ORIGINAL ARTICLE

Serial measurements of midregion proANP and copeptin in ambulatory patients with heart failure: incremental prognostic value of novel biomarkers in heart failure

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ABSTRACT

Background Disease progression in heart failure (HF) reflects derangements in neurohormonal systems, and biomarkers of these systems can help to establish the diagnosis and assess the prognosis. Serial measurements of the precursor peptides of the natriuretic and vasopressin systems (midregional proatrial natriuretic peptide (MR-proANP) and C-terminal provasopressin (copeptin), respectively) should add incremental value to risk stratification in ambulatory patients with HF.

Methods and results A cohort of 187 patients with class III–IV HF was prospectively enrolled, with biomarkers collected every 3 months over 2 years and analysed in relation to death/transplantation. Time-dependent analyses (dichotomous and continuous variables) showed that increases in MR-proANP (HR 7.6, 95% CI 1.85 to 31.15, p<0.01) and copeptin (HR 2.7, 95% CI 1.27 to 5.61, p=0.01) were associated with increased risk, but, in multivariate analysis adjusted for troponin T (cTnT) ≥0.01 ng/ml, only raised MR-proANP remained an independent predictor (HR 5.49, 95% CI 1.31 to 23.01, p=0.02). Combined increases in MR-proANP and copeptin (HR 9.01, 95% CI 1.24 to 65.26, p=0.03) with cTnT (HR 11.1, 95% CI 1.52 to 80.85, p=0.02), and increases ≥30% above already raised values identified the patients at greatest risk (MR-proANP: HR 10.1, 95% CI 2.34 to 43.38, p=0.002; copeptin: HR 11.5, 95% CI 2.74 to 48.08, p<0.001).

Conclusions A strategy of serial monitoring of MR-proANP and, of lesser impact, copeptin, combined with cTnT, may be advantageous in detecting and managing the highest-risk outpatients with HF.

INTRODUCTION

Despite advances in therapy, chronic heart failure (HF) is associated with an adverse prognosis[1 2] and progresses in the absence of overt clinical events.[3 4] Multiple neurohormonal pathways are activated, some of which are causally related and some are an effect of HF itself.[5] One pathway, the natriuretic peptide system, is associated with adverse outcomes.[6–8] Not only are they prognostic when initially measured but we have shown that the combination of B-type natriuretic peptide (BNP) and troponin T (cTnT) measured every 3 months over 2 years provides incremental information about the progression of HF in outpatients.[9] Most previous studies that evaluated changes over time monitored patients for short periods (typically 3–6 months) and/or used only single point-in-time measurements.[8 10–13] Given the importance of risk stratification, additional biomarkers that probe different neurohormonal pathways may further help to define disease progression and prognosis, which is likely to be dynamic and change over time. In addition, such biomarkers may provide novel pathophysiological insights and thus be synergistic with BNP/N-terminal (NT)-proBNP-guided therapy[14] and standard clinical assessment (eg, New York Heart Association (NYHA) class) in making therapeutic decisions.

Midregional proatrial natriuretic peptide (MR-proANP)[15] and C-terminal provasopressin (copeptin), a 39-amino-acid peptide derived from the prohormone preprovasopressin and cosecreted with arginine vasopressin from the posterior pituitary,[16] are precursor peptides related to different pathophysiological mechanisms in HF progression and have prognostic potential.[10 11 17] Recent data suggest that MR-proANP provides comparable diagnostic information to BNP in the acute setting.[18 19]

How much incremental information might be obtained from these new biomarkers, especially when assessed frequently over a prolonged period of time, remains to be evaluated. Accordingly, we measured MR-proANP and copeptin concentrations in outpatients with HF using the methods of design and analysis we reported previously for BNP and cTnT.[9] Our hypotheses were that serial measurements at long-term follow-up would provide independent risk stratification similar, but additive, to that of BNP and cTnT[20 21] and thus enhance identification of outpatients with HF who are at increased risk and would benefit from more intensive management. We pursued alternative analyses, assessing these analytes as continuous variables as well to make sure that we did not place them at a disadvantage by using the same analysis strategy as we previously used for BNP and cTnT.[9]

METHODS

Patients and study design

A cohort of 200 patients with NYHA class III and IV HF was prospectively enrolled from June 2001 to

January 2004. Informed consent was obtained after a primary medical evaluation. Patients were excluded if cardiac revascularisation was anticipated within 6 months of enrolment, they were awaiting cardiac transplantation, or they had experienced an episode of acute HF decompensation within the past 30 days. Patients were followed at prespecified 3-month intervals (±3 weeks) for 24 months. The study was approved by the Mayo Foundation Institutional Review Board and included only patients who provided written consent as required by Minnesota Statute 144.335/ CFR 21 (Part 50).

Study protocol

Blood samples were collected in serum and EDTA, immediately placed on ice, processed and stored at −70°C in divided aliquots until batch analysis was performed for each biomarker. Samples were collected and stored with the pre-hoc specification for multiple analyte evaluations. Precursor peptides (MR-proANP and copeptin) have been shown analytically to be very stable over time when stored at −70°C.[10 11] Clinicians and investigators were blinded to biomarker results. Left ventricular ejection fraction was derived by echocardiography performed up to 3 months before enrolment. An updated patient history and physical examination were completed at each follow-up visit by the primary HF specialist involved, as well as basic laboratory studies. Changes in drugs were recorded. Mean±SD follow-up duration was 18.9±7.8 months.

Biomarker measurements

MR-proANP and copeptin concentrations were measured in EDTA plasma by immunoluminometric assays (CT-proAVP LIA (copeptin) and MR-proANP LIA) provided by BRAHMS Aktiengeselschaft (Henningsdorf, Germany) on a Berthold LB952 Auto CliniLumat Luminescence analyser. Copeptin (interassay and intra-assay CVs <10%) and MR-proANP (interassay CV 2.3%, intra-assay CV 3.6%) were measured with coated-tube immunoluminometric assays. The details for BNP and cTnT measurements have previously been reported.[9]

Raised MR-proANP and BNP concentrations were defined as values >95th centile of a normal population adjusted for age and gender.[10 22] Raised copeptin concentrations were defined as >7.1 pmol/l for women and >9.4 pmol/l for men.[11] Raised cTnT was defined as values ≥0.01 ng/ml.[23 24] Renal function was determined by calculating estimated glomerular filtration rate (ml/min/1.73 m^2) using the modification of diet in renal disease equation.[25]

Statistical analysis

Continuous variables are reported as mean with SD and median with 25th and 75th centile IQRs. Categorical variables are reported as frequency of total values. Logarithmic transformation was performed to achieve approximate normal distribution for the biomarkers. The goal was to evaluate the relationships over time between the time until death or cardiac transplantation and serial MR-proANP and copeptin concentrations in relation to cTnT and BNP using the identical methodology and statistical analysis approach as used in our previous report[9] to ensure consistency in data handling. All modelling was performed with Cox proportional hazards models and executed using two different models. First, we included each biomarker as a continuous variable in univariate and multivariate models. Then, to facilitate the large numbers of cTnT observations that were below the limit of detection (ie, <0.01 ng/ml), we entered two variables, one a dichotomous variable indicating that the cTnT was below detectable levels (normal) and one variable using the logarithmic continuous measurements for cTnT values that were above 0.01 ng/ml. Second, MR-proANP, copeptin and BNP (but not cTnT) data were analysed on the basis of tertiles for risk prediction and to facilitate clinical interpretation. Third, we categorised each of the biomarkers dichotomously as either 'raised' or 'not raised' based on the 95th centile of normal population data and the 0.01 ng/ml cut-off point for cTnT. Results from the Cox proportional hazards models are presented as HRs with corresponding 95% CIs, p values and C-statistics.[26] Because of repeated data collection for all biomarkers and variety of enrolment times, enrolment (single value) and time-dependent (serial values) analysis methods were used. Kaplan–Meier survival curves summarise follow-up outcomes and account for changes in biomarker concentrations during the study period. Differences in survival curves were evaluated with log-rank tests. Likewise, in the time-dependent Cox multivariable models, patients were included in the initial proper risk set, but allowed to move between risk sets (higher risk and lower risk) over time. All other variables in these models are based on values at enrolment. Time-dependent Cox models were also used to evaluate changes in biomarkers between 3-month follow-up periods. To be included in this analysis, patients had to be followed to at least one 3-month visit after enrolment. Because changes in cTnT, but not BNP, were found to be statistically significant in these models, only cTnT was used to assess possible interactive effects. Analyses were carried out using SAS V.9 software[27] or S-plus V.7.[28]

RESULTS

Table 1 shows the clinical and demographic characteristics of the cohort. Of the 200 patients enrolled, 13 had insufficient biomarker data, and therefore results are reported for 187 participants. Mean±SD duration of HF was 41.9±44.2 months (median 31 months). Eight patients died before the initial 3-month follow-up visit and provided only baseline data. Six (3%) patients went on to cardiac transplantation, and 55 (29%) patients died during the study.

At enrolment, MR-proANP and copeptin concentrations were raised in 157 (84%) and 133 (71%) of patients, respectively. Another 17 and 31 patients, respectively, developed new increases during the study. BNP was raised at enrolment in 122 patients (65%), and cTnT concentrations were ≥0.01 ng/ml in 103 patients (55%). During the study, another 31 patients each developed new increases in BNP and cTnT.

Baseline single-sample biomarker analysis

Univariate analysis was undertaken using all baseline variables including standard risk predictors of age, diabetes, hypertension, left ventricular ejection fraction and renal function. The following were shown to be the most significant predictors in this cohort: increasing NYHA class III–IV (HR 3.46, 95% CI 1.75 to 6.83, p<0.001), presence of biventricular pacing (HR 2.79, 95% CI 1.27 to 6.14, p=0.011), myocardial infarction history (HR 1.90, 95% CI 1.13 to 3.19, p=0.015), stroke history (HR 2.29, 95% CI 1.04 to 5.04, p=0.039), raised BNP (HR 2.54, 95% CI 1.35 to 4.78, p=0.004) and raised cTnT (HR 2.69, 95% CI 1.54 to 4.72, p<0.001). Reduced risk was associated with a non-ischaemic aetiology of HF (HR 0.52, 95% CI 0.28 to 0.98, p=0.043) and higher glomerular filtration rate (HR 0.98, 95% CI 0.96 to 0.99, p=0.005). Covariate regression analyses for death/transplantation based on single-sample measurements at study enrolment for MR-proANP and copeptin in an interaction model with cTnT showed that only when increases were combined

Table 1 Baseline demographics and clinical characteristics of patient cohort

Variable	Mean±SD	Median (25–75% IQR)	No of patients (%)
Age (years)	71±10	73 (64–79)	187
Male gender			143 (76.4)
Caucasian/Native American			185/2
NYHA			
Class III			172 (92.0)
Class IV			15 (8.0)
Duration of heart failure (months)	41.9±44.2	31 (6–65)	187
LVEF (%)	27.3±12.2	24 (18–33)	187
Weight (kg)	87±23	82 (73–97)	187
Height (cm)	172±9	174 (166–178)	187
BMI (kg/m^2)	29.2±6.7	28 (25–32.5)	187
Heart rate (beats/min)	71±13	71 (62–80)	187
Systolic BP (mm Hg)	113±21	110 (98–124)	187
Diastolic BP (mm Hg)	63±11	60 (58–70)	187
Haemoglobin (g/dl)	12.5±1.7	12.4 (11.1–13.7)	99
Serum creatinine, mg/dL	1.6±0.6	1.5 (1.3–1.9)	187
GFR (ml/min/1.73 m^2)	46±15	45 (35–57)	187
Potassium (mEq/l)	4.4±0.9	4.4 (4.1–4.7)	187
Sodium (mEq/l)	139±4.1	140 (137–142)	184
MR-proANP (pmol/l)	395±304	333 (202–476)	187
Copeptin (pmol/l)	24.4±24.7	16.6 (7.3–31.1)	187
BNP (pg/ml)	408±423	305 (118–521)	187
Troponin T (ng/ml)	0.074±0.491	0.013 (0.005–0.035)	187
Aetiology of HF			
Ischaemic			101 (54.0)
IDCM			56 (29.9)
Hypertension			5 (2.6)
Valvular			7 (3.7)
Other			18 (9.6)
Diabetes			28 (15)
Hypertension			120 (64)
Hyperlipidaemia			121 (65)
COPD			49 (26)
History of CABG			82 (44)
BiV pacemaker			27 (14)
AICD			50 (27)
History of MI			91 (49)
History of CVA			12 (6)
Never smoker			68 (36)
Atrial fibrillation			86 (46)
Aortic stenosis			20 (11)
Aortic regurgitation			46 (24)
Mitral regurgitation			141 (75)
Tricuspid regurgitation			130 (70)
History of valve replacement surgery			17 (9)
Drugs			
ACE1			140 (75)
ARB			34 (18)
β-blocker			147 (78)
Aldosterone blocker			50 (27)
Digoxin			113 (60)
Diuretic			173 (92)
Aspirin			114 (61)
Nitrates			55 (29)
Antidysrhythmics			40 (21)

ACE1, angiotensin converting enzyme inhibitor; AICD, automatic implantable cardioverter defibrillator; ARB, angiotension receptor blocker; BiV, biventricular; BMI, body mass index; BNP, B-type natriuretic peptide; BP, blood pressure; COPD, chronic obstructive pulmonary disease; CVA, cerebrovascular accident; GFR, glomerular filtration rate; IDCM, idiopathic dilated cardiomyopathy; LVEF, left ventricular ejection fraction; MI, myocardial infarction; MR-proANP, midregional proatrial natriuretic peptide; NYHA, New York Heart Association.

with cTnT did these biomarkers predict worse outcomes (MR-proANP, HR 4.19, 95% CI 2.50 to 11.67, p=0.006; copeptin, HR 3.69, 95% CI 1.56 to 8.73, p=0.003). When baseline peptide values were analysed as continuous variables (log transformed) in regression analysis, all biomarkers were univariate predictors of death/transplantation (table 2).

A model for covariate correction was developed before assessment of MR-proANP, copeptin, cTnT or BNP effects and included the significant univariate variables that were shown to be the most appropriate adjustment for this cohort given the number of events (NYHA class, history of myocardial infarction, and biventricular pacing) and without over-fitting the model. Multivariate analysis with adjustment for these univariate predictors (all data are not shown given that they revealed similar coefficients) showed baseline MR-proANP, copeptin and cTnT to be significant predictors of worse outcome (table 2).

Time-dependent serial biomarker analysis

Univariate time-dependent analyses (over 2 years) for prediction of death/transplantation showed that serial measurements of MR-proANP (HR 7.60, 95% CI 1.85 to 31.15, p=0.0048) and copeptin (HR 2.67, 95% CI 1.27 to 5.61, p=0.0096) were highly predictive. Figure 1A, B illustrates Kaplan–Meier survival analysis for these biomarkers including concentrations obtained at the last follow-up visit or the follow-up visit preceding an event. An increase in MR-proANP was the most potent predictor of poor outcome, and concentrations remaining normal were protective. In multivariate time-dependent modelling with serial MR-proANP and copeptin values adjusted for cTnT ≥0.01 ng/ml, raised MR-proANP (HR 5.49, 95% CI 1.31 to 23.01, p=0.02), but not copeptin (HR 1.89, 95% CI 0.89 to 4.13, p=0.6332), persisted as an independent predictor. Multivariate analysis that included adjustment for the baseline univariate clinical predictors as described above revealed that the HR for cTnT was 2.31 (p=0.003), and, with the separate additions of MR-proANP and copeptin to the model, the HR for MR-proANP was significant (4.56, p=0.039), while copeptin did not contribute to risk prediction in the presence of raised cTnT (table 3).

When analysed in time-dependent models by tertiles, BNP, MR-proANP and copeptin revealed that the risk of death/transplantation was increased with increasing tertile, but risk prediction was not uniform. For BNP, tertile 2 (BNP >115–337 pg/ml) was not different from tertile 1 (≤115 pg/ml), while tertile 3 (>337 pg/ml) was significantly different from tertile 1 (HR 3.06,

Table 2 Univariate and multivariate continuous variable analysis of baseline single-sample biomarkers for risk of death/cardiac transplantation

Variable	HR (75th relative to 25th centile)	Observed 25th and 75th quartiles	p Value	C-statistic
Univariate model				
BNP (pg/ml)	2.10	73–458	<0.001	0.648
MR-proANP (pmol/l)	2.57	191–487	<0.001	0.735
Copeptin (pmol/l)	2.83	8–32	<0.001	0.670
Troponin T (ng/ml)	2.14	<0.01–0.03	<0.001	0.666
Multivariate model				
BNP (pg/ml)	1.88	73–458	0.002	0.679
MR-proANP (pmol/l)	2.38	191–487	<0.001	0.758
Copeptin (pmol/l)	2.56	8–32	<0.001	0.740
Troponin T (ng/ml)	2.24	<0.01–0.03	<0.001	0.709

Biomarkers were added separately to the multivariate model which included NYHA class, history of myocardial infarction, and presence of biventricular pacemaker. Models were fitted using natural log transformations of all continuous variables.
BNP, B-type natriuretic peptide; MR-proBNP, midregional proatrial natriuretic peptide.

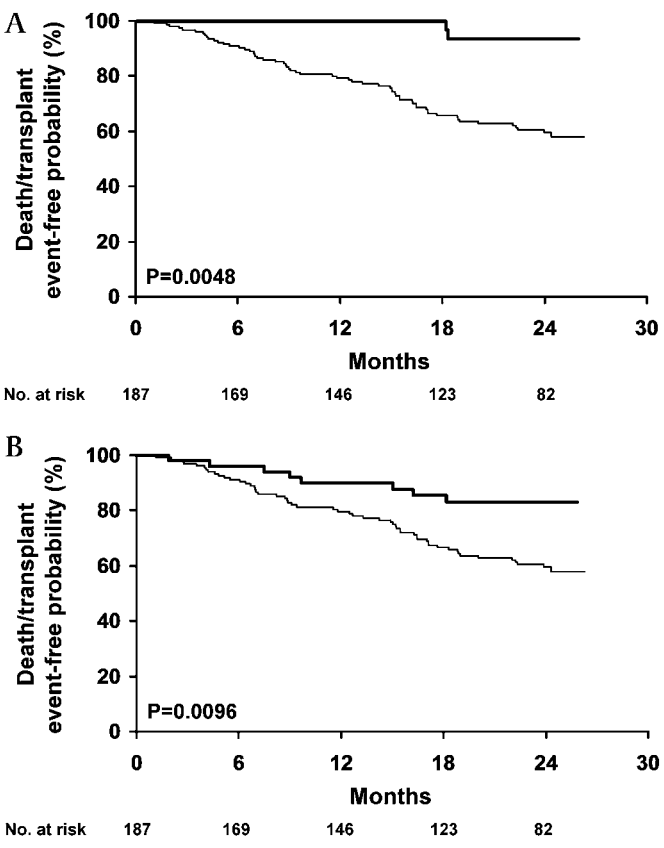

Figure 1 Kaplan–Meier curves for time-dependent event of death or cardiac transplantation for (A) midregional proatrial natriuretic peptide (MR-proANP) and (B) C-terminal provasopressin (copeptin) grouped by category (increased (thin line; >95th centile of normal, adjusted for age and gender, in (A) and >7.1 pmol/l for women and >9.4 pmol/l for men in (B)) or not increased (thick line)) based on serial values over 2 years.

p=0.001). For MR-proANP, tertile 2 (>227–412 pmol/l) was significantly different from tertile 1 (≤227 pmol/l) (HR 4.79, p=0.004), as was tertile 3 (>412 pmol/l) (HR 10.66, p<0.0001). Copeptin was similar to BNP in that tertile 2 (>10.2 to 25 pmol/l) was not different from tertile 1 (≤10.2 pmol/l), while tertile 3 (>25 pmol/l) was significantly different from tertile 1 (HR 4.08, p<0.0001).

Analysis of changes in biomarkers over time

Table 4 shows the predicted risk associated with changes in MR-proANP from one follow-up visit to the next relative to MR-proANP concentrations remaining normal. An increase at any time during follow-up carried a 4.35-fold increase in risk, which was not, however, statistically significant. This finding reflects that the majority of patients had raised concentrations of MR-proANP at study enrolment and thus few patients were in this category of change (from normal to raised). However, modest (<30%) increases or decreases (but not back to normal concentration) from an already raised MR-proANP value were associated with a significant change in risk (HR 5.79, p=0.016). Furthermore, large increases (≥30%; a commonly cited level of significant change[6 8]) from already raised values of MR-proANP carried a very substantial increase in risk (HR 10.07, p=0.002). When the same analysis was carried out for copeptin (table 4), only large increases (≥30%) from already raised values were associated with a further increase in risk (HR 11.48; p<0.001). Raised values returning to normal during follow-up for either peptide were not associated with mitigation of risk, but this may reflect that few patients showed a lowering of values to below the 95th centile during the study; therefore there were too few patients in this category of change for meaningful statistical analysis.

Table 3 Multivariate time-dependent analysis of risk for death/cardiac transplantation (Cox proportional hazard model)

Variable	HR (95% CI)	p Value	C-statistic
Raised BNP	1.39 (0.78 to 2.48)	0.258	
Troponin T ≥0.01 ng/ml	2.18 (1.25 to 3.80)	0.006	
NYHA class (IV vs III)	3.28 (1.63 to 6.61)	<0.001	
History of myocardial infarction	1.36 (0.80 to 2.32)	0.252	
Biventricular pacemaker	3.42 (1.52 to 7.68)	0.003	
Raised MR-proANP*	4.56 (1.08 to 19.32)	0.039	0.709
Raised copeptin*	1.86 (0.84 to 4.12)	0.126	0.705

*Each biomarker added separately to the multivariate model. B=type natriuretic peptide; BNP, NYHA, New York Heart Association; CI, confidence interval; HR, hazard ratio; MR-proANP, midregional proatrial natriuretic peptide.

Time-dependent covariate biomarker analysis

Risk prediction by covariate analysis for MR-proANP and cTnT when analysed for any time point during follow-up demonstrated that an increase in MR-proANP without any increase in cTnT predicted an increased risk of death/transplantation (HR 4.73, 95% CI 1.0 to 20.43, p=0.037). When cTnT was raised (≥0.01 ng/ml) without an increase in MR-proANP, the risk analysis was confounded by too small a number of patients in this category. However, the combined increases in cTnT and MR-proANP augmented risk substantially (HR 10.29, 95% CI 2.48 to 42.67, p=0.0013). A similar analysis for copeptin showed that only the combined increase with cTnT was associated with a significant increase in risk (HR 3.49, 95% CI 1.63 to 7.49, p=0.0013).

Covariate time-dependent models for serial measurements of MR-proANP and copeptin showed that neither biomarker, when increased in isolation, was a significant predictor. Combined increases in both biomarkers, however, were associated with increased risk (HR 9.01, 95% CI 1.24 to 65.26, p=0.03). Of particular interest is the observation in covariate analysis that the individual and combined increases in MR-proANP and copeptin when cTnT was included in the analysis (but cTnT was not raised) were no longer predictive, and the associated HR (5.93, 95% CI 0.78 to 45.11, p=0.086) is lower than that noted when cTnT was not included in the analysis (HR 9.01). The combined increases in MR-proANP, copeptin and cTnT predicted a substantial increase in risk (HR 11.07, 95% CI 1.52 to

Table 4 Effect of change in serial midregional proatrial natriuretic peptide (MR-proANP) and C-terminal provasopressin (copeptin) concentrations over the study period on outcome of death/cardiac transplantation

Variable	HR (95% CI)	p Value	C-Statistic
Normal to raised MR-proANP	4.35 (0.61 to 31.02)	0.143	0.641
Raised MR-proANP to <30% change (increase or decrease but none back to normal value)	5.79 (1.38 to 24.30)	0.016	
Raised MR-proANP to ≥30% further increase in MR-proANP	10.07 (2.34 to 43.38)	0.002	
Normal to raised copeptin	4.11 (0.68 to 24.61)	0.122	0.684
Raised copeptin to <30% change (increase or decrease but none back to normal value)	4.20 (0.95 to 18.47)	0.058	
Raised copeptin to ≥30% further increase in copeptin	11.48 (2.74 to 48.08)	<0.001	

All values are relative to normal values remaining. CI, confidence interval; HR, hazard ratio; MR-proANP, mid-regional proatrial natriuretic peptide.

80.85, p=0.02). This was, however, not much greater than the combined increases in MR-proANP and cTnT when copeptin was excluded (HR 10.29, p=0.0013).

DISCUSSION

Effective risk stratification is a key to success in the long-term management of HF, particularly for outpatients. The results of this study suggest that the conjoint use of MR-proANP with cTnT in outpatients with chronic HF can aid in this strategy. Indeed, in this dataset, MR-proANP provided more robust responses than BNP in most analyses. Copeptin was less robust. All the markers, as in other studies, demonstrated the prognostic value of single-sample increases including BNP and cTnT[6 9 29–31]; however, MR-proANP, and to a lesser extent copeptin, seemed to add support for an incremental value of serial measurements over time.

In categorical analysis, increases in MR-proANP and copeptin at baseline (single samples) or during clinical follow-up (serial samples) were univariately associated with an increased risk of death or cardiac transplantation. New increases from normal concentrations during follow-up did not statistically move patients into a higher risk category, but this is most likely due to the very high percentage (~70%) of patients entering the study with already raised concentrations of these biomarkers by study definition, and therefore relatively few patients were in this category of change to allow analysis. However, once raised, further increases significantly shifted patients to a higher risk category. These large increments (>30%) in MR-proANP and copeptin defined the outpatients who were at highest risk. The large changes necessary to be clinically relevant once values are raised are similar to previous data for BNP and NT-proBNP where only large absolute or percentage changes were associated with clinically meaningful alterations in risk.[32] For these biomarkers, changes up to 80% were required to show significant differences. Values returning to normal for either of the biomarkers did not mitigate risk, most likely because there were small numbers of patients in this category of change.

Of additional importance is the observation that increases in cTnT occurring at baseline or at any time during follow-up dramatically modulate the risk-prediction capacity of MR-proANP or copeptin. While MR-proANP showed an independent contribution to risk stratification in multivariate time-dependent analysis, a substantial incremental effect was also demonstrated when combined with cTnT. The interaction of combined increases in MR-proANP, copeptin and cTnT proved to be the most potent predictor of enhanced risk (HR 11.1, p=0.02), but not substantially different from the combined increases in MR-proANP and cTnT without copeptin (HR 10.3, p=0.001). Increases in cTnT seem to have a potentiating effect on the risk-predicting potential of increases in both MR-proANP and copeptin. Surprisingly, copeptin was less robust in time-dependent analyses with cTnT than MR-proANP, but this may reflect differences in their pathophysiology. For copeptin to be a more potent predictor of risk, more advanced HF reflective of higher vasopressin release may be needed, as observed in decompensated patients. In continuous variable multivariate analysis using single-sample biomarker measurements, both biomarkers were predictive in the presence or absence of raised cTnT. This contrasts with the time-dependent categorical analysis (raised or not raised) where only MR-proANP remained a statistically significant independent predictor after adjustment for cTnT and clinical risk factors. This suggests that defining appropriate cut-off point values is central to the practical clinical use of these biomarkers in individual patients.

These findings suggest that the periodic monitoring of MR-proANP, either separately or in combination with copeptin or particularly with cTnT, after an initial increase may be an effective means of defining meaningful changes in risk and better stratifying outpatients with HF. Our study population is similar to many others, and thus results in both this study and other analyses of other biomarkers should be helpful to physicians in following patients with systolic HF. In addition, insights from the impairment of these neurohormonal regulatory pathways may aid in developing new strategies to preclude new increases in these biomarkers or to intervene more effectively when they occur and thus aid patient prognosis. This may be especially promising for copeptin, serving as a surrogate for arginine vasopressin, to be a reliable biomarker of therapeutic response to vasopressin inhibition.

Limitations of the study

Of the study cohort, complete 2-year follow-up was not accomplished in 18 patients for reasons other than the primary end point. These patients elected not to continue participation in the study, but six had completed at least 1 year of follow-up and were censored at the time of their last visit. Eight patients died after enrolment but before the first 3-month follow-up visit and therefore contributed only enrolment data, but were considered part of the outcome analysis.

In conclusion, our data suggest that both clinically detectable and subclinical events occur in patients with HF based on discrete changes detected by serial biomarker surveillance, and these occur in apparently clinically stable outpatients. In our cohort, standard clinical assessment features such as change in NYHA class or renal function were not altered substantially over the course of the study to be a signal to the clinician of advancing risk. Increases in MR-proANP and copeptin >95th centile of normal population detected by serial monitoring during follow-up in ambulatory patients appear to be common and predictive of increased risk of short-term events including death. The incremental predictive value of monitoring MR-proANP over time alone, in combination with copeptin, and particularly with cTnT, may provide an effective and simple means of identifying ambulatory patients with HF who are at increased risk and allow timely intervention. Overall, our data also suggest that MR-proANP is equivalent, if not superior, to BNP in risk prediction alone and in combination with cTnT. As such, MR-proBNP should also be considered a candidate biomarker in the developing area of natriuretic-peptide-guided therapy in patients with HF.[14 33]

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