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Mid-Regional Pro-Atrial Natriuretic Peptide for Predicting Prognosis in Hypertrophic Cardiomyopathy.

Brief Title: MR Pro-ANP in hypertrophic cardiomyopathy.

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Abstract

Objectives: N-terminal pro-brain natriuretic peptide (NT-proBNP) predicts mortality and the development of heart failure in hypertrophic cardiomyopathy (HCM). Mid-regional pro-atrial natriuretic peptide (MR-proANP) is a stable byproduct of production of atrial natriuretic peptide. We sought to compare the prognostic value of MR-proANP and NT-proBNP in HCM.

Methods: We prospectively enrolled a cohort of patients with HCM from different European centers and followed them. All patients had clinical, ECG and echocardiographic evaluation and measurement of MR-proANP and NT-proBNP at inclusion.

Results: Of 357 patients enrolled, the median age was 52 (IQR: 36-65) years. MR-proANP and NT-proBNP were both independently associated with age, weight, New York Heart Association (NYHA) class, left ventricular ejection fraction (LVEF), wall thickness and left atrial dimension.
During a median follow-up of 23 months, 32 patients had a primary end point defined as death (n=6), heart transplantation (n=8), left ventricular assist device implantation (n=1) or heart failure hospitalisation (n=17). Both NT-proBNP and MR-proANP ($p<10^{-4}$) were strongly associated with the primary endpoint, and the areas under the ROC curves for both peptides were not significantly different. However, in a multiple stepwise regression analysis, the best model for predicting outcome was NYHA 1-2 versus 3-4 (HR=0.35, CI 95% [0.16-0.77], $p < 0.01$), LVEF (HR = 0.96, CI 95% [0.94-0.98], p= 0.0005), and MR-proANP (HR=3.77, CI 95% [2.01-7.08], $p<0.0001$).

Conclusions: MR-proANP emerges as a valuable biomarker for the prediction of death and heart failure related events in HCM patients.

Key Words: natriuretic peptide, MR-proANP, NT-proBNP, hypertrophic cardiomyopathy

Abbreviations: NYHA: New York Heart Association, HCM: hypertrophic cardiomyopathy, NT-proBNP: N terminal pro-brain natriuretic peptide, MR-proANP: mid-regional pro-atrial natriuretic peptide, LVEF: left ventricular ejection fraction, IQR: interquartile range, HR: hazard ratio, CI: confidence interval.

Key questions:

What is already known about this subject?
Predicting prognosis in HCM remains challenging. High levels of BNP and NT-proBNP are associated with cardiovascular events, heart failure and death in patients with HCM and these tests are recommended in the ESC guidelines on HCM.

What does this study add?
This study shows that another biomarker (ie MR-ProANP) might be at least as accurate as NT-proBNP for prognostication in patients with HCM.

How might this impact on clinical practice?

The use of MR-proANP might help to refine risk stratification and to assess the effects of novel interventions in patients with HCM.

INTRODUCTION

Hypertrophic cardiomyopathy (HCM) is a genetic myocardial disease defined by unexplained left ventricular hypertrophy, usually asymmetrical and involving the interventricular septum.[1 2] It is generally associated with a normal or high left ventricular ejection fraction (LVEF) and impaired diastolic function. The clinical pattern is highly heterogeneous: many patients have no or mild symptoms during their whole life, but in some patients, HCM may lead to severe symptoms such as heart failure or sudden death. Heart failure is often associated with left atrial enlargement, which reflects increased left ventricular filling pressures secondary to left ventricular diastolic and, in some cases, systolic dysfunction.[3 4] However predicting prognosis in HCM remains challenging.

Natriuretic peptides are released from the heart due to increased myocardial wall stretch caused by volume and pressure overload.[5 6] The diagnostic and prognostic values of BNP and NT-proBNP are now well-established in heart failure.[7 8] In HCM, increased BNP and NT-proBNP concentrations have been associated with evidence of ventricular dysfunction, exercise intolerance, the development of heart failure and death.[9-12]

MR-proANP is the mid-regional epitope of the ANP prohormone[13] which, like NT-proBNP, has a long circulating half-life. MR-proANP may be equal or superior to BNP or NT-proBNP for diagnosis and prognosis in HF.[14-16] However, there are no published data on the clinical significance, particularly with regard to survival, of MR-proANP in patients with HCM. The aim of the present study was to determine the prognostic value of MR-proANP compared to NT-proBNP in patients with HCM.[17]

METHODS

Patient selection

The population consisted of patients with HCM recruited from the Eurogene Heart Failure Study, an observational multicentre cohort involving 11 European centres in France (Pitié-Salpêtrière Hospital, Paris and Ambroise Paré Hospital, Boulogne-Billancourt), Germany (Marburg, Regensburg, Munster), Italy (Pavia), Portugal (Lisboa), Spain (Barcelona), Sweden (Umea) and the United Kingdom (Hull), evaluating the genetics of patients with familial or sporadic dilated or hypertrophic cardiomyopathy. The study conforms to the principles outlined in the Declaration of Helsinki and was approved by all relevant ethics committees. Data were collected prospectively. The diagnosis of HCM was based on electrocardiogram and echocardiography: maximal left ventricular wall thickness >15 mm for index cases, >13mm or major abnormalities on electrocardiogram (Romhilt-Estes score $\geq$4 and/or pathological Q waves and/or significant ST-T changes) for relatives, without evidence of any other cause for left ventricular hypertrophy.[3] Consecutive patients who fulfilled the criteria were screened to participate.

Patient evaluation

During an outpatient visit, informed consent was obtained. Patients then had a medical history taken, a cardiovascular examination, a 12-lead electrocardiogram and an echocardiography. Previous history of heart failure, previous procedures or surgery, New York Heart Association (NYHA) class, heart rate, sinus rhythm or atrial fibrillation, blood pressure and medical treatments were recorded. M-Mode and two-dimensional transthoracic echocardiography studies were performed in the left lateral decubitus position and measurements made at enrolling sites according to a standard protocol. Left ventricular hypertrophy was assessed with echocardiography according to published criteria.[3] Left ventricular end diastolic and end systolic diameters and left atrial diameters were obtained from M-Mode and two-dimensional images from the parasternal view. LVEF was calculated according to Simpson biplane method. Septal and posterior wall thicknesses were measured from the parasternal view. Left ventricular outflow tract gradient was measured at rest from continuous-wave Doppler and left ventricular outflow obstruction was defined as a maximal gradient > 30 mmHg. Before starting, an echo sample was sent from each centre to an echo core lab.

Measurement of natriuretic peptides

Venous blood samples were collected and transported as whole blood using a fast transport service (TNT company) from recruiting centres to Genethon (Evry, France). Samples were immediately centrifuged. Supernatant serum was stored at -80°C until the samples were analyzed in the Biochemistry Department of Pitie-Salpetriere Hospital, Paris. Serum NT-proBNP was measured by a two-site electrochemiluminescence immunoassay on a Roche Diagnostics E170 analyser. The limit of quantitation is <50 pg/ml, and the coefficients of variation of intra- and inter-assay reproducibility are <5%. MR-proANP was measured by a sandwich chemiluminescence immunoassay on the KRYPTOR system (BRAHMS GmbH, Hennigsdorf, Germany). The limit of quantitation is 4.5 pmol/l, the coefficient of variation of intra-assay reproducibility was <5% and the coefficient of variation of inter-assay reproducibility was <6.5 %. [13]

Follow-up

Patients were followed in their local centres for a median observation time of 23 months. The investigator recorded the following endpoints during a clinic visit or by telephone: death, heart failure hospitalisation, cardiac transplantation or left ventricular assist device, and also intracardiac defibrillator implantation and appropriate shocks, septal myectomy and alcohol septal ablation.

Statistical analysis

Descriptive statistics used median (IQR) for quantitative variables and numbers (percentages) for binary variables. NT-proBNP and MR-proANP were log-transformed (base 10) in order to normalize their probability density functions.

Relationships with NT-proBNP and MR-proANP were assessed in univariate analyses using Spearman rank correlation coefficient tests for quantitative or ordinal variables (such as NYHA), and by Student's t tests for binary variables. Variables with a p-value lower than 0.10 in the univariate analyses were entered into stepwise multiple linear regressions, and only variables significant with a p-value lower than 0.05 were retained in the final models.

The prognostic role of NT-proBNP and MR-proANP was assessed in the framework of survival analysis, with a compound criterion considered as the event. This endpoint

was death, heart transplantation, left ventricular assist device or hospitalisation for heart failure. Missing data were not included in the analysis. The univariate step of the analysis used Kaplan-Meier estimation of the survival functions, and univariate Cox models for testing the relationships of each potential prognostic factor and the criterion. ROC curves were also performed to compare the areas under curve (AUC) of NT-proBNP and MR-proANP. The multivariate step involved the comparison of several Cox models. All computations were performed using the SAS V9.3 statistical package (SAS Institute, New-York).

RESULTS

Of 357 patients enrolled, the median age was 52 (IQR: 36-65), 57 % were male and 88% were in NYHA functional class I or II (Table 1). Median maximal wall thickness was 20 (IQR 17-24) mm and left ventricular outflow tract obstruction was present in 26 % of the patients at baseline. Median NT-proBNP was 550 (IQR: 202-1380) pg/mL and median MR-proANP was 107 (IQR: 58-184) pmol/L. Although all patients were clinically stable, 297 patients had an NT-proBNP concentration >125 pg/mL, 246 a value >300 pg/mL and 172 had an MR-proANP concentration >120 pmol/L, thresholds that have been proposed as useful for the diagnosis of heart failure in various clinical settings.

Factors influencing natriuretic peptides levels in HCM

MR-proANP and NT-proBNP were highly correlated ($r= 0.76$, $p< 0.001$) and both were directly correlated with age, NYHA class, posterior wall thickness and left atrial diameter and indirectly with weight. The correlation between left atrial diameter and MR-proANP was stronger than the correlation between left atrial diameter and NT-proBNP ($p<0.0001$). Values of both markers were higher in women and in patients with atrial fibrillation or paced or if they had a previous hospitalisation for heart failure or a left ventricular outflow tract obstruction. MR-proANP correlated directly with serum creatinine and indirectly with LVEF. NT-proBNP correlated directly with septal and maximal wall thickness and indirectly with systolic blood pressure (Supplementary data, Tables 2a and 2b). Median MR-proANP was 64 pmol/L (IQR: 39-141) in NYHA I patients, 137 pmol/L (IQR: 81-214) in NYHA II and 139 pmol/L (IQR: 95-211) in NYHA III and IV, while median NT-proBNP was 362 pg/mL (IQR:

111-740) in NYHA I, 793 pg/mL (IQR: 307-1549) in NYHA II, 1041 pg/mL (IQR: 362-2112) in NYHA III-IV (Figure 1).

Multivariable linear regression models of log MR-proANP and log NT-proBNP revealed correlations with several variables (Table 2) for both peptides, including weight, age, NYHA class and left atrial diameter. In addition, a higher log MR-proANP was associated with a lower LVEF. Higher log NT-proBNP was associated with lower systolic blood pressure and greater left ventricular wall thickness.

Survival analysis

Patients were followed for a median duration of 23 months (IQR 13-30 months) during which 32 primary endpoints were observed: 6 patients died, 1 patient had a left ventricular assist device, 8 patients underwent cardiac transplantation and 17 patients were hospitalized for heart failure. Patients who reached the primary endpoint had higher concentrations of MR-proANP and NT-proBNP than others (228 (IQR: 135-341) pmol/L versus 101 (IQR:55-171) pmol/L and 2010 (IQR:785-2707) pg/mL versus 505 (IQR:196-1208) pg/mL respectively, both $p< 10^{-5}$). Moreover, during follow-up, 12 patients had an alcohol septal ablation and 2 a septal myectomy. Intracardiac defibrillator were implanted in 12 patients and 5 patients had at least one appropriate shock, which were not predicted by the natriuretic peptides.

Univariate analysis showed that log MR-proANP, log NT-proBNP, heart rate, NYHA, LVEF, posterior left ventricular wall thickness, left atrial diameter and systolic blood pressure were associated with the primary outcome (Table 3). The rate of events was 2.5 % in the lowest tertile (MR-proANP $\leq$ 70 pmol/mL), 5.8 % in the middle tertile (MR-proANP 70-155 pmol/mL), and 18.6 % in the highest tertile (MR-proANP > 155 pmol/mL) (p=0.00002) (Figure 2). Figure 3 shows the comparison of areas under the ROC curve for the prediction of the primary end-point using NT-proBNP (0.7417 [0.6425; 0.8409]) and MR-proANP (0.7703 [0.6820; 0.8586]), which were not significantly different (p=0.42).

In multivariable analysis, log MR-proANP, LVEF and NYHA class III-IV were the only independent prognostic factors. Patients with either NYHA class III-IV or LVEF <50 % had a 27 % (18/66) rate of events compared to only 5 % (14/270) for those in NYHA class 1-2 and LVEF >50 %. In this lower-risk group, MR-proANP in the 2 highest tertiles identified 13 of the 14 patients with events. On the opposite, in the

higher-risk group (NYHA III -IV or LVEF < 50 %), the 2 lowest tertiles of MR-proANP reclassified 31/48 patients without any events.

DISCUSSION

This analysis suggests that MR-proANP may be a useful marker of cardiac dysfunction and predictor of adverse outcomes in patients with HCM, but does not demonstrate definitively its superiority in these respects to NT-proBNP. MR-proANP and NT-proBNP are highly correlated, but some of their determinants are different. MR-proANP is mainly derived from the atria and therefore more closely related to left atrial structure and function. The left atrium may be the best barometer to integrate the effects of left ventricular, both diastolic and systolic, dysfunction. On the other hand, NT-proBNP may be predominantly derived from the ventricular myocardium and more closely related to left ventricular hypertrophy and dysfunction rather. The consequences of left ventricular dysfunction (ie: an increase in LA pressure) may be more important than more direct measures of left ventricular dysfunction.

Many studies have reported increased plasma concentrations of BNP and NT-proBNP in patients with HCM[9-12 18-23] and shown that they are associated with more left ventricular hypertrophy, more left ventricular outflow tract obstruction, left ventricular diastolic and systolic dysfunction, worse symptoms and reduced exercise tolerance. This is not unexpected, as the main source of BNP is cardiomyocytes and increased ventricular wall stress the major stimulus to its secretion.[24] However, few studies have investigated the effects of HCM on plasma concentrations of atrial natriuretic peptide (ANP). Immunohistochemical analyses of endomyocardial biopsies from HCM patients confirm that ANP is expressed in ventricular myocardium in HCM[25] and this expression is associated with myocardial disarray, hypertrophy of myocytes and fibrosis. However, Briguori[18] reported that plasma concentration of ANP in HCM was strongly associated with left atrial function, whereas BNP was strongly associated with obstruction. Only one study, including only 40 patients, has investigated MR-proANP in HCM patients, showing that amongst several biomarkers, MR-proANP was the only one associated with the extent of late gadolinium enhancement on MRI.[26] As previous studies, we found a significant association between NYHA and natriuretic peptides although there was a significant overlap between NYHA 2 and 3-4 (Figure 1).

Several single-centre studies have evaluated the prognostic value of natriuretic peptides in patients with HCM and confirmed that high concentrations of BNP or NT-proBNP are associated with an increase in heart failure events and poorer survival.[11 12]

[20-22] We confirm this finding in a multicentre study for the first time. We also found that MR-proANP was a powerful prognostic marker to predict death, heart transplantation and heart failure events. Similar findings have been reported in heart failure.[15 16 27] As discussed above, this may be because the left atrium is a better marker of overall left ventricular and mitral valve function and also reflects the effects of left atrial hypertension on pulmonary haemodynamics that may ultimately contribute to right heart dysfunction and systemic venous congestion. It is also possible that daily fluctuations in MR-proANP are smaller than for NT-proBNP making it a more stable marker.[28] Finally, its predictive value seems particularly interesting in patients with a low-risk profile based on symptoms and echocardiography.

Currently, there is no evidence that interventions to prevent disease progression are effective for patients with HCM and therefore there is no clinical mandate for early detection of worsening cardiac function unless it is to identify patients at risk of sudden arrhythmic death who required an implantable cardioverter defibrillator. However, MR-proANP could be a valuable research tool to identify patients at increased risk who should be considered for clinical trials of existing or novel treatments that might prevent or reverse progression. It is surprising how little evidence there is that beta-blockers, calcium antagonists or other agents improve outcome in HCM. Recently, clinical trials of a new class of agent, cardiac myosin inhibitors, have begun. MR-proANP might be helpful to identify risk and might also be a measure of a successful intervention.

Study limitations.

Our study is a post-hoc analysis of the multicentre Eurogene Heart Failure cohort. However, enrolment and data-collection were standardized and follow-up was prospective. Some echocardiographic measurements such as left atrial volume, pulmonary pressure, or parameters of diastolic function, were not available for all the patients and therefore were not included in the model for prognostic analysis. Likewise, left ventricular outflow tract obstruction was evaluated only at rest and not during Valsalva or exercise. Patients were enrolled predominantly in tertiary centers with a special interest in HCM for the purposes of genetic evaluation. Thus, most patients did not have advanced disease and had no or few symptoms. This may account for the relatively low event rate. On the opposite, the proportion of patients

needing left ventricular assist device or heart transplantation might appear relatively high. However, our rate of death or heart failure-related events is very comparable to other large cohorts of HCM patients from tertiary centers (around 2.2 %/ year).[11 12] The generalisability of our results to less selected population should be addressed in further studies.

In summary, this multicentre study of patients with HCM suggests that MR-proANP, if not superior to NT-proBNP, is strongly related to cardiac dysfunction and prognosis. MR-proANP might be a useful tool for monitoring patients with HCM to identify patients at increased risk who would be potential candidate to existing or novel interventions.

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Figure 1: Box and whisker plot showing the relationship between New York Association functional class and NT-proBNP and MR-proANP. Data show median, interquartile range (boxes) and full-range.

Figure 2: Kaplan–Meier analysis showing cumulative rates of survival in 357 patients with hypertrophic cardiomyopathy, stratified into tertiles according to MR proANP values. 1st tertile (MR-proANP < 70 pmol/mL), 2nd tertile (MR-proANP 70-155 pmol/mL), 3rd tertile (MR-proANP > 155 pmol/mL).

Figure 3: Areas under the receiver-operating curve (AUC) for NT-proBNP (solid line) and MR-proANP (dashed line) for the prediction of death, heart transplantation, left ventricular assist device implantation and heart failure hospitalisations.

Table 1. Baseline characteristics

Age	52 (36-65)
Male	204 (57)
History	
Syncope	77 (21)
Hospitalisation for Heart failure	49 (13)
Clinical presentation	
Systolic blood pressure, mm Hg	125 (110-140)
Diastolic blood pressure, mmHg	75 (70-80)
Heart rate, beats/min	65 (58-74)
Weight, kg	73 (64-82)
NYHA (%)	
NYHA 1	137 (41)
NYHA 2	160 (47)
NYHA 3-4	41 (12)
Atrial fibrillation (%)	10
Biology	
Serum creatinine (mg/dl)	1.00 (0.89-1.20)
MR-proANP (pmol/L)	107 (58-184)
NT-proBNP (ng/L)	550 (202-1380)
Echocardiography data	
LV end diastolic dimension, mm	46 (41-49)
LV ejection fraction, %	65 (59-71)
LV ejection fraction < 50 %	41 (11)
Posterior wall thickness, mm	11 (10-14)
Septal wall thickness, mm	18 (15-21)
Maximum wall thickness, mm	20 (17-24)
Left atrial diameter, mm	44 (39-50)
Therapy	
Medications	
Beta-receptor antagonist	174 (49)
Calcium channel blocker	78 (22)
Renin angiotensin system inhibitors	53 (15)
Antiarrhythmic drug	50 (14)
Disopyramide	2
Devices	
Pace-maker at baseline	46 (13)
Intracardiac defibrillator at baseline	20 (6)
Intracardiac defibrillator at study end	32 (9)
Procedures	
Septal myectomy at baseline	25 (7)
Septal myectomy at study end	27 (8)
Alcohol septal ablation at baseline	5 (1)

Alcohol septal ablation at study end	17 (5)

Values are median (IQR) or n (%). (NYHA, n=337; Systolic blood pressure, n=350; LV ejection fraction, n= 354; left atrial diameter, n=350; serum creatinine, n=211)

Table 2. Multivariable analysis of the independent determinants of MR-proANP and NT-proBNP

Variable	log MR-proANP			log NT-proBNP		
	Parameter estimate	Standard Error	p	Parameter estimate	Standard error	p
Intercept	4.60			6.30		
NYHA			<0.0001			<0.0001
1	-0.43	0.12		-0.81	0.21	
2	-0.10	0.11		-0.33	0.20	
3-4	0.			0.		
Age (y)	0.009	0.002	<0.001	0.011	0.004	0.0125
Weight (kg)	-0.017	0.003	<0.0001	-0.029	0.005	<0.0001
SBP (mmHg)	-	-	-	-0.011	0.004	0.0046
Max LVWT (mm)	-	-	-	0.085	0.012	<0.0001
LAD (mm)	0.035	0.004	<0.0001	0.038	0.008	<0.0001
LVEF (%)	-0.006	0.003	0.0235	-	-	-

SBP: systolic blood pressure; max LVWT: maximal left ventricular wall thickness; LAD: left atrial diameter; LVEF: left ventricular ejection fraction

ventricular wall thickness; LAD: left atrial diameter; LVEF: left ventricular ejection fraction

Table 3. Univariate and multivariable analysis for prediction of the primary composite endpoint

All-cause mortality, transplantation, LVAD, hospitalisation for heart failure				
	Univariate		Multivariable	
	HR (95% CI)	p-value	HR (95% CI)	p-value
Log MR-proANP	4.25 (2.45-7.38)	<0.0001	3.77 (2.01-7.08)	<0.0001
Log NT-proBNP	2.33 (1.66-3.27)	<0.0001		
Age (years)	1.0 (0.99-1.03)	0.3		
Weight (kg)	0.99 (0.97-1.02)	0.66		
SBP	0.98 (0.96-0.99)	0.02		
Heart rate	1.03 (1.01-1.06)	0.01		
NYHA (1-2 / 3-4)	3.4 (2.1-5.5)	<0.0001	0.35 (0.16-0.77)	0.0086
LVEF (%)	0.95 (0.93-0.97)	<0.0001	0.96 (0.94-0.98)	0.0005
LVEDD (mm)	1.04 (0.99-1.07)	0.055		
PWT (mm)	1.13 (1.01-1.27)	0.033		
SWT (mm)	0.98 (0.92-1.04)	0.55		
Maximal WT (mm)	0.99 (0.93-1.06)	0.91		
LAD (mm)	1.08 (1.04-1.12)	<0.0001		

SBP: systolic blood pressure, LVEF: left ventricular ejection fraction; LVEDD: left ventricular end diastolic diameter, PWT: posterior wall thickness, SWT: septal wall thickness; LAD: left atrial diameter; LVAD: left ventricular assist device. HR= Hazard ratio; CI=confidence interval