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ProANP plasma measurement predicts all cause mortality in acutely hospitalised patients

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Summary

Focus:

- ***To test if proANP associates with short- and long-term mortality in an unselected sample of acutely hospitalized patients.***
- ***To test if proANP provides additional discrimination regarding short- and long-term mortality in an unselected sample of acutely hospitalized patients.***
- ***To test both hypotheses in the full sample and in a subgroup of patients without signs of cardiovascular disease (CVD).***

Key Messages:

- ***This study illustrates that natriuretic peptides in general can be used in the clinical setting to aid in the evaluation of acutely admitted patients short- and long-term prognosis irrespectively of CVD status.***
- ***This study also suggests that combining biomarkers (proANP and proBNP) provides additional information compared to using a single marker strategy.***
- ***This study utilizes a novel and robust proANP measurement technique which opens the possibility for long-term storing of plasma samples for proANP analysis without risk of degradation.***

Strengths and Limitation:

Major strengths: Well characterized cohort with long follow-up and well defined endpoint (mortality). Robust assay for analyzing proANP.

Major limitations: Lack of plasma on subset of cohort, however this subgroup did not differ in survival and baseline values.

Abbreviations and acronyms:

ANP = atrial natriuretic peptide

AP = angina pectoris

BNP = B-type natriuretic peptide

CHF = congestive heart failure

CVD = cardiovascular disease

LVEF = left ventricular ejection fraction

MI = myocardial infarction

NT-proBNP = N-terminal pro–brain natriuretic peptide.

NYHA class = New York Heart Association functional classification

ProANP = pro-atrial natriuretic peptide

ABSTRACT

Importance: The association of natriuretic peptide measurement with all cause mortality in acutely admitted patients has not yet been examined.

Objective: To test the risk and predictive usefulness of proANP with short- and long-term mortality in acutely hospitalised patients.

Design: A prospective cohort study.

Setting: Secondary care at general hospital.

Patients: Participants (n=1337). Amongst these, 1255 (94%) were acutely hospitalised. Medical history, echocardiography, and blood samples were obtained during admission. Vital status after discharge was obtained from national central data registers.

Main Outcome Measure(s): 1-year and long-term mortality.

Results: Median follow-up period was 11.5 years. At the end of follow-up, 926 patients had died, 239 during the first year. ProANP quartiles 2-4 associated with a stepwise increase in risk of 1-year and long-term mortality compared to the first quartile in multivariable adjusted Cox proportional regression models (Hazard Ratio [HR] 1.53 95% Confidence Interval [CI] 1.30-1.81 and HR 1.26 95% CI 1.17-1.36 respectively). Addition of NT-proBNP attenuated proANP's association with mortality in the models (HR 1.24 95% CI 1.01-1.53 and 1.14 95% CI 1.03-1.26 respectively). Most of the effect seemed to associate with the highest proANP levels (fourth quartile). Similar results were observed in subgroups of participants with no evidence of cardiovascular disease (CVD). ProANP in quartiles improved discrimination when added to traditional risk factors in prediction models for 1-year (IDI 0.141 95% CI 0.085-0.197; C-index 0.753 95% CI 0.724-0.783, *P* for

improvement .003) and long-term mortality (IDI 0.053 95% CI 0.032-0.074; C-index 0.736 95% CI 0.720-0.752, *P* for improvement <.001) with similar results in subgroups. Discrimination was best in a combined model with both proANP and NT-proBNP included.

Conclusions and Relevance: Plasma proANP concentrations are associated with and predict short- and long-term all cause mortality in acutely hospitalised patients irrespective of CVD status. Risk stratification using proANP or a combination of proANP and NT-proBNP could lend vital support in the evaluation of the acutely hospitalised patient.

INTRODUCTION

Atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) have important physiological roles in fluid homeostasis and cardiac pathology, including myocardial ischemia and left ventricular dysfunction.[1,2] BNP and the N-terminal precursor fragment (NT-proBNP) have been regarded as the biomarkers of choice when obtaining diagnostic and prognostic information in heart failure patients. Recent development of assays measuring proANP-derived peptides suggests comparable performance with proBNP-derived peptides in heart failure populations.[3-6] Studies have also assessed a possible connection between natriuretic peptide concentrations and the risk of mortality in random populations,[7-9] suggesting an association between plasma concentrations and mortality independently of other risk factors. However, reservations are generally noted due to short-term follow-up and small sample sizes. Accordingly, natriuretic peptide levels and risk of short- and long-term mortality in a larger population of unselected, acutely hospitalised patients have yet to be examined.

In the present study we tested the hypothesis that measurement of total proANP products in plasma may associate with and predict short- and long-term all cause mortality in a large sample of unselected, acutely hospitalised patients irrespective of cardiovascular disease (CVD) status at study entry.

METHODS

DESIGN AND STUDY POPULATION

The present study was based on plasma collected from participants in the Copenhagen Hospital Heart Failure Study. The primary study design has been published previously.[10-12] Briefly stated, the cohort consisted of patients (>40 years of age) admitted sequentially to Amager Hospital in Copenhagen. Enrolment occurred between 1 April 1998 and 31 March 1999. Upon admission, the medical history of all included participants was obtained together with a standard physical examination and a bedside echocardiography (Hewlett Packard Imagepoint, model M2410A; Andover, Massachusetts, USA). During the last 10 months of the study, 80% (n=2230) of the included patients had blood samples collected between 08:00 and 10:00 am. All data collection occurred within 24 hours of admission. Among these, 2193 had a satisfactory echocardiography. Vital status or cause of death during follow-up was collected from national registers. Twenty-five (2%) participants emigrated during the follow-up period and were censored at time of emigration. Written consent was obtained at admission. The ethical committee of Copenhagen approved the study (Trial nr. 01-320/97) prior to enrolment of participants.

SAMPLES

Blood samples were collected in EDTA-containing tubes and centrifuged at 4°C. Plasma was stored at -20°C and only thawed once during the initial investigations.

PROANP MEASUREMENT

Plasma proANP was analysed in 2011 using an in-house method independent of changes in post-translational processing of the ANP precursor.[13] This assay has previously been compared to an automated sandwich assay for mid-regional proANP with an excellent correlation.[14] The coefficient of variation (inter-assay) was 11% at 1240 pmol/L and 6% at 2468 pmol/L. 1337 (61%) of the 2193 participants with blood samples and echocardiographic examinations on record were eligible for proANP measurement.

COVARIATES

A left ventricular ejection fraction (LVEF) <50% was chosen as a cut-off point for defining left ventricular systolic dysfunction. NT-proBNP concentrations were measured at the time of inclusion using a two-step ELISA sandwich assay with streptavidin coated microtitre plates.[15]

STATISTICS

Plasma proANP concentrations were divided into quartiles because of skewed data distribution and presented as medians with interquartile ranges (IQR). Descriptive data are presented as percentages or means with standard deviations (SD). Test for differences were performed using Cochran-Armitage test for trend or Pearson's $\chi 2$-test for categorical data and linear regression or Mann-Whitney U-test for continuous data when appropriate. Comparisons were made between participants with and without proANP measurements on baseline values using Levene's test, and on mortality using survival curves and univariate Cox analysis.

Differences in survival were illustrated using Kaplan-Meier curves based on proANP quartiles and assessed using the log-rank test. Cox proportional regression analysis was used to evaluate the association between proANP concentrations and the risk of all cause mortality, after testing the assumption of proportionality. Initially, a model was fitted using proANP (in quartiles) with age and sex as additional covariates. Subsequently a model consisting of well known predictors of mortality (Table 1) was fitted using backward elimination based on the Akaike information criterion (AIC) defined as AIC = -2 * maximum log-likelihood(model) + 2 * (numbers of covariates).[16] This balances between a model with high likelihood and a reasonable number of variables to achieve the lowest AIC possible. The final model included age, sex, alcohol, smoking, diabetes, history of congestive heart failure, history of pulmonary disease, history of liver disease, haemoglobin, ejection fraction below 50%, and New York Heart Association functional classification 3 or 4. Missing data on covariates were imputed using age and sex as independent variables. ProANP (in quartiles) was then added and the association with mortality was assessed using hazard ratios (HR).

Prediction models for 1-year and long-term mortality were developed using the same covariates as in the multivariable Cox model and then adding proANP. Hence models with traditional risk factors (model 1) were compared to models with traditional risk factors and proANP (model 2). Discrimination was evaluated by calculating the Integrated Discrimination Improvement (IDI).[17] The IDI can be regarded as the difference between improvement in average sensitivity and any potential increase in average 1-specificity when adding proANP to the prediction models. Furthermore, time-dependent C statistics were calculated and differences in the C index were tested between models with and without proANP.[18,19]

Calibration was performed by testing the addition of proANP as an independent variable to the Cox models using the likelihood ratio test. Furthermore, all models were tested using Grønnesby and Borgan goodness-of-fit (GOF) test.[20] Finally, internal validation was performed by bootstrapping the C statistics (resampling with 200 repetitions) to assess the degree of overfitting.

To further test the strength of proANP as an independent predictor of mortality in participants without cardiac impairment, all analyses were repeated using the same model; but excluding participants with evidence of CVD, defined as prior history of congestive heart failure (CHF), myocardial infarction (MI), angina pectoris (AP), valve disease, with LVEF<50% and/or New York Heart Association functional classification (NYHA class) 3 and 4 at admission.

NT-proBNP concentrations (in quartiles), measured in 1998-1999, were used in both the Cox models and the prediction models (model 3) as a quasi-internal validation of the endpoints in the population (with proANP measurements) and for direct comparison against proANP in the Cox- and predictive models. Calibration was achieved in the same manner as proANP. Furthermore, model performances were

tested after the addition of both proANP and NT-proBNP (model 4) to the multivariable predictive models and addition of proANP to predictive models with NT-proBNP included.

Statistical analyses were performed using SPSS version 16.0 (SPSS Inc., Chicago, IL) and STATA version 12 (SataCorp LP, College Station, TX). A two-sided *P*-value <.05 was considered statistically significant.

RESULTS

CLINICAL CHARACTERISTICS

No differences were noted between participants with and without proANP measurement on baseline values or short- and long-term mortality (all $P>.05$; data not shown). All baseline characteristics of the participants are listed in Table 1. Mean age at admission was 70.5 years (SD 14.3 years), 796 (59.5%) of participants were women. 1255 (94%) participants were acutely hospitalised, while the remaining were admitted electively. The median proANP concentration was 780 pmol/L (IQR 912 pmol/L). Median follow-up was 11.5 years (range 11.0-11.9 years). Follow-up was accomplished on 1337 (100%) participants after 1 year and on 1337 (100%) at the end of the study period. 239 (17.9%) participants died within the first year and 926 (69.3%) during the entire follow-up period. 617 (46.1%) participants had a history of CVD, 105 (7.9%) were NYHA class 3 or 4 and 250 (18.7%) had a LVEF<50% recorded at entry.

PROANP AND SURVIVAL

Kaplan-Meier curves displayed stepwise significant differences in survival between the quartiles of proANP within the whole study population, with the highest survival seen amongst participants in the lowest quartile (Figure 2, left panel; log rank test $P<.001$). Similar results were observed in the subgroup of participants, with no evidence of CVD (Figure 2, right panel; log rank test $P<.001$).

PROANP ASSOCIATION WITH MORTALITY

ProANP quartiles two, three, and four displayed a stepwise increase in risk of 1-year mortality compared to the first quartile in the Cox proportional regression models (Table 2a; Trend: Age and sex+proANP: HR 1.61 95% confidence interval [CI] 1.38-1.87; *P*<.001; multivariable+proANP: HR 1.53 95% CI 1.30-1.81; *P*<.001). This stepwise association was still significant, but more modest when regarding long-term mortality (Table 2a; Trend: Age and sex+proANP: HR 1.35 95% confidence interval [CI] 1.26-1.45; *P*<.001; multivariable+proANP: HR 1.26 95% CI 1.17-1.36; *P*<.001). Similar results were observed in subgroups of participants with no evidence of CVD (Table 2b). Results for proANP were attenuated when both proANP and NT-proBNP were included in the models but remained significant on trend (highest *P*=.047).

In most of the Cox regression models, the trend seemed to be carried primarily by the fourth quartile of proANP, which associated significantly with mortality (compared with the first quartile) in all models except association with 1-year mortality in multivariable models with NT-proBNP included (Table 2a and 2b; lowest *P*=.083). Full Cox models with proANP or NT-proBNP included, before selections are located in the supplemental appendix (supplemental Tables 1-4).

PROANP AS A PREDICTOR OF MORTALITY

Addition of proANP to the multivariable models improved discrimination, resulting in an IDI of 0.141 (95% CI 0.085-0.197) and 0.053 (95% CI 0.032-0.074) for 1-year and long-term mortality respectively (Table 3a, model 2; all *P* for improvement <.001). The corresponding IDI's were of the same magnitude in subgroups of participants without evidence of CVD (Table 3b, model 2; highest *P* for improvement .001).

Time dependent C-statistics for both 1-year and long term mortality increased to 0.753 (95% CI 0.724-0.783) and 0.736 (95% CI 0.720-0.752), after adding proANP

to the multivariable models (Table 3a, model 2; *P* for improvement .003 and <.001 respectively). Subgroup analysis, excluding participants with evidence of CVD, yielded similar improvements in C-statistics for 1-year and for long-term mortality (Table 3b, model 2; *P* for improvement .019 and .001 respectively).

NT-proBNP performed similar to proANP in all prediction models (Table 3a and 3b, model 3) except for 1-year mortality in participants without evidence of CVD where proANP consistently performed better although the difference was modest (Table 3b). A combined model including both proANP and NT-proBNP resulted in the best discrimination (Table 3a and 3b, model 4) measured as significant improvement of IDI's and C-index compared to the multivariable model on both 1-year and long-term mortality including subgroups (highest *P* for improvement .022). Models with both proANP and NT-proBNP provided modestly better discrimination compared to models with NT-proBNP included, for long-term mortality (*P*=.015 and *P*=.069 for improvement in IDI and C-index respectively) and in subgroups without evidence of CVD (Table 3a and 4a).

CALIBRATION

The likelihood improved significantly with addition of proANP to all models including multivariable models with NT-proBNP (highest *P*=.042). No models violated the Grønnesby and Borgan test (all *P*>.05), indicating adequate GOF. Bootstrap estimates revealed low degree of overfitting in all models.

DISCUSSION

This study demonstrates that proANP plasma concentrations independently associate with all cause mortality in an unselected population of acutely hospitalised patients. Furthermore, this association persisted in participants with seemingly normal cardiac function. To our knowledge, this is the first study to show such a correlation. Including the proANP measurement to well-established risk factors of short- and long-term mortality also improved discrimination, which underscores the general usefulness of this marker in the prognostic evaluation of the acutely hospitalised patient.

Several other studies have evaluated the association between natriuretic peptide concentrations and death. Most of these have mainly focused on populations with a history of cardiovascular disease.[7,21-23] Others include healthy populations in which the clinical validity of measuring natriuretic peptides regarding predictability of cardiovascular or all cause mortality is debatable.[24-26] In general, the present population has a higher frequency and severity of acute and chronic illnesses compared to outpatients and healthy volunteers. This population thus closely resembles what the clinician encounters in the hospital.

As the majority (94%) of participants was acutely admitted to the hospital, we looked at other studies where the populations had similar backgrounds. Several studies have investigated the diagnostic properties of natriuretic peptide measurement in patients with acute dyspnoea as the primary symptom.[27-29] In the ProBNP Investigation of Dyspnoea in the Emergency Department (PRIDE) trial, NT-proBNP measurement was shown to have valuable diagnostic applications as a rule-out marker of heart failure in a cohort of 599 patients presenting with acute shortness of breath.[30]

A follow-up study using the PRIDE cohort found NT-proBNP to be a strong predictor of 1-year mortality in a multivariate analysis (HR 2.88 95% CI 1.64-5.06; *P*<.001).[31] The conclusion was identical in a follow-up paper evaluating multiple markers.[32]

Even though our study on proANP measurement showed equal prognostic properties, caution must be made when making direct comparisons. The PRIDE cohort consisted of selective patients (with dyspnoea) whereas our cohort consisted of a broad selection of patient categories (supplemental table 5). Of the 599 patients in the PRIDE cohort presenting with acute dyspnoea, 209 (36%) were diagnosed with acute heart failure, and patients with acute severe ischemia were excluded. In our population, 250 (18.7%) had a LVEF<50% with even fewer admitted with symptoms of heart failure. These circumstances further enhance the general findings in our study.

Another large group of participants in the present study were orthopaedic patients (16.1%). Chong et al. measured pre- and postoperative proBNP concentrations in 89 elderly patients (mean age 70.9 years SD +/- 9.6) scheduled for emergency orthopaedic surgery.[33]Their study revealed that pre- and postoperative proBNP measurements were the strongest significant predictors of 1- and 2-year mortality in a multivariable analysis (OR 3.3 95% CI 1.2-9.0 and OR 3.4 95% CI 1.1-11.0 respectively), but not when cardiovascular events before discharge were included in the model. The latter remained the single significant predictor of mortality (OR 4.7 95% CI 1.5-14.9). Nonetheless, the conclusion was that proBNP measurements are useful in identifying surgical patients at risk of cardiac events and later all cause mortality. Since trauma patients were almost non-existent amongst participants in the present study, it is likely that similar circumstances partly contributed to the results in our study population.

The biological explanations for the observed association between increased proANP concentrations and mortality in the present study are numerous. Natriuretic peptides are well-established predictors of cardiovascular mortality and morbidity. Nevertheless, other diverse conditions can lead to elevated peptide concentrations, such as cancer, renal failure, and pulmonary embolism.[34]

Since the (older) 1998-1999 NT-proBNP measurement technique is now discarded, comparison of performance between these biomarkers in the context of the present study must be made with caution, and more studies are needed. However, it can be noted that proANP consistently seemed to associate strongest with long-term mortality patients seemingly free of cardiac impairment. This could be consistent with a more cardiac-oriented sensitivity of NT-proBNP.

STUDY STRENGTHS AND LIMITATIONS

Major strengths of the present study include a large, broad cohort with a well-defined endpoint (all cause mortality) and a long follow-up period (up to 11.5 years). The latter, achievable by using more robust analysis techniques, opens up the possibility of further studies involving similar cohorts with long follow-up. A major limitation in our study is the lack of spare plasma from a large part of the original population which increases the risk of sample bias. However, the baseline values and survival in participants with proANP samples were similar to those without samples.

CONLUSION

In conclusion, our study provides evidence that high plasma proANP concentrations are associated with and predict short- and long-term all cause mortality in acutely hospitalised patients irrespective of CVD status at admission. This could potentially

lead to improved risk stratification using proANP or a combination of proANP and NT-proBNP, which would lend vital support in the evaluation of the acutely hospitalised patient.

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Conflicts of interest: None to declare for all authors.

Contributorship statement

Bo Kobberø Lauridsen: Conducted primary data analysis and wrote first draft.

Kasper Iversen: Participated in data analysis and reviewed the manuscript draft.

Ingrid Hunter: Developed proANP analysis method and reviewed the manuscript.

Morten Bay: Participated in study setup and data collection on participants. Reviewed the manuscript draft.

Vibeke Kirk: Participated in study setup and data collection on participant. Reviewed the manuscript draft.

Olav Wendelboe Nielsen: Participated in study setup. Reviewed the manuscript draft.

Henrik Nielsen: Participated in study setup. Reviewed the manuscript draft.

Søren Boesgaard: Participated in study setup. Reviewed the manuscript draft.

Lars Køber: Reviewed the manuscript draft.

Jens P. Goetze: Developed proANP analysis measured the samples and helped with drafting of the manuscript.

Data sharing*: no additional data available.*

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Figure 1:

Title: Selection of participants from the Copenhagen Hospital Heart Failure Study (1998-1999) for enrolment in the present study.

[a]Blood samples collected from 80% of participants included during the last 10 months of the original study.

Figure 2:

Title: Unadjusted Kaplan-Meier Curves for all cause mortality (in days), by proANP quartiles.

Caption: Left panel: Whole study population. Right panel: Participants with no evidence of cardiovascular disease (see text for details). All *P*<.001for difference in survival tested by log-rank trend test

Table 1: Baseline characteristics according to proANP quartiles.

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P*
N	335	337	332	333	
proANP (pmol/L) (IQR)	336 (138)	594 (161)	990 (258)	2052 (1068)	<.001
1-year mortality (%)	25 (7.5)	33 (9.8)	57 (17.2)	124 (37.2)	<.001
Long-term mortality (%)	147 (44.8)	213 (64.2)	254 (78.4)	312 (95.1)	<.001
Age (SD)	58.5 (12.2)	67.7 (12.6)	75.7 (12.3)	80.3 (9.5)	<.001
Male sex (%)	172 (51.3)	131 (38.9)	108 (32.5)	130 (39.0)	<.001
Smoking (%)	257 (76.7)	251 (74.5)	226 (68.3)	229 (69.6)	.012
Alcohol (%)	60 (18.0)	43 (12.8)	29 (8.8)	19 (5.8)	<.001
Medical history of:					
Diabetes (%)	35 (10.5)	27 (8.0)	47 (14.2)	34 (10.2)	.464
Hypertension (%)	84 (25.1)	77 (22.9)	91 (27.5)	102 (30.9)	.041
Liver disease (%)	9 (2.7)	11 (3.3)	9 (2.7)	11 (3.3)	.739
Pulmonary disease (%)	60 (17.9)	71 (21.1)	62 (18.7)	63 (19.1)	.90
MI (%)	17 (5.1)	27 (8.0)	35 (10.6)	54 (16.4)	<.001
CHF (%)	7 (2.1)	23 (6.8)	43 (13.0)	90 (27.3)	<.001
AP (%)	36 (10.8)	68 (20.2)	88 (26.6)	95 (28.8)	<.001
Valve disease (%)	3 (0.9)	7 (2.1)	9 (2.7)	15 (4.6)	.003
Findings:					
NYHA class:					
3 (%)	3 (0.9)	13 (3.9)	24 (7.3)	58 (18.0)	<.001
4 (%)	0 (0.0)	2 (0.6)	0 (0.0)	5 (1.5)	.025
eGFR (SD)	102.5 (30.4)	90.6 (30.9)	78.5 (26.7)	63.1 (27.5)	<.001
Hgb (SD)	8.5 (1.1)	8.2 (1.2)	8.0 (1.2)	7.8 (1.3)	<.001

LVEF<50 (%)	32 (9.6)	42 (12.5)	62 (18.7)	114 (34.2)	<.001
NT-proBNP (pmol/L) (IQR)	24 (32)	60 (80)	147.5 (160)	477 (740)	<.001

Values are mean ±SD, median with IQR (interquartile range), or n with %.

Differences between quartiles are tested using Cochran-Armitage test for trend or linear regression when appropriate. Abbreviations: AP = Angina pectoris; CHF = Congestive heart failure; eGFR = Estimated glomerual filtration rate; Hgb = Haemoglobin; LVEF = Left ventricular ejection fraction; MI = Myocardial infarction; NYHA class =New York Heart Association functional classification.

Table 2a: Cox proportional regression modelling of risk of 1-year all cause mortality.

	Age and sex + proANP / NT-proBNP			[a]Multivariable + proANP / NT-proBNP			[a]Multivariable + proANP + NT-proBNP		
	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
ProANP quartile (1 = reference)									
2	1.01	0.59-1.72	.967	0.90	0.52-1.55	.704	0.80	0.45-1.43	.445
3	1.42	0.85-2.37	.185	1.24	0.73-2.11	.424	0.91	0.49-1.69	.767
4	3.11	1.90-5.08	<.001	2.63	1.56-4.43	<.001	1.47	0.76-2.83	.248
[b]Trend	1.61	1.38-1.87	<.001	1.53	1.30-1.81	<.001	1.24	1.01-1.53	.040
NT-ProBNP quartile (1 = reference)									
2	1.20	0.68-2.11	.520	1.02	0.58-1.80	.947	1.10	0.60-2.03	.752
3	1.93	1.13-3.30	.017	1.72	1.00-2.97	.051	1.60	0.84-3.06	.154
4	3.82	2.27-6.43	<.001	3.15	1.83-5.42	<.001	2.41	1.22-4.75	.011
[b]Trend	1.68	1.45-1.95	<.001	1.60	1.36-1.88	<.001	1.40	1.14-1.72	.001

Cox proportional regression modelling of risk of long-term, all cause mortality.

	Age and sex + proANP / NT-proBNP			[a]Multivariable + proANP / NT-proBNP			[a]Multivariable + proANP + NT-proBNP		
	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
ProANP quartile (1 = reference)									
2	1.20	0.97-1.49	.095	1.11	0.89-1.38	.350	1.08	0.86-1.36	.521
3	1.31	1.04-1.63	.019	1.12	0.89-1.41	.325	1.01	0.78-1.32	.916
4	2.42	1.93-3.04	<.001	1.98	1.56-2.50	<.001	1.50	1.11-2.02	.008
[b]Trend	1.35	1.26-1.45	<.001	1.26	1.17-1.36	<.001	1.14	1.03-1.26	.010
NT-ProBNP quartile (1 = reference)									
2	1.15	0.92-1.42	.219	1.02	0.82-1.27	.848	1.03	0.81-1.30	.829
3	1.34	1.08-1.68	.009	1.22	0.97-1.53	.082	1.12	0.86-1.46	.406
4	2.36	1.89-2.95	.<001	1.97	1.56-2.49	<.001	1.59	1.18-2.13	.002
[b]Trend	1.35	1.26-1.45	<.001	1.28	1.19-1.38	<.001	1.18	1.07-1.30	.001

Abbreviations: CI, confidence interval; CVD, cardiovascular disease; HR, hazard ratios. [a]Adjusted for age, sex, alcohol, smoking, diabetes, history of congestive heart failure, history of pulmonary disease, history of liver disease, haemoglobin, ejection fraction below 50% and New York Heart Association functional classification 3 or 4. [b]Trend across quartiles.

Table 2b: Cox proportional regression modelling of risk of 1-year all cause mortality in participants without evidence of CVD.

	Age and sex + proANP / NT-proBNP			[a]Multivariable + proANP / NT-proBNP			[a]Multivariable + proANP + NT-proBNP		
	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
ProANP quartile (1 = reference)									
2	1.17	0.63-2.18	.623	0.91	0.47-1.73	.767	0.90	0.45-1.78	.754
3	1.13	0.58-2.20	.720	0.86	0.43-1.72	.676	0.78	0.35-1.74	.545
4	3.56	1.88-6.76	<.001	2.82	1.46-5.46	.002	2.11	0.91-4.88	.083
[b]Trend	1.61	1.30-1.99	<.001	1.54	1.23-1.92	<.001	1.37	1.04-1.82	.027
NT-ProBNP quartile (1 = reference)									
2	1.20	0.63-2.29	.579	0.91	0.48-1.76	.789	1.02	0.50-2.06	.955
3	1.58	0.82-3.04	.173	1.20	0.62-2.34	.586	1.08	0.49-2.41	.847
4	3.83	2.00-7.34	<.001	2.63	1.35-5.12	.004	1.70	0.72-4.04	.228
[b]Trend	1.65	1.33-2.03	<.001	1.48	1.19-1.85	<.001	1.24	0.93-1.64	.140

Cox proportional regression modelling of risk of long-term, all cause mortality in participants without evidence of CVD .

	Age and sex + proANP / NT-proBNP			[a]Multivariable + proANP / NT-proBNP			[a]Multivariable + proANP + NT-proBNP		
	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
ProANP quartile (1 = reference)									
2	1.33	1.03-1.72	.029	1.22	0.94-1.58	.142	1.22	0.92-1.61	.171
3	1.27	0.96-1.68	.091	1.09	0.82-1.45	.544	1.02	0.72-1.42	.931
4	2.47	1.82-3.33	<.001	2.10	1.54-2.85	<.001	1.62	1.10-2.39	.016
[b]Trend	1.30	1.18-1.44	<.001	1.23	1.11-1.37	<.001	1.14	1.00-1.30	.047
NT-ProBNP quartile (1 = reference)									
2	1.12	0.86-1.45	.391	0.96	0.74-1.25	.770	0.94	0.70-1.26	.678
3	1.30	0.98-1.71	.065	1.14	0.86-1.51	.374	1.04	0.74-1.45	.830
4	2.36	1.75-3.17	<.001	2.01	1.49-2.73	<.001	1.63	1.11-2.39	.013
[b]Trend	1.32	1.19-1.45	<.001	1.26	1.14-1.39	<.001	1.18	1.03-1.34	.014

Abbreviations: CI, confidence interval; CVD, cardiovascular disease; HR, hazard ratios. [a]Adjusted for age, sex, alcohol, smoking, diabetes, history of pulmonary disease and haemoglobin. [b]Trend across quartiles.

Table 3a: Comparison between risk prediction models of risk of 1-year all cause mortality.

	Model 1	Model 2	Model 3	Model 4	
Discrimination					
IDI		[a]0.141 (0.085-0.197)	[a]0.176 (0.118-0.234)	[a]0.201 (0.137-0.266)	[b]0.025 (-0.002-0.052)
Relative IDI		[a]0.183	[a]0.229	[a]0.262	[b]0.026
P		[a]<.001	[a].<001	[a].<001	[b].070
C-index	0.731 (0.701-0.760)	0.753 (0.724-0.783)	0.754 (0.725-0.783)	0.759 (0.730-0.788)	
P-difference		[a].003	[a].005	[a].001	[b].141

Comparison between risk prediction models of risk of long-term, all cause mortality.

Discrimination					
IDI		[a]0.053 (0.032-0.074)	[a]0.054 (0.031-0.077)	[a]0.070 (0.045-0.094)	[b]0.015 (0.0029-0.027)
Relative IDI		[a]0.044	[a]0.046	[a]0.059	[b]0.012
P		[a]<.001	[a]<.001	[a]<.001	[b].015
C-index	0.725 (0.709-0.741)	0.736 (0.720-0.752)	0.737 (0.721-0.753)	0.739 (0.724-0.755)	
P-difference		[a]<.001	[a]<.001	[a]<.001	[b].069

Model 1: Adjusted for age, sex, alcohol, smoking, diabetes, history of congestive heart failure, history of pulmonary disease, history of liver disease, haemoglobin, ejection fraction below 50% and New York Heart Association functional classification 3 or 4. Model 2: Model 1 + quartiles of proANP. Model 3: Model 1+ quartiles of NT-proBNP. Model 4: Model 1+ quartiles of proANP + quartiles of NT-proBNP. [a]Versus model 1. [b]Versus model 3.

Table 3b: Comparison between risk prediction models of risk of 1-year all cause mortality in participants without evidence of CVD.

	Model 1	Model 2	Model 3	Model 4	
Discrimination					
IDI		[a]0.185 (0.086-0.284)	[a]0.118 (0.039-0.197)	[a]0.210 (0.102-0.318)	[b]0.092 (0.025-0.160)
Relative IDI		[a]0.192	[a]0.122	[a]0.218	[b]0.085
P		[a]<.001	[a].004	[a]<.001	[b].007
C-index	0.758 (0.718-0.797)	0.780 (0.740-0.821)	0.773 (0.733-0.813)	0.782 (0.741-0.822)	
P-difference		[a].019	[a].070	[a].022	[b].178

Comparison between risk prediction models of risk of long-term, all cause mortality in participants without evidence of CVD.

Discrimination					
IDI		[a]0.045 (0.018-0.072)	[a]0.049 (0.022-0.077)	[a]0.072 (0.039-0.105)	[b]0.023 (0.004-0.041)
Relative IDI		[a]0.039	[a]0.043	[a]0.062	[b]0.019
P		[a].001	[a]<.001	[a]<.001	[b].015
C-index	0.736 (0.715-0.757)	0.747 (0.726-0.768)	0.744 (0.723-0.765)	0.748 (0.727-0.769)	
P-difference		[a].001	[a].013	[a].001	[b].015

Model 1: Adjusted for age, sex, alcohol, smoking, diabetes, history of pulmonary disease, history of liver disease, haemoglobin. Model 2: Model 1 + quartiles of proANP. Model 3: Model 1+ quartiles of NT-proBNP. Model 4: Model 1+ quartiles of proANP + quartiles of NT-proBNP. [a]Versus model 1. [b]Versus model 3.

Figure1:

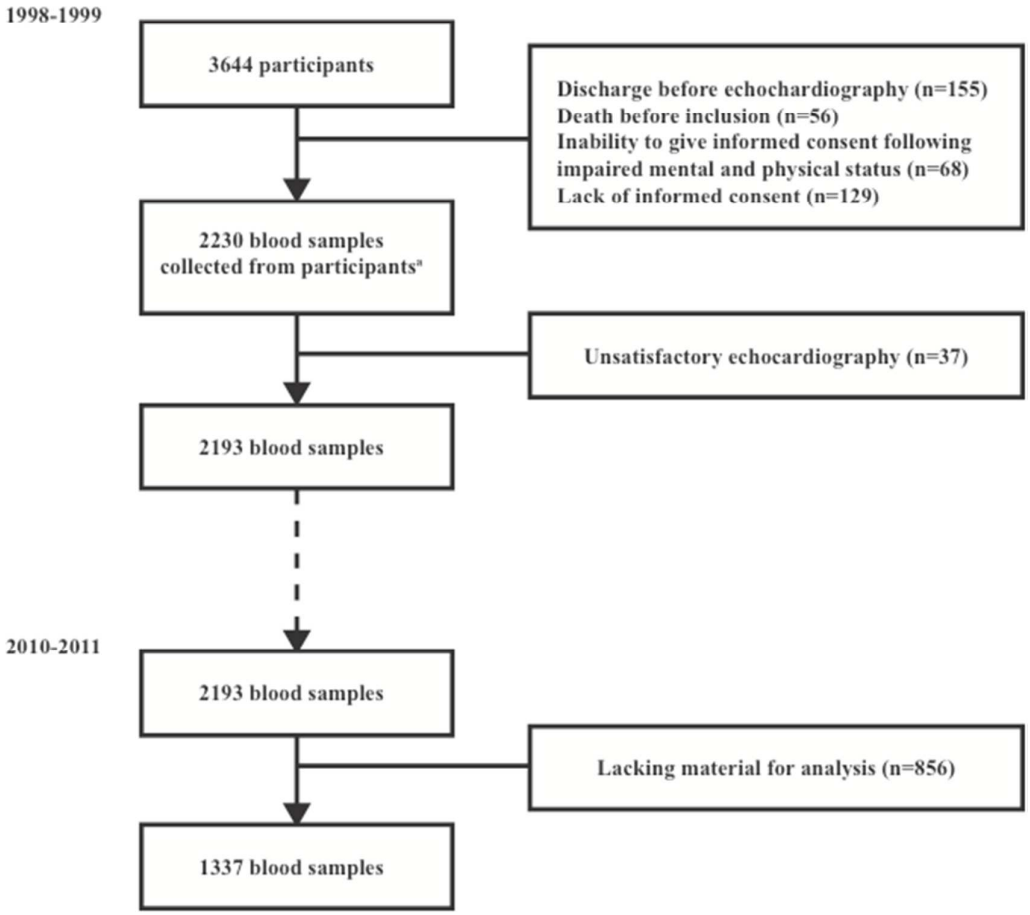
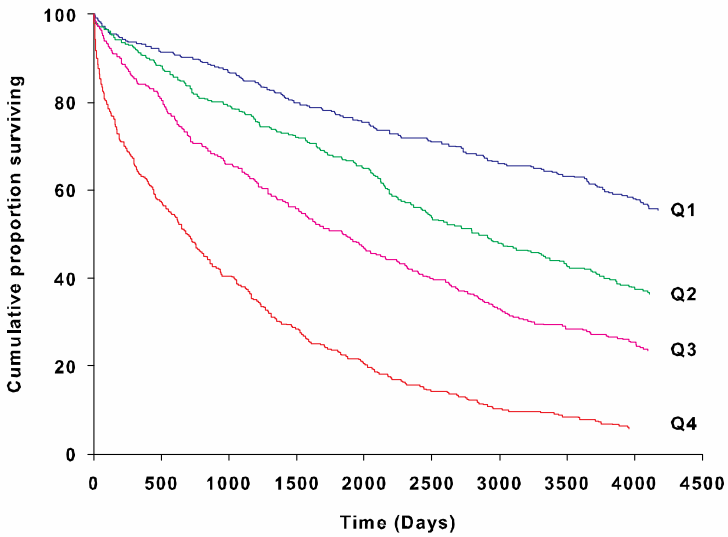
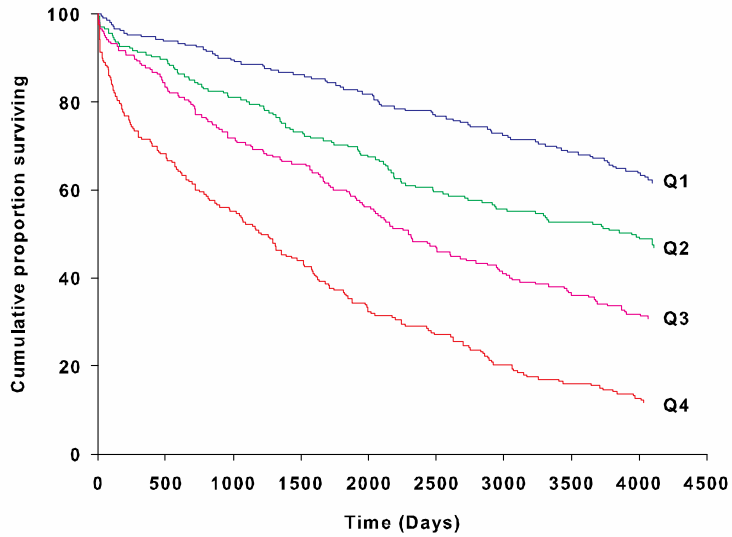


Figure 2:



Quartiles of proANP Numbers at risk

Q1	335	305	290	267	252	237	221	211	194
Q2	337	296	266	242	218	181	161	144	126
Q3	332	267	218	185	155	132	109	94	84
Q4	333	189	134	94	68	47	34	28	19



Quartiles of proANP Numbers at risk

Q1	211	197	188	181	172	161	152	144	134
Q2	205	184	166	150	138	122	114	108	100
Q3	207	171	147	135	115	97	84	74	65
Q4	207	141	114	91	67	56	42	33	26

Supplemental table 1: Full Cox proportional regression modelling of risk of 1-year all cause mortality.

	HR	95% CI	*P*
ProANP quartile (1 = reference)			
2	0.89	0.51-1.54	.669
3	1.21	0.70-2.07	.494
4	2.51	1.47-4.28	.001
Age	1.04	1.02-1.05	<.001
Male sex	1.63	1.21-2.18	.001
Alcohol	0.96	0.56-1.66	.885
Smoking	1.20	0.86-1.68	.281
Diabetes	0.98	0.63-1.52	.928
CHF	0.81	0.54-1.20	.286
MI	1.38	0.85-2.22	.190
AP	0.82	0.56-1.18	.280
Hypertension	0.92	0.68-1.25	.602
Valve disease	1.09	0.47-2.52	.837
Liver disease	1.47	0.67-3.21	.337
Pulmonary disease	1.88	1.38-2.57	<.001
NYHA class 3 or 4	1.08	0.71-1.64	.730
Hgb	0.79	0.71-0.88	<.001
eGFR	1.00	0.99-1.00	.408
LVEF<50	1.06	0.76-1.48	.712

Abbreviations: AP, Angina pectoris; CHF, Congestive heart failure; eGFR = Estimated glomerual filtration rate; Hgb, Haemoglobin; LVEF, Left ventricular ejection fraction; MI, Myocardial infarction; NYHA class, New York Heart Association functional classification.

Trend across proANP quartiles: HR 1.50 95% CI 1.27-1.78; *P*<.001

Supplemental table 2: Full Cox proportional regression modelling of risk of long-term, all cause mortality.

	HR	95% CI	*P*
ProANP quartile (1 = reference)			
2	1.11	0.89-1.38	.357
3	1.12	0.89-1.41	.324
4	2.00	1.57-2.55	<.001
Age	1.05	1.05-1.06	<.001
Male sex	1.31	1.13-1.53	<.001
Alcohol	1.11	0.87-1.42	.385
Smoking	1.25	1.06-1.46	.008
Diabetes	1.24	1.00-1.53	.046
CHF	1.12	0.90-1.38	.304
MI	1.20	0.94-1.54	.141
AP	0.97	0.80-1.16	.716
Hypertension	1.02	0.88-1.18	.814
Valve disease	1.07	0.72-1.60	.735
Liver disease	2.28	1.58-3.28	<.001
Pulmonary disease	1.53	1.29-1.80	<.001
NYHA class 3 or 4	1.16	0.92-1.48	.216
Hgb	0.86	0.81-0.91	<.001
eGFR	1.00	1.00-1.00	.462
LVEF<50	1.16	0.97-1.38	.110

Abbreviations: AP, Angina pectoris; CHF, Congestive heart failure; eGFR = Estimated glomerual filtration rate; Hgb, Haemoglobin; LVEF, Left ventricular ejection fraction; MI, Myocardial infarction; NYHA class, New York Heart Association functional classification.

Trend across proANP quartiles: HR 1.26 95% CI 1.16-1.37; *P*<.001

Supplemental table 3: Full Cox proportional regression modelling of risk of 1-year all cause mortality.

	HR	95% CI	*P*
NT-proBNP quartile (1 = reference)			
2	1.02	0.58-1.81	.946
3	1.70	0.98-2.95	.058
4	3.04	1.75-5.28	<.001
Age	1.04	1.02-1.05	<.001
Male sex	1.72	1.28-2.30	<.001
Alcohol	0.95	0.55-1.65	.865
Smoking	1.17	0.84-1.64	.354
Diabetes	0.89	0.57-1.39	.608
CHF	0.87	0.59-1.29	.483
MI	1.37	0.86-2.20	.186
AP	0.83	0.57-1.20	.318
Hypertension	0.92	0.68-1.25	.601
Valve disease	1.09	0.47-2.52	.846
Liver disease	1.71	0.78-3.73	.178
Pulmonary disease	1.89	1.38-2.58	<.001
NYHA class 3 or 4	1.11	0.73-1.69	.618
Hgb	0.78	0.70-0.87	<.001
eGFR	1.00	0.99-1.00	.320
LVEF<50	0.96	0.69-1.35	.834

Abbreviations: AP, Angina pectoris; CHF, Congestive heart failure; eGFR = Estimated glomerular filtration rate; Hgb, Haemoglobin; LVEF, Left ventricular ejection fraction; MI, Myocardial infarction; NYHA class, New York Heart Association functional classification.

Trend across NT-proBNP quartiles: HR 1.57 95% CI 1.33-1.86; *P*<.001

Supplemental table 4: Full Cox proportional regression modelling of risk of long-term, all cause mortality.

	HR	95% CI	*P*
NT-proBNP quartile (1 = reference)			
2	1.02	0.82-1.27	.877
3	1.21	0.97-1.52	.096
4	1.96	1.55-2.49	<.001
Age	1.05	1.05-1.06	<.001
Male sex	1.33	1.15-1.55	<.001
Alcohol	1.14	0.90-1.46	.271
Smoking	1.21	1.02-1.42	.026
Diabetes	1.16	0.94-1.44	.155
CHF	1.20	0.97-1.48	.087
MI	1.15	0.89-1.47	.280
AP	0.98	0.81-1.17	.803
Hypertension	1.00	0.86-1.16	.996
Valve disease	1.09	0.73-1.63	.681
Liver disease	2.31	1.61-3.32	<.001
Pulmonary disease	1.55	1.31-1.83	<.001
NYHA class 3 or 4	1.19	0.94-1.51	.157
Hgb	0.84	0.80-0.89	<.001
eGFR	1.00	1.00-1.00	.698
LVEF<50	1.10	0.92-1.31	.316

Abbreviations: AP, Angina pectoris; CHF, Congestive heart failure; eGFR = Estimated glomerual filtration rate; Hgb, Haemoglobin; LVEF, Left ventricular ejection fraction; MI, Myocardial infarction; NYHA class, New York Heart Association functional classification.

Trend across NT-proBNP quartiles: HR 1.28 95% CI 1.18-1.38; *P*<.001

Supplemental table 5: Participants by disease categories at discharge.

Disease category	N (%)
Cardiology	260 (19.5)
Orthopaedic	215 (16.1)
Gastroenterology	169 (12.6)
Haematology/oncology	88 (6.6)
Pulmonary	85 (6.4)
Endocrinology	44 (3.3)
Neurology	135 (10.1)
Infectious disease	191 (14.3)
Other	150 (11.2)

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	
Objectives	3	State specific objectives, including any prespecified hypotheses	
Methods			
Study design	4	Present key elements of study design early in the paper	
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	
Bias	9	Describe any efforts to address potential sources of bias	
Study size	10	Explain how the study size was arrived at	
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	
		(*b*) Describe any methods used to examine subgroups and interactions	
		(*c*) Explain how missing data were addressed	
		(*d*) If applicable, explain how loss to follow-up was addressed	
		(*e*) Describe any sensitivity analyses	
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	✓
		(b) Give reasons for non-participation at each stage	✓
		(c) Consider use of a flow diagram	✓
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	✓
		(b) Indicate number of participants with missing data for each variable of interest	✓
		(c) Summarise follow-up time (eg, average and total amount)	✓
Outcome data	15*	Report numbers of outcome events or summary measures over time	✓
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	✓
		(*b*) Report category boundaries when continuous variables were categorized	✓
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	✓
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	✓
Discussion			✓
Key results	18	Summarise key results with reference to study objectives	✓
Limitations			✓
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	✓
Generalisability	21	Discuss the generalisability (external validity) of the study results	✓
Other information			✓
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	✓

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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ProANP plasma measurement predicts all cause mortality in acutely hospitalised patients

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ProANP plasma measurement predicts all cause mortality in acutely hospitalised patients

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Short title: ProANP and all cause mortality.

Word count: Abstract 316, text 2617, references 1017. Three tables, two figures and five supplementary tables.

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Summary

Focus:

- ***To test if proANP associates with short- and long-term mortality in an unselected sample of acutely hospitalized patients.***
- ***To test if proANP provides additional discrimination regarding short- and long-term mortality in an unselected sample of acutely hospitalized patients.***
- ***To test both hypotheses in the full sample and in a subgroup of patients without signs of cardiovascular disease (CVD).***

Key Messages:

- ***This study illustrates that natriuretic peptides in general can be used in the clinical setting to aid in the evaluation of acutely admitted patients short- and long-term prognosis irrespectively of CVD status.***
- ***This study also suggests that combining biomarkers (proANP and proBNP) provides additional information compared to using a single marker strategy.***
- ***This study utilizes a novel and robust proANP measurement technique which opens the possibility for long-term storing of plasma samples for proANP analysis without risk of degradation.***

Strengths and Limitation:

Major strengths: Well characterized cohort with long follow-up and well defined endpoint (mortality). Robust assay for analyzing proANP.

Major limitations: Lack of plasma on subset of cohort, however this subgroup did not differ in survival and baseline values.

Abbreviations and acronyms:

ANP = atrial natriuretic peptide

AP = angina pectoris

BNP = B-type natriuretic peptide

CHF = congestive heart failure

CVD = cardiovascular disease

LVEF = left ventricular ejection fraction

MI = myocardial infarction

NT-proBNP = N-terminal pro–brain natriuretic peptide.

NYHA class = New York Heart Association functional classification

ProANP = pro-atrial natriuretic peptide

ABSTRACT

Importance: The association of natriuretic peptide measurement with all cause mortality in acutely admitted patients has not yet been examined.

Objective: To test the risk and predictive usefulness of proANP with short- and long-term mortality in acutely hospitalised patients.

Design: A prospective cohort study.

Setting: Secondary care at general hospital.

Patients: Participants (n=1337). Amongst these, 1255 (94%) were acutely hospitalised. Medical history, echocardiography, and blood samples were obtained during admission. Vital status after discharge was obtained from national central data registers.

Main Outcome Measure(s): 1-year and long-term mortality.

Results: Median follow-up period was 11.5 years. At the end of follow-up, 926 patients had died, 239 during the first year. ProANP quartiles 2-4 associated with a stepwise increase in risk of 1-year and long-term mortality compared to the first quartile in multivariable adjusted Cox proportional regression models (Hazard Ratio [HR] 1.53 95% Confidence Interval [CI] 1.30-1.81 and HR 1.26 95% CI 1.17-1.36 respectively). Addition of NT-proBNP attenuated proANP's association with mortality in the models (HR 1.24 95% CI 1.01-1.53 and 1.14 95% CI 1.03-1.26 respectively). Most of the effect seemed to associate with the highest proANP levels (fourth quartile). Similar results were observed in subgroups of participants with no evidence of cardiovascular disease (CVD). ProANP in quartiles improved discrimination when added to traditional risk factors in prediction models for 1-year (IDI 0.141 95% CI 0.085-0.197; C-index 0.753 95% CI 0.724-0.783, *P* for

improvement .003) and long-term mortality (IDI 0.053 95% CI 0.032-0.074; C-index 0.736 95% CI 0.720-0.752, *P* for improvement <.001) with similar results in subgroups. Discrimination was best in a combined model with both proANP and NT-proBNP included.

Conclusions and Relevance: Plasma proANP concentrations are associated with and predict short- and long-term all cause mortality in acutely hospitalised patients irrespective of CVD status. Risk stratification using proANP or a combination of proANP and NT-proBNP could lend vital support in the evaluation of the acutely hospitalised patient.

INTRODUCTION

Atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) have important physiological roles in fluid homeostasis and cardiac pathology, including myocardial ischemia and left ventricular dysfunction.[1,2] BNP and the N-terminal precursor fragment (NT-proBNP) have been regarded as the biomarkers of choice when obtaining diagnostic and prognostic information in heart failure patients. Recent development of assays measuring proANP-derived peptides suggests comparable performance with proBNP-derived peptides in heart failure populations.[3-6] Studies have also assessed a possible connection between natriuretic peptide concentrations and the risk of mortality in random populations,[7-9] suggesting an association between plasma concentrations and mortality independently of other risk factors. However, reservations are generally noted due to short-term follow-up and small sample sizes. Accordingly, natriuretic peptide levels and risk of short- and long-term mortality in a larger population of unselected, acutely hospitalised patients have yet to be examined.

In the present study we tested the hypothesis that measurement of total proANP products in plasma may associate with and predict short- and long-term all cause mortality in a large sample of unselected, acutely hospitalised patients irrespective of cardiovascular disease (CVD) status at study entry.

METHODS

DESIGN AND STUDY POPULATION

The present study was based on plasma collected from participants in the Copenhagen Hospital Heart Failure Study. The primary study design has been published previously.[10-12] Briefly stated, the cohort consisted of patients (>40 years of age) admitted sequentially to Amager Hospital in Copenhagen. Enrolment occurred between 1 April 1998 and 31 March 1999. Upon admission, the medical history of all included participants was obtained together with a standard physical examination and a bedside echocardiography (Hewlett Packard Imagepoint, model M2410A; Andover, Massachusetts, USA). During the last 10 months of the study, 80% (n=2230) of the included patients had blood samples collected between 08:00 and 10:00 am. All data collection occurred within 24 hours of admission. Among these, 2193 had a satisfactory echocardiography. Vital status or cause of death during follow-up was collected from national registers. Twenty-five (2%) participants emigrated during the follow-up period and were censored at time of emigration. Written consent was obtained at admission. The ethical committee of Copenhagen approved the study (Trial nr. 01-320/97) prior to enrolment of participants.

SAMPLES

Blood samples were collected in EDTA-containing tubes and centrifuged at 4°C. Plasma was stored at -20°C and only thawed once during the initial investigations.

PROANP MEASUREMENT

Plasma proANP was analysed in 2011 using an in-house method independent of changes in post-translational processing of the ANP precursor.[13] This assay has previously been compared to an automated sandwich assay for mid-regional proANP with an excellent correlation.[14] The coefficient of variation (inter-assay) was 11% at 1240 pmol/L and 6% at 2468 pmol/L. 1337 (61%) of the 2193 participants with blood samples and echocardiographic examinations on record were eligible for proANP measurement.

COVARIATES

A left ventricular ejection fraction (LVEF) <50% was chosen as a cut-off point for defining left ventricular systolic dysfunction. NT-proBNP concentrations were measured at the time of inclusion using a two-step ELISA sandwich assay with streptavidin coated microtitre plates.[15]

STATISTICS

Plasma proANP concentrations were divided into quartiles because of skewed data distribution and presented as medians with interquartile ranges (IQR). Descriptive data are presented as percentages or means with standard deviations (SD). Test for differences were performed using Cochran-Armitage test for trend or Pearson's $\chi 2$-test for categorical data and linear regression or Mann-Whitney U-test for continuous data when appropriate. Comparisons were made between participants with and without proANP measurements on baseline values using Levene's test, and on mortality using survival curves and univariate Cox analysis.

Differences in survival were illustrated using Kaplan-Meier curves based on proANP quartiles and assessed using the log-rank test. Cox proportional regression analysis was used to evaluate the association between proANP concentrations and the risk of all cause mortality, after testing the assumption of proportionality. Initially, a model was fitted using proANP (in quartiles) with age and sex as additional covariates. Subsequently a model consisting of well known predictors of mortality (Table 1) was fitted using backward elimination based on the Akaike information criterion (AIC) defined as AIC = -2 * maximum log-likelihood(model) + 2 * (numbers of covariates).[16] This balances between a model with high likelihood and a reasonable number of variables to achieve the lowest AIC possible. The final model included age, sex, alcohol, smoking, diabetes, history of congestive heart failure, history of pulmonary disease, history of liver disease, haemoglobin, ejection fraction below 50%, and New York Heart Association functional classification 3 or 4. Missing data on covariates were imputed using age and sex as independent variables. ProANP (in quartiles) was then added and the association with mortality was assessed using hazard ratios (HR).

Prediction models for 1-year and long-term mortality were developed using the same covariates as in the multivariable Cox model and then adding proANP. Hence models with traditional risk factors (model 1) were compared to models with traditional risk factors and proANP (model 2). Discrimination was evaluated by calculating the Integrated Discrimination Improvement (IDI).[17] The IDI can be regarded as the difference between improvement in average sensitivity and any potential increase in average 1-specificity when adding proANP to the prediction models. Furthermore, time-dependent C statistics were calculated and differences in the C index were tested between models with and without proANP.[18,19]

Calibration was performed by testing the addition of proANP as an independent variable to the Cox models using the likelihood ratio test. Furthermore, all models were tested using Grønnesby and Borgan goodness-of-fit (GOF) test.[20] Finally, internal validation was performed by bootstrapping the C statistics (resampling with 200 repetitions) to assess the degree of overfitting.

To further test the strength of proANP as an independent predictor of mortality in participants without cardiac impairment, all analyses were repeated using the same model; but excluding participants with evidence of CVD, defined as prior history of congestive heart failure (CHF), myocardial infarction (MI), angina pectoris (AP), valve disease, with LVEF<50% and/or New York Heart Association functional classification (NYHA class) 3 and 4 at admission.

NT-proBNP concentrations (in quartiles), measured in 1998-1999, were used in both the Cox models and the prediction models (model 3) as a quasi-internal validation of the endpoints in the population (with proANP measurements) and for direct comparison against proANP in the Cox- and predictive models. Calibration was achieved in the same manner as proANP. Furthermore, model performances were

tested after the addition of both proANP and NT-proBNP (model 4) to the multivariable predictive models and addition of proANP to predictive models with NT-proBNP included.

Statistical analyses were performed using SPSS version 16.0 (SPSS Inc., Chicago, IL) and STATA version 12 (SataCorp LP, College Station, TX). A two-sided *P*-value <.05 was considered statistically significant.

RESULTS

CLINICAL CHARACTERISTICS

No differences were noted between participants with and without proANP measurement on baseline values or short- and long-term mortality (all $P>.05$; data not shown). All baseline characteristics of the participants are listed in Table 1. Mean age at admission was 70.5 years (SD 14.3 years), 796 (59.5%) of participants were women. 1255 (94%) participants were acutely hospitalised, while the remaining were admitted electively. The median proANP concentration was 780 pmol/L (IQR 912 pmol/L). Median follow-up was 11.5 years (range 11.0-11.9 years). Follow-up was accomplished on 1337 (100%) participants after 1 year and on 1337 (100%) at the end of the study period. 239 (17.9%) participants died within the first year and 926 (69.3%) during the entire follow-up period. 617 (46.1%) participants had a history of CVD, 105 (7.9%) were NYHA class 3 or 4 and 250 (18.7%) had a LVEF<50% recorded at entry.

PROANP AND SURVIVAL

Kaplan-Meier curves displayed stepwise significant differences in survival between the quartiles of proANP within the whole study population, with the highest survival seen amongst participants in the lowest quartile (Figure 2, left panel; log rank test $P<.001$). Similar results were observed in the subgroup of participants, with no evidence of CVD (Figure 2, right panel; log rank test $P<.001$).

PROANP ASSOCIATION WITH MORTALITY

ProANP quartiles two, three, and four displayed a stepwise increase in risk of 1-year mortality compared to the first quartile in the Cox proportional regression models (Table 2a; Trend: Age and sex+proANP: HR 1.61 95% confidence interval [CI] 1.38-1.87; *P*<.001; multivariable+proANP: HR 1.53 95% CI 1.30-1.81; *P*<.001). This stepwise association was still significant, but more modest when regarding long-term mortality (Table 2a; Trend: Age and sex+proANP: HR 1.35 95% confidence interval [CI] 1.26-1.45; *P*<.001; multivariable+proANP: HR 1.26 95% CI 1.17-1.36; *P*<.001). Similar results were observed in subgroups of participants with no evidence of CVD (Table 2b). Results for proANP were attenuated when both proANP and NT-proBNP were included in the models but remained significant on trend (highest *P*=.047).

In most of the Cox regression models, the trend seemed to be carried primarily by the fourth quartile of proANP, which associated significantly with mortality (compared with the first quartile) in all models except association with 1-year mortality in multivariable models with NT-proBNP included (Table 2a and 2b; lowest *P*=.083). Full Cox models with proANP or NT-proBNP included, before selections are located in the supplemental appendix (supplemental Tables 1-4).

PROANP AS A PREDICTOR OF MORTALITY

Addition of proANP to the multivariable models improved discrimination, resulting in an IDI of 0.141 (95% CI 0.085-0.197) and 0.053 (95% CI 0.032-0.074) for 1-year and long-term mortality respectively (Table 3a, model 2; all *P* for improvement <.001). The corresponding IDI's were of the same magnitude in subgroups of participants without evidence of CVD (Table 3b, model 2; highest *P* for improvement .001).

Time dependent C-statistics for both 1-year and long term mortality increased to 0.753 (95% CI 0.724-0.783) and 0.736 (95% CI 0.720-0.752), after adding proANP

to the multivariable models (Table 3a, model 2; *P* for improvement .003 and <.001 respectively). Subgroup analysis, excluding participants with evidence of CVD, yielded similar improvements in C-statistics for 1-year and for long-term mortality (Table 3b, model 2; *P* for improvement .019 and .001 respectively).

NT-proBNP performed similar to proANP in all prediction models (Table 3a and 3b, model 3) except for 1-year mortality in participants without evidence of CVD where proANP consistently performed better although the difference was modest (Table 3b). A combined model including both proANP and NT-proBNP resulted in the best discrimination (Table 3a and 3b, model 4) measured as significant improvement of IDI's and C-index compared to the multivariable model on both 1-year and long-term mortality including subgroups (highest *P* for improvement .022). Models with both proANP and NT-proBNP provided modestly better discrimination compared to models with NT-proBNP included, for long-term mortality (*P*=.015 and *P*=.069 for improvement in IDI and C-index respectively) and in subgroups without evidence of CVD (Table 3a and 4a).

CALIBRATION

The likelihood improved significantly with addition of proANP to all models including multivariable models with NT-proBNP (highest *P*=.042). No models violated the Grønnesby and Borgan test (all *P*>.05), indicating adequate GOF. Bootstrap estimates revealed low degree of overfitting in all models.

DISCUSSION

This study demonstrates that proANP plasma concentrations independently associate with all cause mortality in an unselected population of acutely hospitalised patients. Furthermore, this association persisted in participants with seemingly normal cardiac function. To our knowledge, this is the first study to show such a correlation. Including the proANP measurement to well-established risk factors of short- and long-term mortality also improved discrimination, which underscores the general usefulness of this marker in the prognostic evaluation of the acutely hospitalised patient.

Several other studies have evaluated the association between natriuretic peptide concentrations and death. Most of these have mainly focused on populations with a history of cardiovascular disease.[7,21-23] Others include healthy populations in which the clinical validity of measuring natriuretic peptides regarding predictability of cardiovascular or all cause mortality is debatable.[24-26] In general, the present population has a higher frequency and severity of acute and chronic illnesses compared to outpatients and healthy volunteers. This population thus closely resembles what the clinician encounters in the hospital.

As the majority (94%) of participants was acutely admitted to the hospital, we looked at other studies where the populations had similar backgrounds. Several studies have investigated the diagnostic properties of natriuretic peptide measurement in patients with acute dyspnoea as the primary symptom.[27-29] In the ProBNP Investigation of Dyspnoea in the Emergency Department (PRIDE) trial, NT-proBNP measurement was shown to have valuable diagnostic applications as a rule-out marker of heart failure in a cohort of 599 patients presenting with acute shortness of breath.[30]

A follow-up study using the PRIDE cohort found NT-proBNP to be a strong predictor of 1-year mortality in a multivariate analysis (HR 2.88 95% CI 1.64-5.06; *P*<.001).[31] The conclusion was identical in a follow-up paper evaluating multiple markers.[32]

Even though our study on proANP measurement showed equal prognostic properties, caution must be made when making direct comparisons. The PRIDE cohort consisted of selective patients (with dyspnoea) whereas our cohort consisted of a broad selection of patient categories (supplemental table 5). Of the 599 patients in the PRIDE cohort presenting with acute dyspnoea, 209 (36%) were diagnosed with acute heart failure, and patients with acute severe ischemia were excluded. In our population, 250 (18.7%) had a LVEF<50% with even fewer admitted with symptoms of heart failure. These circumstances further enhance the general findings in our study.

Another large group of participants in the present study were orthopaedic patients (16.1%). Chong et al. measured pre- and postoperative proBNP concentrations in 89 elderly patients (mean age 70.9 years SD +/- 9.6) scheduled for emergency orthopaedic surgery.[33]Their study revealed that pre- and postoperative proBNP measurements were the strongest significant predictors of 1- and 2-year mortality in a multivariable analysis (OR 3.3 95% CI 1.2-9.0 and OR 3.4 95% CI 1.1-11.0 respectively), but not when cardiovascular events before discharge were included in the model. The latter remained the single significant predictor of mortality (OR 4.7 95% CI 1.5-14.9). Nonetheless, the conclusion was that proBNP measurements are useful in identifying surgical patients at risk of cardiac events and later all cause mortality. Since trauma patients were almost non-existent amongst participants in the present study, it is likely that similar circumstances partly contributed to the results in our study population.

The biological explanations for the observed association between increased proANP concentrations and mortality in the present study are numerous. Natriuretic peptides are well-established predictors of cardiovascular mortality and morbidity. Nevertheless, other diverse conditions can lead to elevated peptide concentrations, such as cancer, renal failure, and pulmonary embolism.[34]

Since the (older) 1998-1999 NT-proBNP measurement technique is now discarded, comparison of performance between these biomarkers in the context of the present study must be made with caution, and more studies are needed. However, it can be noted that proANP consistently seemed to associate strongest with long-term mortality patients seemingly free of cardiac impairment. This could be consistent with a more cardiac-oriented sensitivity of NT-proBNP.

STUDY STRENGTHS AND LIMITATIONS

Major strengths of the present study include a large, broad cohort with a well-defined endpoint (all cause mortality) and a long follow-up period (up to 11.5 years). The latter, achievable by using more robust analysis techniques, opens up the possibility of further studies involving similar cohorts with long follow-up. A major limitation in our study is the lack of spare plasma from a large part of the original population which increases the risk of sample bias. However, the baseline values and survival in participants with proANP samples were similar to those without samples.

CONLUSION

In conclusion, our study provides evidence that high plasma proANP concentrations are associated with and predict short- and long-term all cause mortality in acutely hospitalised patients irrespective of CVD status at admission. This could potentially

lead to improved risk stratification using proANP or a combination of proANP and NT-proBNP, which would lend vital support in the evaluation of the acutely hospitalised patient.

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Conflicts of interest: None to declare for all authors.

Contributorship statement

Bo Kobberø Lauridsen: Conducted primary data analysis and wrote first draft.

Kasper Iversen: Participated in data analysis and reviewed the manuscript draft.

Ingrid Hunter: Developed proANP analysis method and reviewed the manuscript.

Morten Bay: Participated in study setup and data collection on participants. Reviewed the manuscript draft.

Vibeke Kirk: Participated in study setup and data collection on participant. Reviewed the manuscript draft.

Olav Wendelboe Nielsen: Participated in study setup. Reviewed the manuscript draft.

Henrik Nielsen: Participated in study setup. Reviewed the manuscript draft.

Søren Boesgaard: Participated in study setup. Reviewed the manuscript draft.

Lars Køber: Reviewed the manuscript draft.

Jens P. Goetze: Developed proANP analysis measured the samples and helped with drafting of the manuscript.

Data sharing*: no additional data available.*

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Figure 1:

Title: Selection of participants from the Copenhagen Hospital Heart Failure Study (1998-1999) for enrolment in the present study.

[a]Blood samples collected from 80% of participants included during the last 10 months of the original study.

Figure 2:

Title: Unadjusted Kaplan-Meier Curves for all cause mortality (in days), by proANP quartiles.

Caption: Left panel: Whole study population. Right panel: Participants with no evidence of cardiovascular disease (see text for details). All *P*<.001for difference in survival tested by log-rank trend test

Table 1: Baseline characteristics according to proANP quartiles.

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P*
N	335	337	332	333	
proANP (pmol/L) (IQR)	336 (138)	594 (161)	990 (258)	2052 (1068)	<.001
1-year mortality (%)	25 (7.5)	33 (9.8)	57 (17.2)	124 (37.2)	<.001
Long-term mortality (%)	147 (44.8)	213 (64.2)	254 (78.4)	312 (95.1)	<.001
Age (SD)	58.5 (12.2)	67.7 (12.6)	75.7 (12.3)	80.3 (9.5)	<.001
Male sex (%)	172 (51.3)	131 (38.9)	108 (32.5)	130 (39.0)	<.001
Smoking (%)	257 (76.7)	251 (74.5)	226 (68.3)	229 (69.6)	.012
Alcohol (%)	60 (18.0)	43 (12.8)	29 (8.8)	19 (5.8)	<.001
Medical history of:					
Diabetes (%)	35 (10.5)	27 (8.0)	47 (14.2)	34 (10.2)	.464
Hypertension (%)	84 (25.1)	77 (22.9)	91 (27.5)	102 (30.9)	.041
Liver disease (%)	9 (2.7)	11 (3.3)	9 (2.7)	11 (3.3)	.739
Pulmonary disease (%)	60 (17.9)	71 (21.1)	62 (18.7)	63 (19.1)	.90
MI (%)	17 (5.1)	27 (8.0)	35 (10.6)	54 (16.4)	<.001
CHF (%)	7 (2.1)	23 (6.8)	43 (13.0)	90 (27.3)	<.001
AP (%)	36 (10.8)	68 (20.2)	88 (26.6)	95 (28.8)	<.001
Valve disease (%)	3 (0.9)	7 (2.1)	9 (2.7)	15 (4.6)	.003
Findings:					
NYHA class:					
3 (%)	3 (0.9)	13 (3.9)	24 (7.3)	58 (18.0)	<.001
4 (%)	0 (0.0)	2 (0.6)	0 (0.0)	5 (1.5)	.025
eGFR (SD)	102.5 (30.4)	90.6 (30.9)	78.5 (26.7)	63.1 (27.5)	<.001
Hgb (SD)	8.5 (1.1)	8.2 (1.2)	8.0 (1.2)	7.8 (1.3)	<.001

LVEF<50 (%)	32 (9.6)	42 (12.5)	62 (18.7)	114 (34.2)	<.001
NT-proBNP (pmol/L) (IQR)	24 (32)	60 (80)	147.5 (160)	477 (740)	<.001

Values are mean ±SD, median with IQR (interquartile range), or n with %.

Differences between quartiles are tested using Cochran-Armitage test for trend or linear regression when appropriate. Abbreviations: AP = Angina pectoris; CHF = Congestive heart failure; eGFR = Estimated glomerual filtration rate; Hgb = Haemoglobin; LVEF = Left ventricular ejection fraction; MI = Myocardial infarction; NYHA class =New York Heart Association functional classification.

Table 2a: Cox proportional regression modelling of risk of 1-year all cause mortality.

	Age and sex + proANP / NT-proBNP			[a]Multivariable + proANP / NT-proBNP			[a]Multivariable + proANP + NT-proBNP		
	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
ProANP quartile (1 = reference)									
2	1.01	0.59-1.72	.967	0.90	0.52-1.55	.704	0.80	0.45-1.43	.445
3	1.42	0.85-2.37	.185	1.24	0.73-2.11	.424	0.91	0.49-1.69	.767
4	3.11	1.90-5.08	<.001	2.63	1.56-4.43	<.001	1.47	0.76-2.83	.248
[b]Trend	1.61	1.38-1.87	<.001	1.53	1.30-1.81	<.001	1.24	1.01-1.53	.040
NT-ProBNP quartile (1 = reference)									
2	1.20	0.68-2.11	.520	1.02	0.58-1.80	.947	1.10	0.60-2.03	.752
3	1.93	1.13-3.30	.017	1.72	1.00-2.97	.051	1.60	0.84-3.06	.154
4	3.82	2.27-6.43	<.001	3.15	1.83-5.42	<.001	2.41	1.22-4.75	.011
[b]Trend	1.68	1.45-1.95	<.001	1.60	1.36-1.88	<.001	1.40	1.14-1.72	.001

Cox proportional regression modelling of risk of long-term, all cause mortality.

	Age and sex + proANP / NT-proBNP			[a]Multivariable + proANP / NT-proBNP			[a]Multivariable + proANP + NT-proBNP		
	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
ProANP quartile (1 = reference)									
2	1.20	0.97-1.49	.095	1.11	0.89-1.38	.350	1.08	0.86-1.36	.521
3	1.31	1.04-1.63	.019	1.12	0.89-1.41	.325	1.01	0.78-1.32	.916
4	2.42	1.93-3.04	<.001	1.98	1.56-2.50	<.001	1.50	1.11-2.02	.008
[b]Trend	1.35	1.26-1.45	<.001	1.26	1.17-1.36	<.001	1.14	1.03-1.26	.010
NT-ProBNP quartile (1 = reference)									
2	1.15	0.92-1.42	.219	1.02	0.82-1.27	.848	1.03	0.81-1.30	.829
3	1.34	1.08-1.68	.009	1.22	0.97-1.53	.082	1.12	0.86-1.46	.406
4	2.36	1.89-2.95	.<001	1.97	1.56-2.49	<.001	1.59	1.18-2.13	.002
[b]Trend	1.35	1.26-1.45	<.001	1.28	1.19-1.38	<.001	1.18	1.07-1.30	.001

Abbreviations: CI, confidence interval; CVD, cardiovascular disease; HR, hazard ratios. [a]Adjusted for age, sex, alcohol, smoking, diabetes, history of congestive heart failure, history of pulmonary disease, history of liver disease, haemoglobin, ejection fraction below 50% and New York Heart Association functional classification 3 or 4. [b]Trend across quartiles.

Table 2b: Cox proportional regression modelling of risk of 1-year all cause mortality in participants without evidence of CVD.

	Age and sex + proANP / NT-proBNP			[a]Multivariable + proANP / NT-proBNP			[a]Multivariable + proANP + NT-proBNP		
	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
ProANP quartile (1 = reference)									
2	1.17	0.63-2.18	.623	0.91	0.47-1.73	.767	0.90	0.45-1.78	.754
3	1.13	0.58-2.20	.720	0.86	0.43-1.72	.676	0.78	0.35-1.74	.545
4	3.56	1.88-6.76	<.001	2.82	1.46-5.46	.002	2.11	0.91-4.88	.083
[b]Trend	1.61	1.30-1.99	<.001	1.54	1.23-1.92	<.001	1.37	1.04-1.82	.027
NT-ProBNP quartile (1 = reference)									
2	1.20	0.63-2.29	.579	0.91	0.48-1.76	.789	1.02	0.50-2.06	.955
3	1.58	0.82-3.04	.173	1.20	0.62-2.34	.586	1.08	0.49-2.41	.847
4	3.83	2.00-7.34	<.001	2.63	1.35-5.12	.004	1.70	0.72-4.04	.228
[b]Trend	1.65	1.33-2.03	<.001	1.48	1.19-1.85	<.001	1.24	0.93-1.64	.140

Cox proportional regression modelling of risk of long-term, all cause mortality in participants without evidence of CVD .

	Age and sex + proANP / NT-proBNP			[a]Multivariable + proANP / NT-proBNP			[a]Multivariable + proANP + NT-proBNP		
	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
ProANP quartile (1 = reference)									
2	1.33	1.03-1.72	.029	1.22	0.94-1.58	.142	1.22	0.92-1.61	.171
3	1.27	0.96-1.68	.091	1.09	0.82-1.45	.544	1.02	0.72-1.42	.931
4	2.47	1.82-3.33	<.001	2.10	1.54-2.85	<.001	1.62	1.10-2.39	.016
[b]Trend	1.30	1.18-1.44	<.001	1.23	1.11-1.37	<.001	1.14	1.00-1.30	.047
NT-ProBNP quartile (1 = reference)									
2	1.12	0.86-1.45	.391	0.96	0.74-1.25	.770	0.94	0.70-1.26	.678
3	1.30	0.98-1.71	.065	1.14	0.86-1.51	.374	1.04	0.74-1.45	.830
4	2.36	1.75-3.17	<.001	2.01	1.49-2.73	<.001	1.63	1.11-2.39	.013
[b]Trend	1.32	1.19-1.45	<.001	1.26	1.14-1.39	<.001	1.18	1.03-1.34	.014

Abbreviations: CI, confidence interval; CVD, cardiovascular disease; HR, hazard ratios. [a]Adjusted for age, sex, alcohol, smoking, diabetes, history of pulmonary disease and haemoglobin. [b]Trend across quartiles.

Table 3a: Comparison between risk prediction models of risk of 1-year all cause mortality.

	Model 1	Model 2	Model 3	Model 4	
Discrimination					
IDI		[a]0.141 (0.085-0.197)	[a]0.176 (0.118-0.234)	[a]0.201 (0.137-0.266)	[b]0.025 (-0.002-0.052)
Relative IDI		[a]0.183	[a]0.229	[a]0.262	[b]0.026
P		[a]<.001	[a].<001	[a].<001	[b].070
C-index	0.731 (0.701-0.760)	0.753 (0.724-0.783)	0.754 (0.725-0.783)	0.759 (0.730-0.788)	
P-difference		[a].003	[a].005	[a].001	[b].141

Comparison between risk prediction models of risk of long-term, all cause mortality.

Discrimination					
IDI		[a]0.053 (0.032-0.074)	[a]0.054 (0.031-0.077)	[a]0.070 (0.045-0.094)	[b]0.015 (0.0029-0.027)
Relative IDI		[a]0.044	[a]0.046	[a]0.059	[b]0.012
P		[a]<.001	[a]<.001	[a]<.001	[b].015
C-index	0.725 (0.709-0.741)	0.736 (0.720-0.752)	0.737 (0.721-0.753)	0.739 (0.724-0.755)	
P-difference		[a]<.001	[a]<.001	[a]<.001	[b].069

Model 1: Adjusted for age, sex, alcohol, smoking, diabetes, history of congestive heart failure, history of pulmonary disease, history of liver disease, haemoglobin, ejection fraction below 50% and New York Heart Association functional classification 3 or 4. Model 2: Model 1 + quartiles of proANP. Model 3: Model 1+ quartiles of NT-proBNP. Model 4: Model 1+ quartiles of proANP + quartiles of NT-proBNP. [a]Versus model 1. [b]Versus model 3.

Table 3b: Comparison between risk prediction models of risk of 1-year all cause mortality in participants without evidence of CVD.

	Model 1	Model 2	Model 3	Model 4	
Discrimination					
IDI		[a]0.185 (0.086-0.284)	[a]0.118 (0.039-0.197)	[a]0.210 (0.102-0.318)	[b]0.092 (0.025-0.160)
Relative IDI		[a]0.192	[a]0.122	[a]0.218	[b]0.085
P		[a]<.001	[a].004	[a]<.001	[b].007
C-index	0.758 (0.718-0.797)	0.780 (0.740-0.821)	0.773 (0.733-0.813)	0.782 (0.741-0.822)	
P-difference		[a].019	[a].070	[a].022	[b].178

Comparison between risk prediction models of risk of long-term, all cause mortality in participants without evidence of CVD.

Discrimination					
IDI		[a]0.045 (0.018-0.072)	[a]0.049 (0.022-0.077)	[a]0.072 (0.039-0.105)	[b]0.023 (0.004-0.041)
Relative IDI		[a]0.039	[a]0.043	[a]0.062	[b]0.019
P		[a].001	[a]<.001	[a]<.001	[b].015
C-index	0.736 (0.715-0.757)	0.747 (0.726-0.768)	0.744 (0.723-0.765)	0.748 (0.727-0.769)	
P-difference		[a].001	[a].013	[a].001	[b].015

Model 1: Adjusted for age, sex, alcohol, smoking, diabetes, history of pulmonary disease, history of liver disease, haemoglobin. Model 2: Model 1 + quartiles of proANP. Model 3: Model 1+ quartiles of NT-proBNP. Model 4: Model 1+ quartiles of proANP + quartiles of NT-proBNP. [a]Versus model 1. [b]Versus model 3.

Figure1:

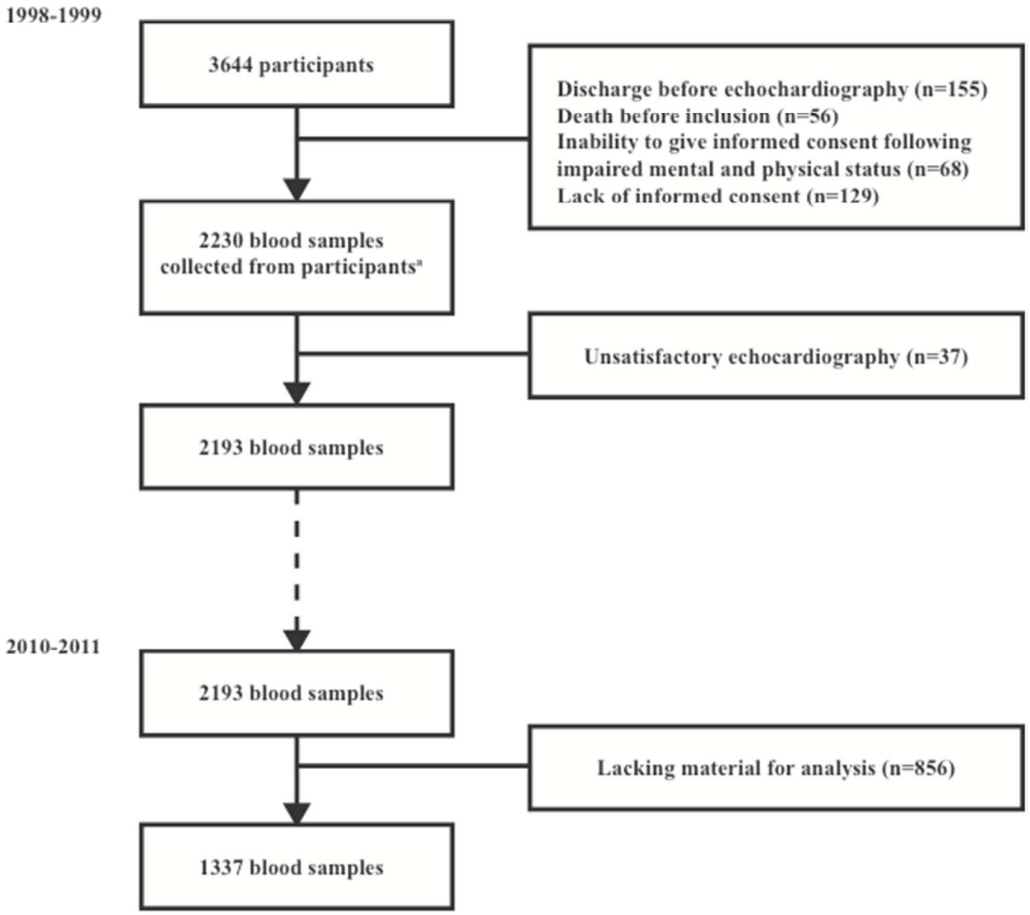
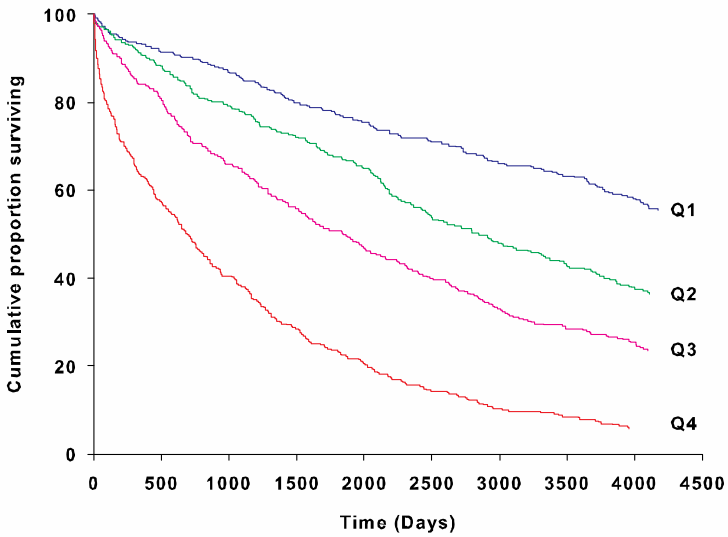
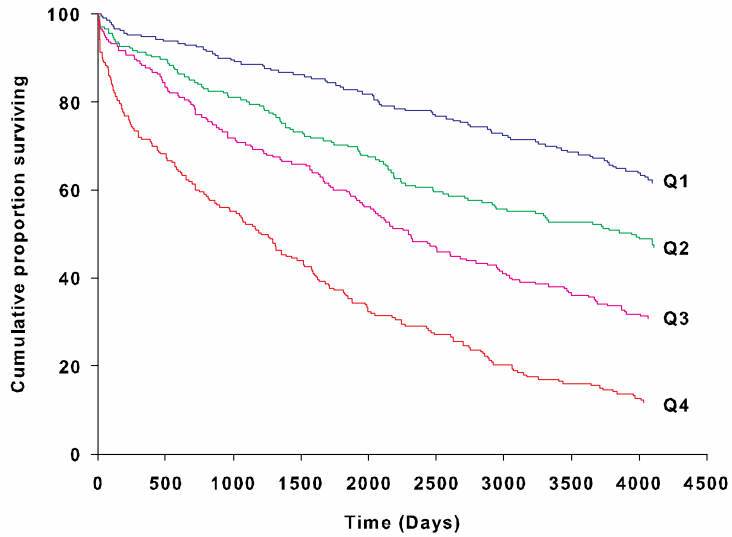


Figure 2:



Quartiles of proANP Numbers at risk

Q1	335	305	290	267	252	237	221	211	194
Q2	337	296	266	242	218	181	161	144	126
Q3	332	267	218	185	155	132	109	94	84
Q4	333	189	134	94	68	47	34	28	19



Quartiles of proANP Numbers at risk

Q1	211	197	188	181	172	161	152	144	134
Q2	205	184	166	150	138	122	114	108	100
Q3	207	171	147	135	115	97	84	74	65
Q4	207	141	114	91	67	56	42	33	26

Supplemental table 1: Full Cox proportional regression modelling of risk of 1-year all cause mortality.

	HR	95% CI	*P*
ProANP quartile (1 = reference)			
2	0.89	0.51-1.54	.669
3	1.21	0.70-2.07	.494
4	2.51	1.47-4.28	.001
Age	1.04	1.02-1.05	<.001
Male sex	1.63	1.21-2.18	.001
Alcohol	0.96	0.56-1.66	.885
Smoking	1.20	0.86-1.68	.281
Diabetes	0.98	0.63-1.52	.928
CHF	0.81	0.54-1.20	.286
MI	1.38	0.85-2.22	.190
AP	0.82	0.56-1.18	.280
Hypertension	0.92	0.68-1.25	.602
Valve disease	1.09	0.47-2.52	.837
Liver disease	1.47	0.67-3.21	.337
Pulmonary disease	1.88	1.38-2.57	<.001
NYHA class 3 or 4	1.08	0.71-1.64	.730
Hgb	0.79	0.71-0.88	<.001
eGFR	1.00	0.99-1.00	.408
LVEF<50	1.06	0.76-1.48	.712

Abbreviations: AP, Angina pectoris; CHF, Congestive heart failure; eGFR = Estimated glomerual filtration rate; Hgb, Haemoglobin; LVEF, Left ventricular ejection fraction; MI, Myocardial infarction; NYHA class, New York Heart Association functional classification.

Trend across proANP quartiles: HR 1.50 95% CI 1.27-1.78; *P*<.001

Supplemental table 2: Full Cox proportional regression modelling of risk of long-term, all cause mortality.

	HR	95% CI	*P*
ProANP quartile (1 = reference)			
2	1.11	0.89-1.38	.357
3	1.12	0.89-1.41	.324
4	2.00	1.57-2.55	<.001
Age	1.05	1.05-1.06	<.001
Male sex	1.31	1.13-1.53	<.001
Alcohol	1.11	0.87-1.42	.385
Smoking	1.25	1.06-1.46	.008
Diabetes	1.24	1.00-1.53	.046
CHF	1.12	0.90-1.38	.304
MI	1.20	0.94-1.54	.141
AP	0.97	0.80-1.16	.716
Hypertension	1.02	0.88-1.18	.814
Valve disease	1.07	0.72-1.60	.735
Liver disease	2.28	1.58-3.28	<.001
Pulmonary disease	1.53	1.29-1.80	<.001
NYHA class 3 or 4	1.16	0.92-1.48	.216
Hgb	0.86	0.81-0.91	<.001
eGFR	1.00	1.00-1.00	.462
LVEF<50	1.16	0.97-1.38	.110

Abbreviations: AP, Angina pectoris; CHF, Congestive heart failure; eGFR = Estimated glomerual filtration rate; Hgb, Haemoglobin; LVEF, Left ventricular ejection fraction; MI, Myocardial infarction; NYHA class, New York Heart Association functional classification.

Trend across proANP quartiles: HR 1.26 95% CI 1.16-1.37; *P*<.001

Supplemental table 3: Full Cox proportional regression modelling of risk of 1-year all cause mortality.

	HR	95% CI	*P*
NT-proBNP quartile (1 = reference)			
2	1.02	0.58-1.81	.946
3	1.70	0.98-2.95	.058
4	3.04	1.75-5.28	<.001
Age	1.04	1.02-1.05	<.001
Male sex	1.72	1.28-2.30	<.001
Alcohol	0.95	0.55-1.65	.865
Smoking	1.17	0.84-1.64	.354
Diabetes	0.89	0.57-1.39	.608
CHF	0.87	0.59-1.29	.483
MI	1.37	0.86-2.20	.186
AP	0.83	0.57-1.20	.318
Hypertension	0.92	0.68-1.25	.601
Valve disease	1.09	0.47-2.52	.846
Liver disease	1.71	0.78-3.73	.178
Pulmonary disease	1.89	1.38-2.58	<.001
NYHA class 3 or 4	1.11	0.73-1.69	.618
Hgb	0.78	0.70-0.87	<.001
eGFR	1.00	0.99-1.00	.320
LVEF<50	0.96	0.69-1.35	.834

Abbreviations: AP, Angina pectoris; CHF, Congestive heart failure; eGFR = Estimated glomerular filtration rate; Hgb, Haemoglobin; LVEF, Left ventricular ejection fraction; MI, Myocardial infarction; NYHA class, New York Heart Association functional classification.

Trend across NT-proBNP quartiles: HR 1.57 95% CI 1.33-1.86; *P*<.001

Supplemental table 4: Full Cox proportional regression modelling of risk of long-term, all cause mortality.

	HR	95% CI	*P*
NT-proBNP quartile (1 = reference)			
2	1.02	0.82-1.27	.877
3	1.21	0.97-1.52	.096
4	1.96	1.55-2.49	<.001
Age	1.05	1.05-1.06	<.001
Male sex	1.33	1.15-1.55	<.001
Alcohol	1.14	0.90-1.46	.271
Smoking	1.21	1.02-1.42	.026
Diabetes	1.16	0.94-1.44	.155
CHF	1.20	0.97-1.48	.087
MI	1.15	0.89-1.47	.280
AP	0.98	0.81-1.17	.803
Hypertension	1.00	0.86-1.16	.996
Valve disease	1.09	0.73-1.63	.681
Liver disease	2.31	1.61-3.32	<.001
Pulmonary disease	1.55	1.31-1.83	<.001
NYHA class 3 or 4	1.19	0.94-1.51	.157
Hgb	0.84	0.80-0.89	<.001
eGFR	1.00	1.00-1.00	.698
LVEF<50	1.10	0.92-1.31	.316

Abbreviations: AP, Angina pectoris; CHF, Congestive heart failure; eGFR = Estimated glomerual filtration rate; Hgb, Haemoglobin; LVEF, Left ventricular ejection fraction; MI, Myocardial infarction; NYHA class, New York Heart Association functional classification.

Trend across NT-proBNP quartiles: HR 1.28 95% CI 1.18-1.38; *P*<.001

Supplemental table 5: Participants by disease categories at discharge.

Disease category	N (%)
Cardiology	260 (19.5)
Orthopaedic	215 (16.1)
Gastroenterology	169 (12.6)
Haematology/oncology	88 (6.6)
Pulmonary	85 (6.4)
Endocrinology	44 (3.3)
Neurology	135 (10.1)
Infectious disease	191 (14.3)
Other	150 (11.2)

ProANP plasma measurement predicts all cause mortality in acutely hospitalised patients

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Contributorship statement

Bo Kobberø Lauridsen: Conducted primary data analysis and wrote first draft.

Kasper Iversen: Participated in data analysis and reviewed the manuscript draft.

Ingrid Hunter: Developed proANP analysis method and reviewed the manuscript.

Morten Bay: Participated in study setup and data collection on participants. Reviewed the manuscript draft.

Vibeke Kirk: Participated in study setup and data collection on participant. Reviewed the manuscript draft.

Olav Wendelboe Nielsen: Participated in study setup. Reviewed the manuscript draft.

Henrik Nielsen: Participated in study setup. Reviewed the manuscript draft.

Søren Boesgaard: Participated in study setup. Reviewed the manuscript draft.

Lars Køber: Reviewed the manuscript draft.

Jens P. Goetze: Developed proANP analysis measured the samples and helped with drafting of the manuscript.

Summary

Focus:

- *To test if proANP associates with short- and long-term mortality in an unselected sample of acutely hospitalized patients.*
- *To test if proANP provides additional discrimination regarding short- and long-term mortality in an unselected sample of acutely hospitalized patients.*
- *To test both hypotheses in the full sample and in a subgroup of patients without signs of cardiovascular disease (CVD).*

Key Messages:

- *This study illustrates that natriuretic peptides in general can be used in the clinical setting to aid in the evaluation of acutely admitted patients short- and long-term prognosis irrespectively of CVD status.*
- *This study also suggests that combining biomarkers (proANP and proBNP) provides additional information compared to using a single marker strategy.*
- *This study utilizes a novel and robust proANP measurement technique which opens the possibility for long-term storing of plasma samples for proANP analysis without risk of degradation.*

Strengths and Limitation:

Major strengths: Well characterized cohort with long follow-up and well defined endpoint (mortality). Robust assay for proANP measurement.

Major limitations: Lack of plasma on subset of cohort, however this subgroup did not differ in survival and baseline values.

- Please include a data sharing statement which either says where extra data can be accessed (e.g. "Extra data is available by emailing XYZ") or simply "There is no additional data available".

There is no additional data available.

- It is a good idea to include statements relating to ethics, funding, data sharing, etc within the main document file so that they are easily accessible to peer reviewers.

This information is stated on the title page.

Abbreviations and acronyms:

ANP = atrial natriuretic peptide

AP = angina pectoris

BNP = B-type natriuretic peptide

CHF = congestive heart failure

CVD = cardiovascular disease

LVEF = left ventricular ejection fraction

MI = myocardial infarction

NT-proBNP = N-terminal pro–brain natriuretic peptide.

NYHA class = New York Heart Association functional classification

ProANP = pro-atrial natriuretic peptide

ABSTRACT

Importance: The association of natriuretic peptide measurement with all cause mortality in a broad selection of acutely admitted patients has not yet been examined.

Objective: ~~To test the risk association and predictive usefulness of proANP measurement with short- and long-term mortality in acutely hospitalised patients~~ To test the risk association between proANP and short- and long-term mortality and its predictive value in acutely hospitalised patients and compare this to NT-proBNP.

Design, Setting and Patients: Participants ~~(n=1337)~~ were selected from the Copenhagen Hospital Heart Failure Study (n=3644) ~~were included~~. Medical history, satisfactory echocardiography, and blood samples were ~~obtained during admission~~ available on 2193 participants in 1998-1999 were NT-proBNP was measured. Vital status after discharge was obtained from national central data registers. 1337 participants with eligible blood samples in were selected in 2010-2011 for proANP measurement. Amongst these, 1255 (94%) were acutely hospitalised in 1998-1999.

Main Outcome Measure(s): 1-year and long-term mortality.

Results: Median follow-up period was 11.5 years. At the end of follow-up, 926 patients had died, 239 during the first year. ProANP quartiles to 2-4 (median proANP levels: 594 pmol/L, 990 pmol/L and 2052 pmol/L respectively) associated with a stepwise increase in risk of 1-year and long-term mortality compared to the first quartile (336 pmol/L) in multivariable adjusted Cox proportional regression models (Hazard Ratio [HR] 1.53 95% Confidence Interval [CI] 1.30-1.81 and HR 1.26 95% CI 1.17-1.36 respectively). Addition of NT-proBNP attenuated proANP's association with mortality in the models (HR 1.24 95% CI 1.01-1.53 and 1.14 95% CI 1.03-1.26 respectively). ~~Most of the effect seemed to associate~~ The increased risk was observed

in participants with the highest proANP levels (fourth quartile). Similar results were observed in subgroups of participants with no evidence of cardiovascular disease (CVD). ProANP in quartiles improved discrimination when added to traditional risk factors in prediction models for 1-year (IDI 0.141 95% CI 0.085-0.197; C-index 0.753 95% CI 0.724-0.783, *P* for improvement .003) and long-term mortality (IDI 0.053 95% CI 0.032-0.074; C-index 0.736 95% CI 0.720-0.752, *P* for improvement <.001) with similar results in subgroups. Discrimination was best in a combined model with both proANP and NT-proBNP included.

Conclusions and Relevance: High plasma proANP concentrations are associated with and predict short- and long-term all cause mortality in acutely hospitalised patients irrespective of CVD status at admission. ~~Risk stratification using proANP or a combination of proANP and NT-proBNP could lend vital support in the evaluation of the acutely hospitalised patient.~~

INTRODUCTION

Atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) have important physiological roles in fluid homeostasis and cardiac pathology, including myocardial ischemia and left ventricular dysfunction.[1,2] BNP and the N-terminal precursor fragment (NT-proBNP) have been regarded as the biomarkers of choice when obtaining diagnostic and prognostic information in heart failure patients. Recent development of assays measuring proANP-derived peptides suggests comparable performance with proBNP-derived peptides in heart failure populations.[3-6] Studies have also assessed a possible connection between natriuretic peptide concentrations and the risk of mortality in random populations,[7-9] suggesting an association between plasma concentrations and mortality independently of other risk factors. However, reservations are generally noted due to short-term follow-up and small sample sizes. Accordingly, natriuretic peptide levels and risk of short- and long-term mortality in a larger population of unselected, acutely hospitalised patients have yet to be examined.

In the present study we tested the hypothesis that measurement of total proANP products in plasma may associate with and predict short- and long-term all cause mortality in a large sample of unselected, acutely hospitalised patients irrespective of cardiovascular disease (CVD) status at study entry.

METHODS

DESIGN AND STUDY POPULATION

The present study was based on plasma collected from participants in the Copenhagen Hospital Heart Failure Study. The primary study design has been published previously.[10-12] Briefly stated, the cohort consisted of patients (>40 years of age) admitted sequentially to Amager Hospital in Copenhagen. Enrolment occurred between 1 April 1998 and 31 March 1999. Upon admission, the medical history of all included participants was obtained together with a standard physical examination and a bedside echocardiography (Hewlett Packard Imagepoint, model M2410A; Andover, Massachusetts, USA). During the last 10 months of the study, 80% (n=2230) of the included patients in that period had blood samples collected between 08:00 and 10:00 am. All data collection occurred within 24 hours of admission. ~~Among the 2230 patients,~~ 2193 of the 2230 patients had a satisfactory echocardiography. 1337 (61%) of the 2193 participants with blood samples from 1998-1999 and echocardiographic examinations on record were eligible for proANP measurement in 2010-2011 (Figure 1). Vital status or cause of death during follow-up was collected from national registers. Twenty-five (2%) participants emigrated during the follow-up period and were censored at time of emigration. Written consent was obtained at admission. The ethical committee of Copenhagen approved the study (Trial nr. 01-320/97) prior to enrolment of participants.

SAMPLES

Blood samples were collected in EDTA-containing tubes and centrifuged at 4°C. Plasma was stored at -20°C and only thawed once during the initial investigations.

PROANP MEASUREMENT

Plasma proANP was analysed in 2011 using an in-house method independent of changes in post-translational processing of the ANP precursor.[13] This assay has previously been compared to an automated sandwich assay for mid-regional proANP with an excellent correlation.[14] Notably, this assay measures and internal epitope in the N-terminal proANP fragment that only is released after trypsin treatment of plasma; hence the assay is extremely robust in terms of degradation in frozen plasma.[15]

The coefficient of variation (inter-assay) was 11% at 1240 pmol/L and 6% at 2468 pmol/L. ~~1337 (61%) of the 2193 participants with blood samples and echocardiographic examinations on record were eligible for proANP measurement.~~

COVARIATES

A left ventricular ejection fraction (LVEF) <50% was chosen as a cut-off point for defining left ventricular systolic dysfunction. NT-proBNP concentrations were measured at the time of inclusion using a two-step ELISA sandwich assay with streptavidin coated microtitre plates.[16]

STATISTICS

Plasma proANP concentrations were divided into quartiles or log-transformed because of skewed data distribution and presented as medians with interquartile ranges (IQR). Descriptive data are presented as percentages or means with standard deviations (SD). Test for differences were performed using Cochran-Armitage test for trend or Pearson's $\chi 2$-test for categorical data and analysis-of-variance or Mann-Whitney U-test for continuous data when appropriate. Comparisons were made between participants with and without proANP measurements on baseline values using Levene's test, and on mortality using survival curves and univariate Cox analysis.

Differences in survival were illustrated using Kaplan-Meier curves based on proANP quartiles and assessed using the log-rank test. Cox proportional regression analysis was used to evaluate the association between proANP concentrations and the risk of all cause mortality, after testing the assumption of proportionality. Initially, a model was fitted using proANP (in quartiles) with age and sex as additional covariates. Subsequently a model consisting of well known predictors of mortality (Table 1) was fitted using backward elimination based on the Akaike information criterion (AIC) defined as AIC = -2 * maximum log-likelihood(model) + 2 * (numbers of covariates).[17] This balances between a model with high likelihood and a reasonable number of variables to achieve the lowest AIC possible. The final model included age, sex, alcohol, smoking, diabetes, history of congestive heart failure, history of pulmonary disease, history of liver disease, haemoglobin, ejection fraction below 50%, and New York Heart Association functional classification 3 or 4. Missing data on covariates were imputed using age and sex as independent variables. ProANP (in quartiles) was then added and the association with mortality was assessed using

hazard ratios (HR). Furthermore log-transformed values of proANP were used in all Cox models.

Prediction models for 1-year and long-term mortality were developed using the same covariates as in the multivariable Cox model and then adding proANP. Hence models with traditional risk factors (model 1) were compared to models with traditional risk factors and proANP (model 2). Discrimination was evaluated by calculating the Integrated Discrimination Improvement (IDI).[18] The IDI can be regarded as the difference between improvement in average sensitivity and any potential increase in average 1-specificity when adding proANP to the prediction models. Furthermore, time-dependent C statistics were calculated and differences in the C index were tested between models with and without proANP.[19,20]

Calibration was performed by testing the addition of proANP as an independent variable to the Cox models using the likelihood ratio test. Furthermore, all models were tested using Grønnesby and Borgan goodness-of-fit (GOF) test.[21] Finally, internal validation was performed by bootstrapping the C statistics (resampling with 200 repetitions) to assess the degree of overfitting.

To further test the strength of proANP as an independent predictor of mortality in participants without cardiac impairment, all analyses were repeated using the same model; but excluding participants with evidence of CVD, defined as prior history of congestive heart failure (CHF), myocardial infarction (MI), angina pectoris (AP), valve disease, with LVEF<50% and/or New York Heart Association functional classification (NYHA class) 3 and 4 at admission.

NT-proBNP concentrations ~~(in quartiles)~~, measured in 1998-1999, were used in both the Cox models and the prediction models (model 3) as a quasi-internal validation of the endpoints in the population (with proANP measurements) and for

direct comparison against proANP in the Cox- and predictive models. Calibration was achieved in the same manner as proANP. Furthermore, model performances were tested after the addition of both proANP and NT-proBNP (model 4) to the multivariable predictive models and addition of proANP to predictive models with NT-proBNP included.

Statistical analyses were performed using SPSS version 16.0 (SPSS Inc., Chicago, IL) and STATA version 12 (SataCorp LP, College Station, TX). A two-sided *P*-value <.05 was considered statistically significant.

RESULTS

CLINICAL CHARACTERISTICS

No differences were noted between participants with and without proANP measurement on baseline values or short- and long-term mortality (all $P>.05$; data not shown). All baseline characteristics of the participants are listed in Table 1. Mean age at admission was 70.5 years (SD 14.3 years), 796 (59.5%) of participants were women. 1255 (94%) participants were acutely hospitalised, while the remaining were admitted electively. The median proANP concentration was 780 pmol/L (IQR 912 pmol/L). Median follow-up was 11.5 years (range 11.0-11.9 years). Follow-up was accomplished on 1337 (100%) participants after 1 year and on 1337 (100%) at the end of the study period. 239 (17.9%) participants died within the first year and 926 (69.3%) during the entire follow-up period. 617 (46.1%) participants had a history of CVD, 105 (7.9%) were NYHA class 3 or 4 and 250 (18.7%) had a LVEF<50% recorded at entry.

PROANP AND SURVIVAL

Kaplan-Meier curves displayed stepwise significant differences in survival between the quartiles of proANP within the whole study population, with the highest survival seen amongst participants in the lowest quartile (Figure 2, left panel; log rank test $P<.001$). Similar results were observed in the subgroup of participants, with no evidence of CVD (Figure 2, right panel; log rank test $P<.001$).

PROANP ASSOCIATION WITH MORTALITY

ProANP quartiles two, three, and four displayed a stepwise increase in risk of 1-year mortality compared to the first quartile in the Cox proportional regression models (Table 2a; Trend: Age and sex+proANP: HR 1.61 95% confidence interval [CI] 1.38-1.87; *P*<.001; multivariable+proANP: HR 1.53 95% CI 1.30-1.81; *P*<.001). This stepwise association was still significant, but more modest when regarding long-term mortality (Table 2a; Trend: Age and sex+proANP: HR 1.35 95% confidence interval [CI] 1.26-1.45; *P*<.001; multivariable+proANP: HR 1.26 95% CI 1.17-1.36; *P*<.001). Similar results were observed in subgroups of participants with no evidence of CVD (Table 2b). Results for proANP were attenuated when both proANP and NT-proBNP were included in the models but remained significant on trend (highest *P*=.047).

In most of the Cox regression models, the trend seemed to be carried primarily by the fourth quartile of proANP, which associated significantly with mortality (compared with the first quartile) in all models except association with 1-year mortality in multivariable models with NT-proBNP included (Table 2a and 2b; lowest *P*=.083). The log transformed values of proANP and NT-proBNP also associated with short and long-term mortality (HRs for 1 log unit change are seen in table 2 and 3). Log transformed proANP performed modestly better than log transformed NT-proBNP in most analysis but proANP was also slightly skewed after log transformation.

Full Cox models with proANP or NT-proBNP included, before selections are located in the supplemental appendix (supplemental Tables 1-4).

PROANP AS A PREDICTOR OF MORTALITY

Addition of proANP to the multivariable models improved discrimination, resulting in an IDI of 0.141 (95% CI 0.085-0.197) and 0.053 (95% CI 0.032-0.074) for 1-year and

long-term mortality respectively (Table 3a, model 2; all *P* for improvement <.001). The corresponding IDI's were of the same magnitude in subgroups of participants without evidence of CVD (Table 3b, model 2; highest *P* for improvement .001).

Time dependent C-statistics for both 1-year and long term mortality increased to 0.753 (95% CI 0.724-0.783) and 0.736 (95% CI 0.720-0.752), after adding proANP to the multivariable models (Table 3a, model 2; *P* for improvement .003 and <.001 respectively). Subgroup analysis, excluding participants with evidence of CVD, yielded similar improvements in C-statistics for 1-year and for long-term mortality (Table 3b, model 2; *P* for improvement .019 and .001 respectively).

NT-proBNP performed similar to proANP in all prediction models (Table 3a and 3b, model 3) except for 1-year mortality in participants without evidence of CVD where proANP consistently performed better although the difference was modest (Table 3b). A combined model including both proANP and NT-proBNP resulted in the best discrimination (Table 3a and 3b, model 4) measured as significant improvement of IDI's and C-index compared to the multivariable model on both 1-year and long-term mortality including subgroups (highest *P* for improvement .022). Models with both proANP and NT-proBNP provided modestly better discrimination compared to models with NT-proBNP included, for long-term mortality (*P*=.015 and *P*=.069 for improvement in IDI and C-index respectively) and in subgroups without evidence of CVD (Table 3a and 4a).

CALIBRATION

The likelihood improved significantly with addition of proANP to all models including multivariable models with NT-proBNP (highest *P*=.042). No models

violated the Grønnesby and Borgan test (all $P>.05$), indicating adequate GOF. Bootstrap estimates revealed low degree of overfitting in all models.

DISCUSSION

This study demonstrates that proANP plasma concentrations independently associate with all cause mortality in an unselected population of acutely hospitalised patients. Furthermore, this association persisted in participants with seemingly normal cardiac function. To our knowledge, this is the first study to show such a correlation. Including the proANP measurement to well-established risk factors of short- and long-term mortality also improved discrimination, which underscores the general usefulness of this marker in the prognostic evaluation of the acutely hospitalised patient.

Several other studies have evaluated the association between natriuretic peptide concentrations and death. Most of these have mainly focused on populations with a history of cardiovascular disease.[7,22-24] Others include healthy populations in which the clinical validity of measuring natriuretic peptides regarding predictability of cardiovascular or all cause mortality is debatable.[25-27] In general, the present population has a higher frequency and severity of acute and chronic illnesses compared to outpatients and healthy volunteers. This population thus closely resembles what the clinician encounters in the hospital.

As the majority (94%) of participants was acutely admitted to the hospital, we looked at other studies where the populations had similar backgrounds. Several studies have investigated the diagnostic properties of natriuretic peptide measurement in patients with acute dyspnoea as the primary symptom.[28-30] In the ProBNP

Investigation of Dyspnoea in the Emergency Department (PRIDE) trial, NT-proBNP measurement was shown to have valuable diagnostic applications as a rule-out marker of heart failure in a cohort of 599 patients presenting with acute shortness of breath.[31] A follow-up study using the PRIDE cohort found NT-proBNP to be a strong predictor of 1-year mortality in a multivariate analysis (HR 2.88 95% CI 1.64-5.06; *P*<.001).[32] The conclusion was identical in a follow-up paper evaluating multiple markers.[33]

Even though our study on proANP measurement showed equal prognostic properties, caution must be made when making direct comparisons. The PRIDE cohort consisted of selective patients (with dyspnoea) whereas our cohort consisted of a broad selection of patient categories (supplemental table 5). Of the 599 patients in the PRIDE cohort presenting with acute dyspnoea, 209 (36%) were diagnosed with acute heart failure, and patients with acute severe ischemia were excluded. In our population, 250 (18.7%) had a LVEF<50% with even fewer admitted with symptoms of heart failure. These circumstances further enhance the general findings in our study.

Another large group of participants in the present study were orthopaedic patients (16.1%). Chong et al. measured pre- and postoperative proBNP concentrations in 89 elderly patients (mean age 70.9 years SD +/- 9.6) scheduled for emergency orthopaedic surgery.[34] Their study revealed that pre- and postoperative proBNP measurements were the strongest significant predictors of 1- and 2-year mortality in a multivariable analysis (OR 3.3 95% CI 1.2-9.0 and OR 3.4 95% CI 1.1-11.0 respectively), but not when cardiovascular events before discharge were included in the model. The latter remained the single significant predictor of mortality (OR 4.7 95% CI 1.5-14.9). Nonetheless, the conclusion was that proBNP measurements are useful in identifying surgical patients at risk of cardiac events and later all cause

mortality. Since trauma patients were almost non-existent amongst participants in the present study, it is likely that similar circumstances partly contributed to the results in our study population.

The biological explanations for the observed association between increased proANP concentrations and mortality in the present study are numerous. Natriuretic peptides are well-established predictors of cardiovascular mortality and morbidity. Nevertheless, other diverse conditions can lead to elevated peptide concentrations, such as cancer, renal failure, and pulmonary embolism.[35]

Since the (older) 1998-1999 NT-proBNP measurement technique is now discarded, comparison of performance between these biomarkers in the context of the present study must be made with caution, and more studies are needed. However, it can be noted that proANP consistently seemed to associate strongest with long-term mortality patients seemingly free of cardiac impairment. This could be consistent with a more cardiac-oriented sensitivity of NT-proBNP.

STUDY STRENGTHS AND LIMITATIONS

Major strengths of the present study include a large, broad cohort with a well-defined endpoint (all cause mortality) and a long follow-up period (up to 11.5 years). The latter, achievable by using more robust analysis techniques, opens up the possibility of further studies involving similar cohorts with long follow-up. A major limitation in our study is the lack of spare plasma from a large part of the original population which increases the risk of sample bias. However, the baseline values and survival in participants with proANP samples were similar to those without samples.

We also lacked detailed information on additional predictors of mortality such as body mass index, cholesterol levels, which could also be used in a clinical setting.

Furthermore the lack of repeated measurements prohibited us from using time dependent covariates. However this could be a minor issue, since part of the pathophysiology behind the elevated natriuretic peptides inevitable leads to death.

CONLUSION

In conclusion, our study provides evidence that high plasma proANP concentrations are associated with and predict short- and long-term all cause mortality in acutely hospitalised patients irrespective of CVD status at admission. This could potentially lead to improved risk stratification using proANP or a combination of proANP and NT-proBNP, which would lend vital support in the evaluation of the acutely hospitalised patient.

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Figure 1:

Title: Selection of participants from the Copenhagen Hospital Heart Failure Study (1998-1999) for enrolment in the present study.

[a]Blood samples collected from 80% of participants included during the last 10 months of the original study.

Figure 2:

Title: Unadjusted Kaplan-Meier Curves for all cause mortality (in days), by proANP quartiles.

Caption: Left panel: Whole study population. Right panel: Participants with no evidence of cardiovascular disease (see text for details). All *P*<.001for difference in survival tested by log-rank trend test

Table 1: Baseline characteristics according to proANP quartiles.

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P*
N	335	337	332	333	
proANP (pmol/L) (IQR)	336 (138)	594 (161)	990 (258)	2052 (1068)	<.001
1-year mortality (%)	25 (7.5)	33 (9.8)	57 (17.2)	124 (37.2)	<.001
Long-term mortality (%)	147 (44.8)	213 (64.2)	254 (78.4)	312 (95.1)	<.001
Age (SD)	58.5 (12.2)	67.7 (12.6)	75.7 (12.3)	80.3 (9.5)	<.001
Male sex (%)	172 (51.3)	131 (38.9)	108 (32.5)	130 (39.0)	<.001
Smoking (%)	257 (76.7)	251 (74.5)	226 (68.3)	229 (69.6)	.012
Alcohol (%)	60 (18.0)	43 (12.8)	29 (8.8)	19 (5.8)	<.001
Medical history of:					
Diabetes (%)	35 (10.5)	27 (8.0)	47 (14.2)	34 (10.2)	.464
Hypertension (%)	84 (25.1)	77 (22.9)	91 (27.5)	102 (30.9)	.041
Liver disease (%)	9 (2.7)	11 (3.3)	9 (2.7)	11 (3.3)	.739
Pulmonary disease (%)	60 (17.9)	71 (21.1)	62 (18.7)	63 (19.1)	.90
MI (%)	17 (5.1)	27 (8.0)	35 (10.6)	54 (16.4)	<.001
CHF (%)	7 (2.1)	23 (6.8)	43 (13.0)	90 (27.3)	<.001
AP (%)	36 (10.8)	68 (20.2)	88 (26.6)	95 (28.8)	<.001
Valve disease (%)	3 (0.9)	7 (2.1)	9 (2.7)	15 (4.6)	.003
Findings:					
NYHA class:					
3 (%)	3 (0.9)	13 (3.9)	24 (7.3)	58 (18.0)	<.001
4 (%)	0 (0.0)	2 (0.6)	0 (0.0)	5 (1.5)	.025
eGFR (SD)	102.5 (30.4)	90.6 (30.9)	78.5 (26.7)	63.1 (27.5)	<.001
Hgb (SD)	8.5 (1.1)	8.2 (1.2)	8.0 (1.2)	7.8 (1.3)	<.001

LVEF<50 (%)	32 (9.6)	42 (12.5)	62 (18.7)	114 (34.2)	<.001
NT-proBNP (pmol/L) (IQR)	24 (32)	60 (80)	147.5 (160)	477 (740)	<.001

Values are mean ±SD, median with IQR (interquartile range), or n with %.

Differences between quartiles are tested using Cochran-Armitage test for trend or analysis-of-variance when appropriate. Abbreviations: AP = Angina pectoris; CHF = Congestive heart failure; eGFR = Estimated glomerual filtration rate; Hgb = Haemoglobin; LVEF = Left ventricular ejection fraction; MI = Myocardial infarction; NYHA class =New York Heart Association functional classification.

Table 2a: Cox proportional regression modelling of risk of 1-year all cause mortality.

	Age and sex + proANP / NT-proBNP			[a]Multivariable + proANP / NT-proBNP			[a]Multivariable + proANP + NT-proBNP		
	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
ProANP quartile (1 = reference)									
2	1.01	0.59-1.72	.967	0.90	0.52-1.55	.704	0.80	0.45-1.43	.445
3	1.42	0.85-2.37	.185	1.24	0.73-2.11	.424	0.91	0.49-1.69	.767
4	3.11	1.90-5.08	<.001	2.63	1.56-4.43	<.001	1.47	0.76-2.83	.248
[b]Trend	1.61	1.38-1.87	<.001	1.53	1.30-1.81	<.001	1.24	1.01-1.53	.040
Pr. log unit change	2.16	1.77-2.64	<.001	2.05	1.64-2.56	<.001	1.39	1.02-1.9	.039
NT-ProBNP quartile (1 = reference)									
2	1.20	0.68-2.11	.520	1.02	0.58-1.80	.947	1.10	0.60-2.03	.752
3	1.93	1.13-3.30	.017	1.72	1.00-2.97	.051	1.60	0.84-3.06	.154
4	3.82	2.27-6.43	<.001	3.15	1.83-5.42	<.001	2.41	1.22-4.75	.011
[b]Trend	1.68	1.45-1.95	<.001	1.60	1.36-1.88	<.001	1.40	1.14-1.72	.001
Pr. log unit change	1.52	1.37-1.69	<.001	1.52	1.34-1.71	<.001	1.34	1.13-1.58	.001

Cox proportional regression modelling of risk of long-term, all cause mortality.

	Age and sex + proANP / NT-proBNP			[a]Multivariable + proANP / NT-proBNP			[a]Multivariable + proANP + NT-proBNP		
	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
ProANP quartile (1 = reference)									
2	1.20	0.97-1.49	.095	1.11	0.89-1.38	.350	1.08	0.86-1.36	.521
3	1.31	1.04-1.63	.019	1.12	0.89-1.41	.325	1.01	0.78-1.32	.916
4	2.42	1.93-3.04	<.001	1.98	1.56-2.50	<.001	1.50	1.11-2.02	.008
[b]Trend	1.35	1.26-1.45	<.001	1.26	1.17-1.36	<.001	1.14	1.03-1.26	.010
Pr. log unit change	1.70	1.53-1.90	<.001	1.54	1.37-1.74	<.001	1.27	1.08-1.48	.003
NT-ProBNP quartile (1 = reference)									
2	1.15	0.92-1.42	.219	1.02	0.82-1.27	.848	1.03	0.81-1.30	.829
3	1.34	1.08-1.68	.009	1.22	0.97-1.53	.082	1.12	0.86-1.46	.406
4	2.36	1.89-2.95	.<001	1.97	1.56-2.49	<.001	1.59	1.18-2.13	.002
[b]Trend	1.35	1.26-1.45	<.001	1.28	1.19-1.38	<.001	1.18	1.07-1.30	.001
Pr. log unit change	1.31	1.24-1.38	<.001	1.26	1.19-1.34	<.001	1.16	1.07-1.26	<.001

Abbreviations: CI, confidence interval; CVD, cardiovascular disease; HR, hazard ratios. [a]Adjusted for age, sex, alcohol, smoking, diabetes, history of congestive heart failure, history of pulmonary disease, history of liver disease, haemoglobin, ejection fraction below 50% and New York Heart Association functional classification 3 or 4. [b]Trend across quartiles.

Table 2b: Cox proportional regression modelling of risk of 1-year all cause mortality in participants without evidence of CVD.

	Age and sex + proANP / NT-proBNP			[a]Multivariable + proANP / NT-proBNP			[a]Multivariable + proANP + NT-proBNP		
	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
ProANP quartile (1 = reference)									
2	1.17	0.63-2.18	.623	0.91	0.47-1.73	.767	0.90	0.45-1.78	.754
3	1.13	0.58-2.20	.720	0.86	0.43-1.72	.676	0.78	0.35-1.74	.545
4	3.56	1.88-6.76	<.001	2.82	1.46-5.46	.002	2.11	0.91-4.88	.083
[b]Trend	1.61	1.30-1.99	<.001	1.54	1.23-1.92	<.001	1.37	1.04-1.82	.027
Pr. log unit change	2.19	1.62-2.96	<.001	2.04	1.48-2.82	<.001	1.53	0.99-2.38	.058
NT-ProBNP quartile (1 = reference)									
2	1.20	0.63-2.29	.579	0.91	0.48-1.76	.789	1.02	0.50-2.06	.955
3	1.58	0.82-3.04	.173	1.20	0.62-2.34	.586	1.08	0.49-2.41	.847
4	3.83	2.00-7.34	<.001	2.63	1.35-5.12	.004	1.70	0.72-4.04	.228
[b]Trend	1.65	1.33-2.03	<.001	1.48	1.19-1.85	<.001	1.24	0.93-1.64	.140
Pr. log unit change	1.59	1.34-1.89	<.001	1.49	1.25-1.79	<.001	1.27	0.99-1.62	.060

Cox proportional regression modelling of risk of long-term, all cause mortality in participants without evidence of CVD .

	Age and sex + proANP / NT-proBNP			[a]Multivariable + proANP / NT-proBNP			[a]Multivariable + proANP + NT-proBNP		
	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
ProANP quartile (1 = reference)									
2	1.33	1.03-1.72	.029	1.22	0.94-1.58	.142	1.22	0.92-1.61	.171
3	1.27	0.96-1.68	.091	1.09	0.82-1.45	.544	1.02	0.72-1.42	.931
4	2.47	1.82-3.33	<.001	2.10	1.54-2.85	<.001	1.62	1.10-2.39	.016
[b]Trend	1.30	1.18-1.44	<.001	1.23	1.11-1.37	<.001	1.14	1.00-1.30	.047
Pr. log unit change	1.56	1.33-1.82		1.46	1.24-1.71	<.001	1.16	0.93-1.44	.18
NT-ProBNP quartile (1 = reference)									
2	1.12	0.86-1.45	.391	0.96	0.74-1.25	.770	0.94	0.70-1.26	.678
3	1.30	0.98-1.71	.065	1.14	0.86-1.51	.374	1.04	0.74-1.45	.830
4	2.36	1.75-3.17	<.001	2.01	1.49-2.73	<.001	1.63	1.11-2.39	.013
[b]Trend	1.32	1.19-1.45	<.001	1.26	1.14-1.39	<.001	1.18	1.03-1.34	.014
Pr. log unit change	1.30	1.20-1.42	<.001	1.27	1.16-1.38	<.001	1.20	1.07-1.35	.002

Abbreviations: CI, confidence interval; CVD, cardiovascular disease; HR, hazard ratios. [a]Adjusted for age, sex, alcohol, smoking, diabetes, history of pulmonary disease and haemoglobin. [b]Trend across quartiles.

Table 3a: Comparison between risk prediction models of risk of 1-year all cause mortality.

	Model 1	Model 2	Model 3	Model 4	
Discrimination					
IDI		[a]0.141 (0.085-0.197)	[a]0.176 (0.118-0.234)	[a]0.201 (0.137-0.266)	[b]0.025 (-0.002-0.052)
Relative IDI		[a]0.183	[a]0.229	[a]0.262	[b]0.026
P		[a]<.001	[a].<001	[a].<001	[b].070
C-index	0.731 (0.701-0.760)	0.753 (0.724-0.783)	0.754 (0.725-0.783)	0.759 (0.730-0.788)	
P-difference		[a].003	[a].005	[a].001	[b].141

Comparison between risk prediction models of risk of long-term, all cause mortality.

Discrimination					
IDI		[a]0.053 (0.032-0.074)	[a]0.054 (0.031-0.077)	[a]0.070 (0.045-0.094)	[b]0.015 (0.0029-0.027)
Relative IDI		[a]0.044	[a]0.046	[a]0.059	[b]0.012
P		[a]<.001	[a]<.001	[a]<.001	[b].015
C-index	0.725 (0.709-0.741)	0.736 (0.720-0.752)	0.737 (0.721-0.753)	0.739 (0.724-0.755)	
P-difference		[a]<.001	[a]<.001	[a]<.001	[b].069

Model 1: Adjusted for age, sex, alcohol, smoking, diabetes, history of congestive heart failure, history of pulmonary disease, history of liver disease, haemoglobin, ejection fraction below 50% and New York Heart Association functional classification 3 or 4. Model 2: Model 1 + quartiles of proANP. Model 3: Model 1+ quartiles of NT-proBNP. Model 4: Model 1+ quartiles of proANP + quartiles of NT-proBNP. [a]Versus model 1. [b]Versus model 3.

Table 3b: Comparison between risk prediction models of risk of 1-year all cause mortality in participants without evidence of CVD.

	Model 1	Model 2	Model 3	Model 4	
Discrimination					
IDI		[a]0.185 (0.086-0.284)	[a]0.118 (0.039-0.197)	[a]0.210 (0.102-0.318)	[b]0.092 (0.025-0.160)
Relative IDI		[a]0.192	[a]0.122	[a]0.218	[b]0.085
P		[a]<.001	[a].004	[a]<.001	[b].007
C-index	0.758 (0.718-0.797)	0.780 (0.740-0.821)	0.773 (0.733-0.813)	0.782 (0.741-0.822)	
P-difference		[a].019	[a].070	[a].022	[b].178

Comparison between risk prediction models of risk of long-term, all cause mortality in participants without evidence of CVD.

Discrimination					
IDI		[a]0.045 (0.018-0.072)	[a]0.049 (0.022-0.077)	[a]0.072 (0.039-0.105)	[b]0.023 (0.004-0.041)
Relative IDI		[a]0.039	[a]0.043	[a]0.062	[b]0.019
P		[a].001	[a]<.001	[a]<.001	[b].015
C-index	0.736 (0.715-0.757)	0.747 (0.726-0.768)	0.744 (0.723-0.765)	0.748 (0.727-0.769)	
P-difference		[a].001	[a].013	[a].001	[b].015

Model 1: Adjusted for age, sex, alcohol, smoking, diabetes, history of pulmonary disease, history of liver disease, haemoglobin. Model 2: Model 1 + quartiles of proANP. Model 3: Model 1+ quartiles of NT-proBNP. Model 4: Model 1+ quartiles of proANP + quartiles of NT-proBNP. [a]Versus model 1. [b]Versus model 3.

Figure1:

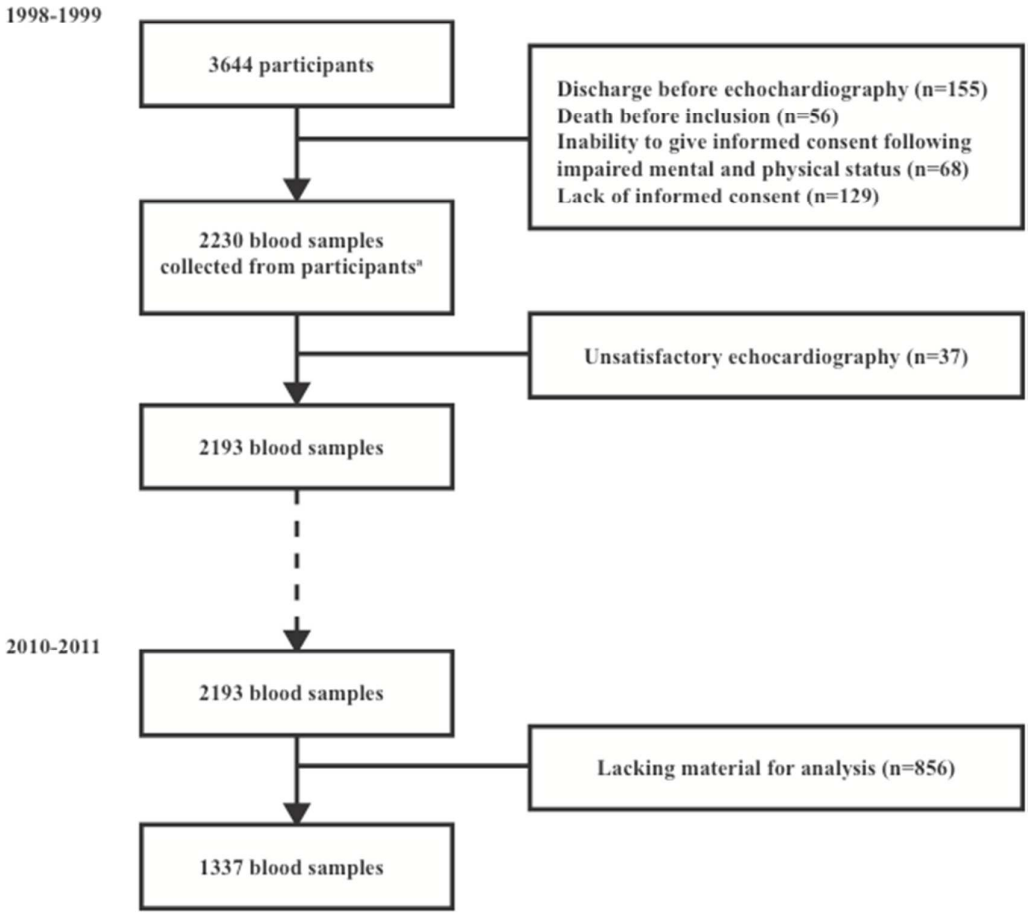
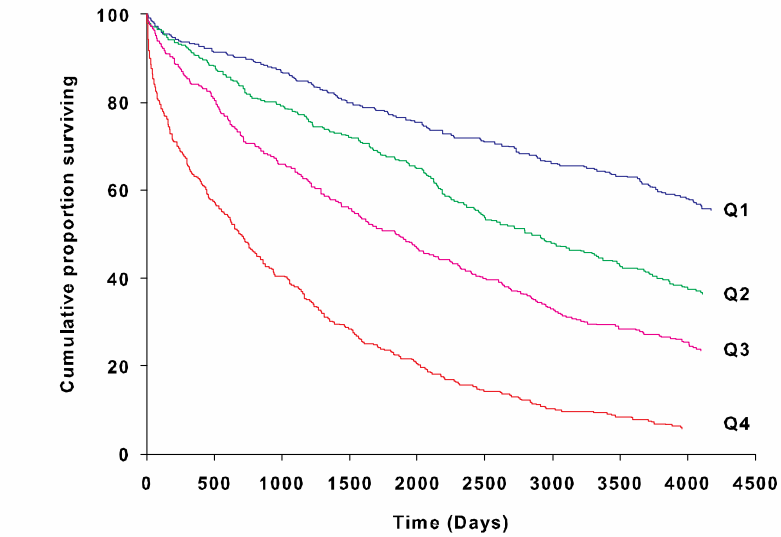
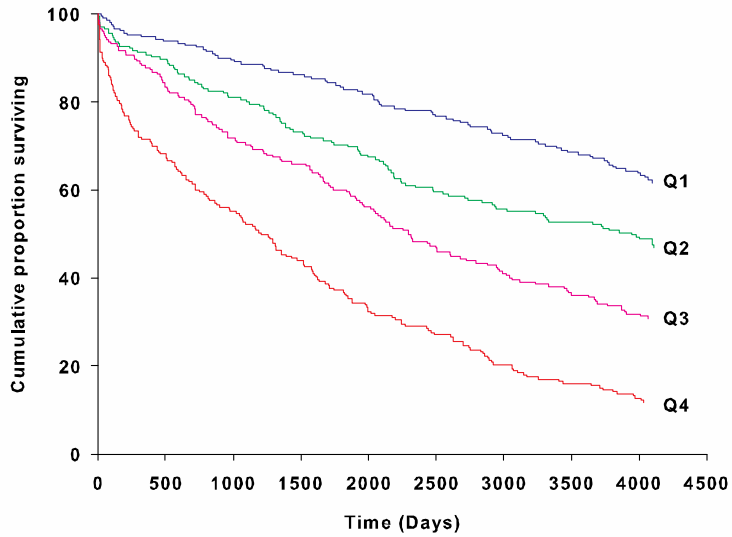


Figure 2:



Quartiles of proANP — Numbers at risk

Q1	335	305	290	267	252	237	221	211	194
Q2	337	296	266	242	218	181	161	144	126
Q3	332	267	218	185	155	132	109	94	84
Q4	333	189	134	94	68	47	34	28	19



Quartiles of proANP — Numbers at risk

Q1	211	197	188	181	172	161	152	144	134
Q2	205	184	166	150	138	122	114	108	100
Q3	207	171	147	135	115	97	84	74	65
Q4	207	141	114	91	67	56	42	33	26

Supplemental table 1: Full Cox proportional regression modelling of risk of 1-year all cause mortality.

	HR	95% CI	*P*
ProANP quartile (1 = reference)			
2	0.89	0.51-1.54	.669
3	1.21	0.70-2.07	.494
4	2.51	1.47-4.28	.001
Age	1.04	1.02-1.05	<.001
Male sex	1.63	1.21-2.18	.001
Alcohol	0.96	0.56-1.66	.885
Smoking	1.20	0.86-1.68	.281
Diabetes	0.98	0.63-1.52	.928
CHF	0.81	0.54-1.20	.286
MI	1.38	0.85-2.22	.190
AP	0.82	0.56-1.18	.280
Hypertension	0.92	0.68-1.25	.602
Valve disease	1.09	0.47-2.52	.837
Liver disease	1.47	0.67-3.21	.337
Pulmonary disease	1.88	1.38-2.57	<.001
NYHA class 3 or 4	1.08	0.71-1.64	.730
Hgb	0.79	0.71-0.88	<.001
eGFR	1.00	0.99-1.00	.408
LVEF<50	1.06	0.76-1.48	.712

Abbreviations: AP, Angina pectoris; CHF, Congestive heart failure; eGFR = Estimated glomerual filtration rate; Hgb, Haemoglobin; LVEF, Left ventricular ejection fraction; MI, Myocardial infarction; NYHA class, New York Heart Association functional classification.

Trend across proANP quartiles: HR 1.50 95% CI 1.27-1.78; *P*<.001

HR pr. log unit change og proANP: HR 2.05 95% CI 1.61-2.60; *P*<.001

Supplemental table 2: Full Cox proportional regression modelling of risk of long-term, all cause mortality.

	HR	95% CI	*P*
ProANP quartile (1 = reference)			
2	1.11	0.89-1.38	.357
3	1.12	0.89-1.41	.324
4	2.00	1.57-2.55	<.001
Age	1.05	1.05-1.06	<.001
Male sex	1.31	1.13-1.53	<.001
Alcohol	1.11	0.87-1.42	.385
Smoking	1.25	1.06-1.46	.008
Diabetes	1.24	1.00-1.53	.046
CHF	1.12	0.90-1.38	.304
MI	1.20	0.94-1.54	.141
AP	0.97	0.80-1.16	.716
Hypertension	1.02	0.88-1.18	.814
Valve disease	1.07	0.72-1.60	.735
Liver disease	2.28	1.58-3.28	<.001
Pulmonary disease	1.53	1.29-1.80	<.001
NYHA class 3 or 4	1.16	0.92-1.48	.216
Hgb	0.86	0.81-0.91	<.001
eGFR	1.00	1.00-1.00	.462
LVEF<50	1.16	0.97-1.38	.110

Abbreviations: AP, Angina pectoris; CHF, Congestive heart failure; eGFR = Estimated glomerual filtration rate; Hgb, Haemoglobin; LVEF, Left ventricular ejection fraction; MI, Myocardial infarction; NYHA class, New York Heart Association functional classification.

Trend across proANP quartiles: HR 1.26 95% CI 1.16-1.37; *P*<.001

HR pr. log unit change og proANP: HR 1.57 95% CI 1.39-1.78; *P*<.001

Supplemental table 3: Full Cox proportional regression modelling of risk of 1-year all cause mortality.

	HR	95% CI	*P*
NT-proBNP quartile (1 = reference)			
2	1.02	0.58-1.81	.946
3	1.70	0.98-2.95	.058
4	3.04	1.75-5.28	<.001
Age	1.04	1.02-1.05	<.001
Male sex	1.72	1.28-2.30	<.001
Alcohol	0.95	0.55-1.65	.865
Smoking	1.17	0.84-1.64	.354
Diabetes	0.89	0.57-1.39	.608
CHF	0.87	0.59-1.29	.483
MI	1.37	0.86-2.20	.186
AP	0.83	0.57-1.20	.318
Hypertension	0.92	0.68-1.25	.601
Valve disease	1.09	0.47-2.52	.846
Liver disease	1.71	0.78-3.73	.178
Pulmonary disease	1.89	1.38-2.58	<.001
NYHA class 3 or 4	1.11	0.73-1.69	.618
Hgb	0.78	0.70-0.87	<.001
eGFR	1.00	0.99-1.00	.320
LVEF<50	0.96	0.69-1.35	.834

Abbreviations: AP, Angina pectoris; CHF, Congestive heart failure; eGFR = Estimated glomerual filtration rate; Hgb, Haemoglobin; LVEF, Left ventricular ejection fraction; MI, Myocardial infarction; NYHA class, New York Heart Association functional classification.

Trend across NT-proBNP quartiles: HR 1.57 95% CI 1.33-1.86; *P*<.001

HR pr. log unit change og proANP: HR 1.51 95% CI 1.31-1.72; *P*<.001

Supplemental table 4: Full Cox proportional regression modelling of risk of long-term, all cause mortality.

	HR	95% CI	*P*
NT-proBNP quartile (1 = reference)			
2	1.02	0.82-1.27	.877
3	1.21	0.97-1.52	.096
4	1.96	1.55-2.49	<.001
Age	1.05	1.05-1.06	<.001
Male sex	1.33	1.15-1.55	<.001
Alcohol	1.14	0.90-1.46	.271
Smoking	1.21	1.02-1.42	.026
Diabetes	1.16	0.94-1.44	.155
CHF	1.20	0.97-1.48	.087
MI	1.15	0.89-1.47	.280
AP	0.98	0.81-1.17	.803
Hypertension	1.00	0.86-1.16	.996
Valve disease	1.09	0.73-1.63	.681
Liver disease	2.31	1.61-3.32	<.001
Pulmonary disease	1.55	1.31-1.83	<.001
NYHA class 3 or 4	1.19	0.94-1.51	.157
Hgb	0.84	0.80-0.89	<.001
eGFR	1.00	1.00-1.00	.698
LVEF<50	1.10	0.92-1.31	.316

Abbreviations: AP, Angina pectoris; CHF, Congestive heart failure; eGFR = Estimated glomerual filtration rate; Hgb, Haemoglobin; LVEF, Left ventricular ejection fraction; MI, Myocardial infarction; NYHA class, New York Heart Association functional classification.

Trend across NT-proBNP quartiles: HR 1.28 95% CI 1.18-1.38; *P*<.001

HR pr. log unit change og proANP: HR 1.27 95% 1.19-1.35; *P*<.001

Supplemental table 5: Participants by disease categories at discharge.

Disease category	N (%)
Cardiology	260 (19.5)
Orthopaedic	215 (16.1)
Gastroenterology	169 (12.6)
Haematology/oncology	88 (6.6)
Pulmonary	85 (6.4)
Endocrinology	44 (3.3)
Neurology	135 (10.1)
Infectious disease	191 (14.3)
Other	150 (11.2)

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	
Objectives	3	State specific objectives, including any prespecified hypotheses	
Methods			
Study design	4	Present key elements of study design early in the paper	
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	
Bias	9	Describe any efforts to address potential sources of bias	
Study size	10	Explain how the study size was arrived at	
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	
		(*b*) Describe any methods used to examine subgroups and interactions	
		(*c*) Explain how missing data were addressed	
		(*d*) If applicable, explain how loss to follow-up was addressed	
		(*e*) Describe any sensitivity analyses	
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	✓
		(b) Give reasons for non-participation at each stage	✓
		(c) Consider use of a flow diagram	✓
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	✓
		(b) Indicate number of participants with missing data for each variable of interest	✓
		(c) Summarise follow-up time (eg, average and total amount)	✓
Outcome data	15*	Report numbers of outcome events or summary measures over time	✓
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	✓
		(*b*) Report category boundaries when continuous variables were categorized	✓
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	✓
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	✓
Discussion			✓
Key results	18	Summarise key results with reference to study objectives	✓
Limitations			✓
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	✓
Generalisability	21	Discuss the generalisability (external validity) of the study results	✓
Other information			✓
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	✓

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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