertainty remains regarding the presence of heart disease in a geriatric outpatient BNP testing may help to exclude severe cardiac abnormalities.

CHAPTER 5: EFFECT OF LARGE ARTERY STIFFNESS ON NT-PROBNP

When the aorta and large elastic arteries become stiffer with increasing age, their cushioning function decreases. This results in adverse hemodynamic effects, including an increase in systolic blood pressure and pulse pressure with increased systolic cardiac load and decreased myocardial perfusion pressure. As increased left ventricular wall stretch and myocardial ischemia are both important stimuli for the release of NT-proBNP, we hypothesized that arterial stiffness is a determinant of circulating levels of NT-proBNP.

In a large community-dwelling older population (n = 6211, mean age 69.2 years) serum NT-proBNP, brachial pulse pressure and carotid-femoral pulse wave velocity were measured. In subjects without cardiovascular disease median NT-proBNP was 6.7 pmol/l in men (n = 2073) and 10.1 pmol/l in women (n = 3085) ($p < 0.001$). In these subjects, indices of arterial stiffness correlated with NT-proBNP with beta coefficients for brachial pulse pressure and carotid-femoral pulse wave velocity of 0.315 and 0.255 in men and 0.233 and 0.232 in women (all $p < 0.001$). After multivariable adjustment (age, weight, height, mean arterial pressure, heart rate, smoking, diabetes, estimated glomerular filtration rate, total and high density lipoprotein cholesterol and use of lipid lowering and antihypertensive medication) these associations remained significant for brachial pulse pressure and carotid-femoral pulse wave velocity in men and for brachial pulse pressure in women. In multivariable adjusted models brachial pulse pressure explained 20.3% and carotid-femoral pulse wave velocity 10.7% of the variation of NT-proBNP in men and respectively 10.8% and 9.4% in women. In subjects with

prevalent cardiovascular disease indices of arterial stiffness and NT-proBNP were unrelated in multivariable adjusted models.

Our findings show that arterial stiffness is independently associated with elevated NT-proBNP levels in subjects without prevalent cardiovascular disease. We also demonstrated that the association between arterial stiffness and NT-proBNP is confounded by several factors of which age is most important. The association between vascular stiffness and NT-proBNP is stronger in men than in women and absent in individuals with prevalent cardiovascular disease.

CHAPTER 6: BNP AND NT-PROANP IN PERITONEAL DIALYSIS

Mortality in patients with end stage renal disease is largely determined by cardiovascular disease. In chapter 6 we report on the prognostic value of BNP and amino-terminal pro-atrial natriuretic peptide (NT-proANP) for overall mortality in patients with end stage renal disease on peritoneal dialysis. From a large, prospective multi-center study (n = 1464) which follows patients with end stage renal disease from the time of initiation of dialysis up to transplantation, end of follow-up or death, we selected a random sample of 68 peritoneal dialysis patients without clinically overt heart failure. Six months after the start of dialysis blood was collected for measurement of NT-proANP and BNP. During a follow-up ranging from 1.5 to 4.5 years mortality was 15%. An NT-proANP and a BNP level above the median was associated with an increased mortality risk (NT-proANP: hazard ratio 11.3 [95% CI 1.4 to 91.9]; BNP: hazard ratio 11.3 [95% CI 1.4 to 91.4]). This association remained significant for BNP after adjustment for age, co-morbidity and residual glomerular filtration rate (hazard ratio 8.5 [95% CI 1.0 to 73.8]). BNP is a predictor of survival in peritoneal dialysis patients even after adjustment for age, co-morbidity and residual glomerular filtration.

CHAPTER 7 AND 8: PROGNOSTIC VALUE OF NT-PROBNP IN THE GENERAL POPULATION

With advancing age the predictive value of traditional risk factors decreases and markers of vascular disease, inflammation, and end organ damage may

have additional prognostic value. Increased circulating NT-proBNP levels are a marker of cardiac dysfunction but also associate with coronary heart disease and stroke. In chapter 7 we investigated whether increased circulating NT-proBNP levels have additional prognostic value for first cardio- and cerebrovascular events beyond classical risk factors. In chapter 8 we compared NT-proBNP, pulse wave velocity, ankle-brachial index, creatinine clearance, intima media thickness and C-reactive protein to investigate which risk marker has the highest additional predictive value above the traditional risk factors in older subjects free of cardiovascular disease.

These studies were performed within the framework of the Rotterdam study, a large community-based cohort of 5063 participants free of cardiovascular disease, aged 55 years or older, in which circulating NT-proBNP levels, the other risk markers and cardiovascular risk factors were measured. Participants were followed for the occurrence of first major fatal or non-fatal cardiovascular event. 420 participants developed a first cardiovascular event (108 fatal). After adjustment for classical risk factors the hazard ratio for cardiovascular events was 2.32 (95% CI 1.55 to 2.70) in men, and 3.08 (95% CI 1.91 to 3.74) in women for participants with NT-proBNP in the upper compared to the lowest tertile. Corresponding hazard ratio's for coronary heart disease, heart failure and ischemic stroke were 2.01 (95% CI 1.14 to 2.59), 2.90 (95% CI 1.33 to 4.34) and 2.06 (95% CI 0.91 to 3.18) for men and 2.95 (95% CI 1.30 to 4.55), 5.93 (95% CI 2.04 to 11.2) and 2.07 (95% CI 1.00 to 2.97) for women. Incorporation of NT-proBNP in the classical risk model significantly improved the C statistic both in men and women and resulted in a net reclassification improvement of 9.2% (95% CI 3.5% to 14.9%, $p = 0.001$) in men and 13.3% (95% CI 5.9% to 20.8%, $p < 0.001$) in women.

In men, after adjusting for age an increase in pulse wave velocity, creatinine clearance, NT-proBNP and intima media thickness was related to an increased risk for cardiovascular disease. An increase in ankle brachial index was related to a decreased hazard ratio of cardiovascular disease. In women, NT-proBNP, ankle brachial index and intima media thickness were related to future cardiovascular events. C-reactive protein was not associated with an increased risk for cardiovascular disease, neither in men, nor women. No significant improvement of C statistic or reclassification was seen beyond the traditional cardiovascular risk markers with any of the risk markers, except NT-proBNP.

We conclude that in an asymptomatic older population NT-proBNP improves risk prediction not only of heart failure, but of cardiovascular disease in general beyond classical risk factors, resulting in a substantial reclassification of participants. Pulse wave velocity, ankle-brachial index, creatinine clearance, intima media thickness and C-reactive protein have no additional predictive value above the traditional risk factors in older subjects free of CVD.

CHAPTER 9: GENERAL DISCUSSION

Chapter 9 represents a general discussion, in which the results of the various studies are discussed in more detail within their past and current scientific perspective. Also possible directions for future research are provided.

Nederlandse samenvatting

HOOFDSTUK 1 EN 2: INTRODUCTIE EN DOELSTELLING

De familie van natriuretische peptiden omvat drie eiwitten: atriaal natriuretisch peptide (ANP), B-type natriuretisch peptide en C-type natriuretisch peptide (CNP). Deze drie eiwitten hebben allen een centrale intramoleculaire ringstructuur van 17 aminozuren. Deze ringstructuur is ook aanwezig bij natriuretische peptiden van andere diersoorten. De biologisch actieve peptiden (ANP, BNP en CNP) worden afgesplitst van de drie afzonderlijke propeptiden (proANP, proBNP en proCNP) alvorens hun afgifte aan de circulatie. Zowel de actieve eiwitten als de amino-terminale afscheidingsproducten zijn meetbaar in het plasma en serum (bijvoorbeeld BNP en amino-terminaal pro-B-type natriuretisch peptide [NT-proBNP]). Twee typen receptoren, beide gekoppeld aan guanylylcyclase, zijn geïdentificeerd: natriuretisch peptide receptor A en B (NPR-A en NPR-B). NPR-A bindt met name ANP en BNP, NPR-B heeft de hoogste selectiviteit voor CNP. Binding van ANP of BNP aan de NPR-A receptor verhoogt de glomerulaire filtratie snelheid en de renale zoutexcretie, vandaar de naam natriuretische peptiden, en remt de productie van renine en aldosteron. De natriuretisch peptide receptor C heeft een hoge affiniteit voor alle drie de natriuretische peptiden en klaart de peptiden uit de circulatie door binding, gevolgd door internalisatie in de cel. Daarnaast worden de natriuretische peptiden afgebroken door het enzym neutraalendopeptidase. Bij mensen wordt het grootste gedeelte van BNP gevormd in de ventriculaire cardiomyocyten. De belangrijkste determinant van de expressie van het BNP-gen is de mate van mechanische rek van de cardiomyocyt. Ook neurohormonen, pro-inflammatoire eiwitten en schildklierhormoon beïnvloeden de BNP-genexpressie.

Bij patiënten met hartfalen is de plasmaconcentratie van BNP en NT-proBNP verhoogd. Zowel BNP als NT-proBNP hebben een hoge sensitiviteit voor het aantonen van verminderde linkerventrikelfunctie. De specificiteit is echter te laag om BNP of NT-proBNP te gebruiken als screeningstest voor asymptomatische populaties. De diagnostische karakteristieken verbeteren als de incidentie van hartfalen hoger is, zoals bij personen die zich presenteren bij de huisarts met kortademigheid. Sensitiviteit en specificiteit zijn het hoogst bij patiënten met acute kortademigheid die zich presenteren op de spoedeisende hulp van een ziekenhuis. Verwacht werd dat frequente meting van BNP of NT-proBNP en hierop gebaseerde therapie bij poliklinische patienten met hartfalen de prognose sterk zou verbeteren. Recent onderzoek liet

evenwel zien dat poliklinische follow-up middels meting van natriuretische peptiden in het algemeen geen voordeel heeft ten opzichte van de tot op heden gebruikelijke controles zonder meting van BNP of NT-proBNP. De enige patiëntengroep die mogelijk baat heeft bij dergelijke metingen zijn hartfalenpatiënten jonger dan 65 jaar. Omdat een verhoogde plasmaconcentratie van BNP en NT-proBNP niet alleen is geassocieerd met hartfalen maar ook met hart- en vaatziekten, is het te verwachten dat beide eiwitten voorspellende waarde hebben voor het ontstaan van hart- en vaatziekten.

De studies waarop dit proefschrift is gebaseerd hadden de volgende doelstellingen: 1) het onderzoeken van het effect van de introductie van de NT-proBNP-bepaling op de Spoedeisende Hulp bij patiënten met acute kortademigheid op beslissingen omtrent opname en ontslag; 2) het onderzoeken van het verband tussen natriuretisch peptide plasmaconcentraties en echocardiografische afwijkingen bij poliklinische geriatrische patiënten; 3) het bepalen van de invloed van toenemende arteriële stijfheid op de plasmaconcentratie van natriuretische peptiden; 4) het onderzoeken van de prognostische waarde van natriuretische peptiden bij patiënten met eindstadium nierfalen; 5) het onderzoeken van de voorspellende waarde van natriuretische peptiden voor hart- en vaatziekten bij personen zonder hart- en vaatziekten in de voorgeschiedenis en het vergelijken van de voorspellende waarde van natriuretische peptiden met andere indicatoren voor hart- en vaatziekten.

HOOFDSTUK 3: NT-PROBNP OP DE SPOEDEISENDE HULP

In verschillende studies is aangetoond dat voor patiënten die zich presenteren op de spoedeisende hulp NT-proBNP een goede biomarker is voor het aantonen of uitsluiten van hartfalen. Bepaling van NT-proBNP zou daarmee kunnen leiden tot een verbetering van de patiëntenzorg en een afname van de zorgkosten. In een Zwitserse studie verbeterde de patiëntenzorg na introductie van NT-proBNP-bepalingen op de spoedeisende hulp. Echter, omdat het effect van introductie van NT-proBNP op patiëntenzorg en kosten sterk afhankelijk is van de manier waarop de gezondheidszorg is georganiseerd, onderzochten wij of de introductie van NT-proBNP-bepalingen bij patiënten met acute kortademigheid op de spoedeisende hulp van het Erasmus MC leidde tot een verbetering van het diagnostisch proces met minder zorgkosten zon-

der toename van de sterfte. In een prospectief onderzoek werden patiënten die zich presenteerden met acute kortademigheid op de spoedeisende hulp van het Erasmus MC gerandomiseerd voor directe bepaling van NT-proBNP of geen bepaling van NT-proBNP. De afkapwaarde om hartfalen uit te sluiten was 11 pmol/l voor mannen en 17 pmol/l voor vrouwen. Hartfalen was aanwezig bij een afkapwaarde ≥120 pmol/l. De primaire eindpunten waren de tijd tot ontslag uit het ziekenhuis in dagen en de totale kosten gerelateerd aan het ziekenhuisbezoek. Het verschil in kosten en 30-dagen-mortaliteit tussen de NT-proBNP-groep en de controlegroep werd geanalyseerd middels *bootstrapanalyse*. In totaal werden 477 patiënten (54% mannen) geïncludeerd. De gemiddelde leeftijd van de deelnemers was 59 jaar, waarbij 44% van de deelnemers bekend was met een hartziekte. De mediane tijd tot ontslag was 1.9 (interkwartielafstand 0.12 tot 8.4) dagen in de NT-proBNP-groep (n = 236) en 3.9 (interkwartielafstand 0.16 tot 11.0) dagen in de controlegroep (n = 241) (p = 0.04). Er was een trend tot kostendaling na introductie van NT-proBNP-bepaling van 1096 euro per patiënt (95% betrouwbaarheidsinterval –197 tot 2574 euro), zonder dat er een verschil was in sterfte (15 overledenen in de NT-proBNP-groep en 18 overledenen in de controlegroep).

Uit de resultaten van onze studie kan worden afgeleid dat introductie van snelle NT-proBNP-bepalingen bij patiënten, die zich presenteren op de spoedeisende hulp met acute kortademigheid, zeer waarschijnlijk leidt tot een kostendaling, veroorzaakt door de kortere opnameduur in het ziekenhuis. Belangrijk is dat deze kortere opnameduur niet leidt tot een grotere sterftekans.

HOOFDSTUK 4: ANP, BNP EN ECHOCARDIOGRAFISCHE AFWIJKINGEN

De diagnostiek van hartfalen is lastiger bij oudere patiënten, omdat deze patiënten zich kunnen presenteren met atypische klachten. Omdat medicamenteuze behandeling van hartfalen de ziektelast beperkt en de overleving verbetert, is het belangrijk om de patiënten op te sporen die voordeel zouden kunnen hebben van behandeling. In onze studie hebben wij de relatie is tussen de plasmaconcentratie van ANP, BNP en echocardiografische afwijkingen onderzocht.

Bij 209 personen, gerekruteerd op de polikliniek Geriatrie van het Erasmus MC, werd een transthoracale echocardiografie verricht. Alle deelnemers waren 65 jaar of ouder en hadden geen ernstige cognitieve beperking. De gemiddelde ANP en BNP plasmaconcentraties waren respectievelijk 11.0 en 10.8 pmol/l. De plasmaconcentratie van BNP, maar niet die van ANP, steeg met het aantal echocardiografische afwijkingen. Alleen BNP correleerde met linkerventrikelfunctie en linkerventrikelmassa. In vergelijking met een lagere concentratie was een hogere natriuretisch peptideconcentratie geassocieerd met een grotere kans op echocardiografische afwijkingen (odds ratio [derde tertiel versus eerste tertiel]; BNP, 7.15 [95% betrouwbaarheidsinterval 2.15 tot 23.71]; ANP, 3.07 [95% betrouwbaarheidsinterval 1.15 tot 8.16]). Met een afkapwaarde van 6.85 pmol/l voor BNP was de sensitiviteit 92%, de specificiteit 31%, de negatief voorspellende waarde 97% en de positief voorspellende waarde 16%.

Bij geriatrische patiënten is een verhoogde plasmaconcentratie van BNP geassocieerd met echocardiografische afwijkingen. In vergelijking met ANP is BNP een betere biomarker. Indien bij een geriatrische poliklinische patiënt na zorgvuldige anamnese en lichamelijk onderzoek onduidelijkheid bestaat over de aanwezigheid van hartziekten kan een BNP-bepaling gezien de grote negatief voorspellende waarde vooral helpen om ernstige echocardiografische afwijkingen uit te sluiten.

HOOFDSTUK 5: INVLOED VAN VAATWANDSTIJFHEID OP NT-PROBNP

Met het stijgen van de leeftijd worden de aorta en de andere grote arteriën stijver. De polsgolf wordt daardoor minder gedempt, resulterend in een hogere systolische bloeddruk, een lagere diastolische bloeddruk en een verhoogde polsdruk. Hierdoor neemt de belasting van het hart toe en de myocardiale perfusie af. Zowel een toename van de wandspanning van de linkerventrikel van het hart, als myocardiale ischemie stimuleren de aanmaak van NT-proBNP. Onze hypothese was dat vaatwandstijfheid een belangrijke determinant is van de plasmaconcentratie van NT-proBNP.

De NT-proBNP-plasmaconcentratie, arteria brachialis polsdruk en carotis-femoralis polsgolfsnelheid werden bij 6211 personen gemeten (gemiddelde leeftijd 69.2 jaar). Bij deelnemers zonder hart- en vaatziekten was de me-

diane NT-proBNP-plasmaconcentratie 6.7 pmol/l bij mannen (n = 2073) en 10.1 pmol/l bij vrouwen (n = 3085) (p < 0.001). Bij deze deelnemers was zowel polsdruk (β coëfficiënt; mannen: 0.315; vrouwen: 0.233) als polsgolfsnelheid (β coëfficiënt; mannen: 0.255; vrouwen: 0.232) gecorreleerd met NT-proBNP. Na correctie voor leeftijd, gewicht, lengte, gemiddelde arteriële bloeddruk, hartslag, roken, diabetes mellitus, geschatte glomerulaire filtratie snelheid, totaal cholesterol, HDL en gebruik van cholesterolverlagers en bloeddrukverlagende medicatie bleven polsdruk en polsgolfsnelheid gecorreleerd met NT-proBNP bij mannen. Bij vrouwen bleef de relatie tussen NT-proBNP en polsdruk significant. In het multivariate model verklaarde polsdruk 20,3% en polsgolfsnelheid 10,7 % van de variatie in NT-proBNP bij mannen en respectievelijk 10.8% en 9.4% van de variatie bij vrouwen. Bij patiënten met hart- en vaatziekten in de voorgeschiedenis waren polsdruk en polsgolfsnelheid na correctie voor bovengenoemde variabelen niet meer gecorreleerd met NT-proBNP.

Het verband tussen NT-proBNP en vaatwandstijfheid wordt bepaald door meerdere factoren waarvan leeftijd de belangrijkste is. Na correctie voor bekende factoren is arteriële vaatwandstijfheid onafhankelijk geassocieerd met de NT-proBNP-plasmaconcentratie bij personen zonder hart- en vaatziekten. De relatie tussen vaatwandstijfheid en NT-proBNP is sterker bij mannen dan bij vrouwen en wordt niet teruggevonden bij personen met hart- en vaatziekten in de voorgeschiedenis.

HOOFDSTUK 6: BNP EN NT-PROANP BIJ PERITONEAAL DIALYSE

Hart- en vaatziekten zijn een belangrijke doodsoorzaak bij patiënten met eindstadium nierfalen. In hoofdstuk 6 rapporteren wij de resultaten van een studie naar de voorspellende waarde voor mortaliteit van BNP en amino-terminaal atriaal natriuretisch peptide (NT-proANP) bij peritoneaal dialysepatiënten. Uit een prospectief verzameld cohort van dialysepatiënten (n = 1464), gevolgd van start van dialyse tot transplantatie of overlijden, werden 68 patiënten zonder aanwijzingen voor hartfalen willekeurig geselecteerd. BNP en NT-proANP werd gemeten zes maanden na start van dialyse. Tijdens de follow-up (spreiding 1.5 tot 4.5 jaar) was de mortaliteit 15%. Zowel een NT-proANP als een BNP boven de mediaan was geassocieerd met een ver-

hoogde mortaliteit (NT-proANP: hazard ratio 11.3 [95% betrouwbaarheidsinterval 1.4 tot 91.9]; BNP: hazard ratio 11.3 [95% betrouwbaarheidsinterval 1.4 tot 91.4]). Deze associatie bleef bestaan voor BNP na aanpassing voor leeftijd, co-morbiditeit en nierfunctie (hazard ratio 8.5 [95% betrouwbaarheidsinterval 1.0 tot 73.8]).

HOOFDSTUK 7 EN 8: PROGNOSTISCHE WAARDE VAN NT-PROBNP IN DE ALGEMENE POPULATIE

Op oudere leeftijd neemt de voorspellende waarde van de traditionele risicofactoren af. Mogelijk hebben markers van vaatziekte, ontsteking en eindorgaanschade dan nog wel voorspellende waarde. Een verhoogde NT-proBNP-plasmaconcentratie is een marker van cardiale disfunctie maar is ook geassocieerd met coronarialijden en beroerten. In hoofdstuk 7 onderzoeken wij of een verhoogde NT-proBNP plasmaconcentratie aanvullende voorspellende waarde heeft voor hart- en vaatziekten onafhankelijk van de klassieke risicofactoren bij personen zonder ziektegeschiedenis van een hartinfarct, ischemische beroerte of hartfalen. In hoofdstuk 8 vergelijken we NT-proBNP met polsgolfsnelheid, enkel-arm index, kreatinineklaring, intima-media-dikte en C-reactief proteïne om te zien welke risicoindicator de hoogste additionele waarde heeft voor de voorspelling van hart- en vaatziekten bij mensen van 55 jaar en ouder zonder hart- en vaatziekten in de voorgeschiedenis boven op de klassieke risicofactoren.

De analyses werden verricht binnen de Rotterdam studie, een groot prospectief cohort-onderzoek binnen de algemene populatie. Bij 5063 deelnemers zonder hart- en vaatziekten werd de NT-proBNP-plasmaconcentratie gemeten en vergeleken met de andere risicomarkers. Alle deelnemers werden gevolgd voor hart- en vaatziekten. 420 deelnemers ontwikkelden hart- en vaatziekten, waarvan 108 dodelijk. Na aanpassing voor de klassieke risicofactoren was de hazard ratio voor hart- en vaatziekten 2.32 (95% betrouwbaarheidsinterval 1.55 tot 2.70) voor mannen en 3.08 (95% betrouwbaarheidsinterval 1.91 tot 3.74) voor vrouwen, bij vergelijk van deelnemers met een NT-proBNP plasmaconcentratie in het hoogste versus het laagste tertiel. De bijpassende hazard ratio's met 95% betrouwbaarheidsinterval voor coronaire hartziekten, hartfalen en ischemische beroerten waren 2.01 (1.14 tot 2.59), 2.90 (1.33 tot 4.34), 2.06 (0.91 tot 3.18) voor mannen en 2.95 (1.30 tot 4.55),

5.93 (2.04 tot 11.2), 2.07 (1.00 tot 2.97) voor vrouwen. Toevoeging van NT-proBNP aan het klassieke risicomodel verbeterde significant de C-statistiek in beide geslachten en resulteerde in een netto reclassificatieverbetering van 9.2% (95% betrouwbaarheidsinterval 3.5% tot 14.9%) bij mannen en van 13.3% (95% betrouwbaarheidsinterval 5.9% tot 20.8%) bij vrouwen.

Bij mannen was na correctie voor leeftijd een verhoging van polsgolfsnelheid, kreatinineklaring, NT-proBNP en intima-media-dikte gerelateerd aan hart- en vaatziekten. Een verhoging van de enkel-armindex was gerelateerd aan een verminderd risico van hart- en vaatziekten. Bij vrouwen was NT-proBNP, enkel-armindex en intima-media-dikte gerelateerd aan hart- en vaatziekten. Een verhoging van C-reactief proteïne was niet geassocieerd met een verhoogd risico van hart- en vaatziekten, noch bij mannen, noch bij vrouwen. Voor geen van de risicomarkers, behalve NT-proBNP werd een verbetering gezien van de C-statistiek of reclassificatie na toevoeging aan het klassieke risicomodel voor hart- en vaatziekten.

We concluderen dat in een asymptomatische oudere populatie NT-proBNP de voorspelling niet alleen van hartfalen maar ook van hart- en vaatziekten in het algemeen verbetert boven op de klassieke risicofactoren, uiteindelijk leidend tot een significante reclassificatie van deelnemers. Polsgolfsnelheid, enkel-armindex, kreatinineklaring, intima- media dikte en C-reactief proteïne daarentegen hebben geen additionele voorspellende waarde voor hart- en vaatziekten boven op de klassieke risicofactoren bij ouderen zonder hart- en vaatziekten.

HOOFDSTUK 9: ALGEMENE DISCUSSIE

Hoofdstuk 9 omvat een algemene discussie, waarin de resultaten van de verschillende onderzoeken worden besproken in hun historische en huidige wetenschappelijke perspectief. Tevens worden aanbevelingen gedaan voor vervolgonderzoek.

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PHD PORTFOLIO

Natriuretic Peptides, Diagnostic and Prognostic Biomarkers
J.H.W. Rutten

Erasmus MC Department:	Internal Medicine
Research School:	Cardiovascular Research School Erasmus University Rotterdam (COEUR), Department of Vascular Pharmacology
Promotor:	Prof.dr. A.H.J. Danser
Supervisor:	dr. A.H. van den Meiracker
PhD period:	2004 – 2010

PhD training	**Year**	**ETCS**
General academic skills and research skills		
Consultatiecentrum Patientgebonden onderzoek	2005	0.3
Modern Statistical Methods, NIHES	2006	4.3
Biomedical English Writing and Communication	2008	1.5
In-depth courses		
COEUR Courses, Research Seminars* and Lectures	2004-2009	15.9
Young Cardiovascular Master Class	2005	0.6
Cardiac Function and Adaptation	2005	2.0
Course Dutch Society for Vascular Medicine	2005	0.6
Introduction to R	2008	1.2
Hypertension Summer School, ESH, Annecy	2008	1.5
Symposia and conferences		
Symposium Vascular Medicine, Rotterdam	2005	0.2
Internistendagen*	2005-2010	3.0
Wetenschapsdagen Inwendige Geneeskunde, Erasmus MC*§	2005-2010	3.6
16th Meeting on Hypertension, ESH, Madrid§	2006	1.2
Cardiology and Vascular Medicine, ESC, Rotterdam	2006	0.9
15th International Vascular Biology Meeting, Noordwijkerhout	2006	0.3

International Society of Peritoneal Dialysis, Hong Kong*	2006	1.5
12th Amine Oxidase and Trace Amines Workshop	2006	1.5
18th Meeting on Hypertension, ESH, Berlin	2008	1.2
Internisten Symposium Vasculaire Geneeskunde Nefrologie	2008-2010	0.6
NHG / MIVAB Meeting*	2009	0.6
19th Meeting on Hypertension, ESH, Milan*	2009	1.2
Artery Meeting, Cambridge§	2009	0.9
Rotterdamse Internistendag*	2007-2009	0.9
20th Meeting on Hypertension, ESH, Stockholm§	2010	1.2

Teaching

Supervising and teaching MSc students, Erasmus MC	2007	3.0
Supervising and teaching MSc students, Maasstad Ziekenhuis	2009	2.4

Grants, prizes

Netherlands Foundation for Cardiovascular Excellence	2006	€ 50.000
Travel grant 19th Meeting on Hypertension, ESH	2008	
Poster Prize Wetenschapsdagen Interne Geneeskunde	2010	
Poster Prize 20th Meeting on Hypertension, ESH	2010	
Netherlands Foundation for Cardiovascular Excellence	2010	€ 92.000

* oral presentation; § poster presentation
Abbreviations: NIHES, Netherlands Institute for Health Sciences; NHG, Nederlands Hypertensie Genootschap; ESH, European Society of Hypertension; ESC, European Society of Cardiology

CURRICULUM VITAE

De auteur van dit proefschrift werd geboren op 2 augustus 1977 te Boxmeer. Na het behalen van het Gymnasium diploma, cum laude, in 1996 aan het Bernardinuscollege te Heerlen, studeerde hij Geneeskunde aan de Universiteit Maastricht. Zijn laatste keuze co-schap Interne Geneeskunde liep hij in het Moi Teaching and Referral Hospital te Eldoret, Kenia. In 2003 behaalde hij zijn artsexamen, cum laude, waarna hij begon als arts-assistent op de afdeling Intensive Care van het toenmalige Dijkzigt Ziekenhuis te Rotterdam, het huidige Erasmus MC. Na bijna twee jaar te hebben gewerkt in de directe patiëntenzorg, startte hij eind 2004 onder begeleiding van dr. A.H. van den Meiracker met wetenschappelijk onderzoek naar natriuretische peptiden, hetgeen de basis zou vormen voor dit proefschrift. Begin 2007 ving hij aan met zijn opleiding tot internist in het Erasmus MC te Rotterdam (opleider: Prof. dr. J.L.C.M. van Saase). Sindsdien wisselde hij zijn wetenschappelijk werk af met het klinische werk in het kader van zijn opleiding. Sinds 2009 is hij werkzaam als arts in opleiding tot specialist in het Maasstad ziekenhuis te Rotterdam (opleider: dr. M.A. van den Dorpel).

DANKWOORD

"Wetenschap is de titanische poging van het menselijk intellect zich uit zijn kosmische isolement te verlossen door te begrijpen." Uit: Nooit meer slapen, W.F. Hermans, 1966.

Mijn persoonlijke titanische poging was niet mogelijk zonder het intellect en de steun van velen. Daarnaast zorgden mijn collega's, vrienden en familie ervoor dat in deze zes jaar het kosmisch isolement minder op mij drukte. In dit laatste deel van mijn proefschrift wil ik het woord richten tot degenen die er mede voor hebben gezorgd dat mijn promotie een succes is geworden.

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Prof.dr. J. Lindemans, beste Jan, onderzoek naar een biomarker is natuurlijk niet mogelijk zonder de hulp van een ervaren klinisch chemicus. Hartelijk dank voor alle ondersteuning en voor het plaatsnemen in de kleine commissie.

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Beste Nathalie, de combinatie vallende ouderen en natriuretische peptiden lijkt niet echt logisch, maar inventieve onderzoekers bedenken altijd wel een hypothese. We hebben daarna vaak uitgesproken nog samen onderzoek te doen, en nu je eenmaal in het AMC zit gaat dat zeker zorgen voor Rot-

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