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RESEARCH ARTICLE

The effect of diabetes on the diagnostic and prognostic performance of mid-region pro-atrial natriuretic peptide and mid-region pro-adrenomedullin in patients with acute dyspnea

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Abstract
Serum mid-regional pro-atrial natriuretic peptide (MR-proANP) and pro-adrenomedullin (MR-proADM) are novel biomarkers for acute heart failure (AHF). Like other AFH biomarkers, the performance of these tests are affected by the presence of clinical variables such as renal failure and obesity. In a substudy of the Biomarkers from Acute Heart Failure Study, we show that diabetes did not influence the performance of these markers with regards to AHF diagnosis or 90-day all cause death. However, in patients without AHF, increased MR-proADM alone was associated with the presence of diabetes.

Keywords: Mid-regional pro-adrenomedullin, mid-regional pro-atrial natriuretic peptide, heart failure, risk stratification

Introduction

The natriuretic peptide are part of a family of peptides that regulate water and electrolyte balance (Vanderheyden, Bartunek, & Goethals, 2004). B-type natriuretic peptide (BNP) is a 32-amino acid peptide that has the highest tissue distribution in the ventricules of the heart. Atrial natriuretic peptide (ANP) is a 28-amino acid peptide that has the highest tissue distribution in the atria. ProBNP and proANP are the larger precursor protein that are enzymatically cleaved to form the free biologically active peptides BNP and ANP, respectively, and the inactive N-terminal remnant peptide NT-proBNP and NT-proANP, respectively. Adrenomedulin is 52-amino acid peptide that originates from a variety of human tissues and also has vasodilatory properties (Eto, 2001).

All of these peptides are increased in patients with acute heart failure (AHF) as a compensatory mechanism

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to vasoconstriction, fluid and volume overload. In clinical practice, only BNP and NT-proBNP is routinely used (Maisel et al. 2002, Januzzi et al. 2005). Part of the reluctance of using ANP and adrenomedullin is due to the analyte's instability in blood. The midregions (MR) of pro-atrial natriuretic peptide (MR-ANP) and pro-adrenomedulin (MR-proADM) are more stable in blood and have been shown to be alternative biomarkers in AHF for diagnosis and risk stratification of respectively (Morgenthaler et al. 2004, Morgenthaler et al. 2005). In the Biomarkers in Acute Heart Failure (BACH) trial, MR-proANP was shown to have equivalent diagnostic utility to BNP in most patient groups. However, in patients with renal insufficiency, obesity, advanced age or presence of edema, MR-proANP added to the utility of BNP in patients with intermediate BNP levels (Maisel et al. 2010, Daniels et al. 2006). If this test is to be adopted in addition to or in lieu of BNP/NT-proBNP testing, further characterization of clinical performance is necessary. Previous reports have documented that MR-proANP is higher in older vs. younger patients and lower in obese vs. lean patients (Daniels et al. 2011), similar to findings for BNP/NT-proBNP (Wieczorek et al. 2002). However in contrast to the B-type peptides, MR-proANP levels are higher in men than women. With regards to co-morbidities, renal failure affects BNP/NTproBNP (McCullough et al. 2003), but not diabetes (Wu et al. 2004), or anemia (Wu et al. 2005). In this study, we determined if the presence of diabetes alters the diagnostic or prognostic performance of MR-proANP or MR-proADM for AHF.

Research design and methods

Study population

The BACH trial was a prospective 15-center multinational clinical trial of patients presenting to the emergency department with acute dyspnea (Daniels et al. 2006). The study population consisted of 1641 patients enrolled from March 2007 to February 2008. The final diagnosis of AHF, heart failure (HF) history, or no AHF was adjudicated by two board-certified cardiologists who reviewed all medical records pertaining to the patient, but remained blinded to the MR-proANP and MR-proADM results, but not BNP or NT-proBNP (whichever was used locally). In the event of disagreement between adjudicators, a third board-certified cardiologist intervened. This study was approved by the institutional review boards of each participating center, and all patients provided written consent for participation. The main outcomes of trial have been published elsewhere (Maisel et al. 2010).

Blood was collected at the time of emergency department presentation into plastic phlebotomy tubes containing EDTA, centrifuged, and plasma immediately stored frozen at −70°C. EDTA was used because it is the only tube approved for assay of MR-proADM and BNP. MR-proANP and NT-proBNP can be tested on serum, or heparinized and EDTA plasma. All samples were immediately frozen and later sent to a core laboratory (University of Maryland) for testing. MR-proANP and MR-proADM were tested using an automated sandwich chemiluminescence immunoassay on the Kryptor Sytstem (Thermo Fisher, Henningsdorf/Berlin Germany). BNP was tested on using the Triage System, (Alere Inc., San Diego, CA), and NT-proBNP was tested using the Elecsys 2010 Analyzer (Roche Diagnostics, Indianapolis). The limit of quantitation and upper dynamic range of these assays were 4.5–1000 pmol/L for MR-proANP, 0.23–10 nmol/L for MR-proADM, 5.0–5000 pg/mL for BNP and 10–35,000 pg/mL for NT-proBNP. Total imprecision of these assays were 5.4%, 9.8%, 3.0% and 3.0%, for these assays, respectively. Results for the local laboratory for BNP and NT-proBNP were only used in the adjudication of the final diagnosis, and not in the statistical analysis of this work. Clinical outcomes at 90 days were determined by telephone calls to patients and/or their family members and medical records review. The primary endpoint was all-cause death.

Patient groups

Clinical variables and medical history were determined by clinical study coordinators through a retrospective review of medical records. A history of active smoking, wheezing, coughing, weight gain, night sweats, night sweats, orthopnea, and dyspnea on rest were determined from documented medical history, and self-reporting by each participant. The eGFR was calculated using the Cockcroft-Gault equation (Cockcroft & Gault, 1976). The presence of chronic renal failure or diabetes were determined by review of the medical history at the time of their index presentation. This trial was not specifically aimed at diabetes, therefore there was no objective diagnostic tests performed, such as a fasting blood glucose or hemoglobin A1c. As such, this is a study limitation related to the design of the study.

The diabetes status was known in 1621 of the 1641 patients at baseline and for 1596 information about both diabetes status and history of HF is present. There were 831 patients with no current HF or previous history of HF (164 with diabetes), 565 patients with HF (218 with diabetes) and 200 with no current HF but a previous history of HF (70 with diabetes). Results of this trial were compared to the Breathing Not Properly Trial for BNP (Maisel et al. 2002), which had a similar distribution of patients among these groups.

With patient consent at enrollment, we contacted patients and their surviving families where appropriate at 90 days of enrollment to determine clinical outcomes. Outcomes include death, rehospitalization, and revisit to the emergency department. For the purpose of this study, the endpoint was all-cause death.

Statistics

To adjust for non-normal distribution, all biomarker values were log10 transformed prior to analysis. An analysis of variance (ANOVA) was performed to determine the linear regression models used to analyze

the effect of possible covariates on biomarker values. The model used was: log10(biomarker)~age+gender+BMI +eGFR+diabetes status (presence/absence). Statistical variables that were obtained included the degree of freedom, partial sum of squares (SS), mean square, F and *p* values. Proportions of patients who died within 90 days are compared using Fisher's exact test with simulated *p* values (2000 replications). *p* values were computed by Monte Carlo simulation, in larger than 2 × 2 tables using the software R, version 2.5.1. (The R Project for Statistical Computing, Institute for Statistics and Mathematics Resources, Vienna, Austria). Simulation was done conditional on the row and column marginal. For risk stratification, the cutoff concentration was optimized using receiver operating characteristic curve analysis to 539 pmol/L for MR-proANP, 1.985 pmol/L for MR-proADM, 1021 pg/mL for BNP and 6310 pg/mL for NT-proBNP (Maisel et al. 2010). Kaplan–Meier curves were constructed for these biomarkers versus death at 90-days. Multivariate logistic regression models were constructed for outcomes using biomarkers alone, diabetes alone, and interactions between biomarkers and diabetes.

Results

Table 1 shows the results of demographic variables, recent history, physical examination, clinical history, and baseline biomarker results for the three patient groups (i.e. all patients, with and without diabetes mellitus). The analysis of variance showed that an increase in MR-proADM was an independent variable for diabetic patients with no HF or HF history (Table 2, $p < 0.0005$). None of the other biomarkers achieved statistical significance for diabetes. Age, gender, BMI, and eGFR with biomarker levels were associated with these biomarker results in many of these patient categories as shown in previously published studies (Daniels et al. 2011) (Table 2).

Figure 1 shows the receiver operating characteristic (ROC) curves indicating the ability of each biomarker to discriminate acute heart failure, in the presence and absence of diabetes. No significant difference in the areas under the ROC curves were observed when data from diabetic and non-diabetic patients were compared.

Figures 2A and B shows the proportion of patients dead or alive at 90 days at the predefined cutoff points of baseline MR-proANP and MR-proADM. In all groups, increased concentrations of MR-proANP and MR-proADM were associated with a higher rate of death at 90 days. The highest proportion of patients who died was non-AHF patients with and without diabetes for MR-proANP (third and sixth column of Figure 2A) and non-AHF and non-diabetics for MR-proADM (sixth column of Figure 2B). These data suggest that these are potential biomarkers of mortality independent of AHF.

Increases in MR-proANP or MR-proADM were not associated with acute myocardial infarction and recurrent HF outcomes (data not shown). As the BACH trial was focused on AHF, the vascular complications of diabetes such as retinopathy and nephropathy were not recorded. Table 3 shows the multivariate logistic regression for HF outcomes for biomarkers alone and with diabetes status. All biomarkers were associated with AHF outcomes ($p < 0.0001$). However, only MR-proADM showed an interaction when diabetes was added to the model ($p = 0.0053$, Table 3). Figure 3 shows the Kaplan–Meier survival curves. All biomarker concentration above the cutoff concentration was associated with higher mortality ($p < 0.0001$). There was no difference when diabetes was added to the model.

Discussion

Atrial and B-type natriuretic peptides and adrenomedullin are potent vasodilators that are released into the circulation with hypertension and volume overload. Circulating natriuretic peptides primarily originate from the atria and ventricles of the heart (Vanderheyden, Bartunek, & Goethals, 2004). Both BNP and NT-proBNP are well established as a

Table 1. Demographic variables for diabetics vs. non-diabetics in the BACH Trial

Variables	N	All patients	DM absent	DM present	*p* value
Demographics					
Age (y)	1621	63.7 ± 16.9	62.6 ± 18	66.7 ± 13.3	0.0004
Male gender	1621	847 (52)[a]	601 (52)	246 (53)	0.6205
Race	1606				
White		1075 (67)	777 (67)	298 (65)	
Black		444 (28)	331 (29)	142 (31)	
Other		58 (3.6)	42 (3.6)	16 (3.5)	
Recent history					
Smoking	1583	462 (29)	345 (30)	117 (25)	0.0759
Wheezing	1530	467 (30)	336 (29)	131 (28)	0.5925
Cough	1589	940 (59)	674 (58)	266 (57)	0.693
Weight gain	1426	249 (17)	157 (14)	92 (20)	0.0035
Night sweats	1483	322 (22)	228 (20)	94 (20)	0.0035
Orthopnea	1521	685 (45)	457 (39)	228 (49)	0.0003

(Continued)

Table 1. *(Continued)*.

Variables	N	All patients	DM absent	DM present	*p* value
Dyspnea at rest	1588	786 (49)	556 (4.8)	230 (50)	0.4034
Exam variables					
Heart rate (bpm)	1612	91.5 ± 22.8	92.7 ± 23.7	88.4 ± 22	0.0032
Systolic BP	1611	140.8 ± 28.6	139.3 ± 27.9	144.5 ± 29.9	0.0032
Diastolic BP	1610	80.8 ± 17.3	81.3 ± 17.8	79.6 ± 16	0.1177
BMI (kg/m^2)	1383	29.2 ± 8.8	28 ± 8.7	32.3 ± 9.4	<0.0001
Temperature (°C)	1559	36.8 ± 0.7	36.8 ± 0.7	36.8 ± 0.7	0.2931
Pulse oximetry (%)	1589	95.1 ± 5.3	95.2 ± 5.4	94.9 ± 5.1	0.0256
Respiratory rate	1519	21.5 ± 5.9	21.4 ± 5.9	21.7 ± 5.9	0.162
Rales	1605	518 (32)	363 (31)	155 (34)	0.3436
S3[b]	1565	44 (2.8)	26 (2.2)	18 (3.8)	0.0882
Murmur	1587	252 (16)	179 (15)	73 (16)	0.8791
Elevated JVP	1524	269 (18)	175 (15)	94 (20)	0.007
Edema	1597	586 (37)	361 (31)	225 (49)	<.0001
Ascities	1565	41 (2.6)	25 (2.2)	16 (3.5)	0.1168
Wheezing	1600	450 (28)	328 (28)	122 (26)	0.4979
History variables					
Arrhythmias	1548	402 (26)	273 (24)	129 (28)	0.0458
Asthma	1587	315 (20)	240 (21)	75 (16)	0.0591
CRI	1581	245 (15)	136 (12)	109 (24)	<0.0001
Heart failure	1591	565 (36)	339 (29)	226 (49)	<0.0001
CAD	1583	501 (32)	302 (26)	199 (43)	<0.0001
COPD	1589	468 (29)	335 (29)	133 (29)	0.6668
Hyperlipidemia	1546	567 (37)	325 (29.2)	242 (52)	<0.0001
Hypertension	1601	1067 (67)	666(57)	401 (87)	<0.0001
MI	1581	299 (19)	166 (14)	133 (29)	<0.0001
Pneumonia	1530	259 (17)	187 (16)	72 (16)	0.9393
Pulmonary embolism	1598	84 (5.2)	59 (5.1)	25 (5.4)	0.8033
CABG	1610	156 (9.8)	85 (7.4)	71 (15)	<0.0001
Angioplasty/stent	1597	202 (12.6)	118 (10)	84 (18)	<0.0001
Stroke/CVA	1600	161 (10)	92 (8)	69 (15)	<0.0001
Pacemaker/ICD	1611	160 (10)	96 (8.3)	64 (13)	0.0008
Prosthetic valve	1608	42 (2.6)	26 (2.2)	16 (3.5)	0.1637
Outpatient medications					
Aspirin	1598	568 (36)	359 (30)	209 (45)	<0.0001
Clopidogrel	1601	129 (8.1)	65 (5.6)	65 (14)	<0.0001
Warfarin	1599	256 (16)	168 (15)	88 (19)	0.0288
Beta blockers	1597	624 (39)	370 (32)	254 (55)	<0.0001
ACEI or ATRB	1600	675 (42)	396 (34)	279 (60)	<0.0001
Ca channel blockers	1598	364 (23)	201 (17)	163 (35)	<0.0001
Statins	1601	515 (32)	271 (23)	244 (53)	<0.0001
Diuretics	1602	766 (48)	464 (40)	302 (65)	<0.0001
Digoxin	1601	120 (7.5)	82 (7.1)	38 (8.2)	0.4631
Aldosterone inhibitor	1599	148 (9.3)	91 (7.9)	57 (12)	0.0056
Anti-arrhythmics	1600	90 (5.6)	62 (5.4)	28 (6.1)	0.6321
Nebulizer/inhaler	1597	556 (35)	407 (35)	149 (32)	0.2953
Steroids	1566	389 (25)	295 (25)	94 (20)	0.0277
Antibiotics	1598	200 (12.5)	541 (47)	46 (10)	0.0657
Smoking cessation tx	1561	28 (1.8)	22 (1.9)	6 (1.3)	0.5276
eGFR[c]	1298	83.4 ± 68	83.6 ± 62.9	83.1 ± 78.4	0.3529
MR-proANP	1615	175 (67–369)	151 (59–346)	241 (98–428)	<0.0001
MR-proADM	1615	0.9 (0.6–1.4)	0.8 (0.5–1.3)	1.1 (0.7–1.8)	<0.0001
BNP	1618	165 (36–574)	123 (29–497)	276 (60–736)	<0.0001
NT-proBNP	1603	841 (112–4082)	617 (90–3626)	1639 (265–5493)	<0.0001

Abbreviations: DM, diabetes mellitus; BMI, body mass index; JVP, Juglar venous pressure; CRI, chronic renal insufficiency; HF, heart failure; CAD, coronary artery disease; COPD, chronic obstructive pulmonary disease; MI, Myocardial infarction; CABG, coronary artery bypass graft; CVA cerebrovascular accident; ICD, implantable defribrillator; ACEI, angiotensin converting enzyme inhibitor; ATRB, angiotensin receptor blocker; eGFR, estimated glomerular filtration rate.

[a]For non-numeric data, the percentage with the group (No DM or DM) is given in parenthesis.

[b]The presence of a third heart sound by auscultation.

[c]Used the Cockcroft–Gault equation (Cockroft & Gault, 1976).

Table 2. Multivariate linear regression models for various patient groups and biomarker analysis (p values).[a]

	MR-proANP Partial SS/P[b]	NT-proBNP Partial SS/P	MR-proADM Partial SS/P	BNP Partial SS/P
No HF or history of HF				
Diabetes	0.0144/0.693	1.64/0.0621	**0.586/<0.0005**	0.760/0.080
Gender	0.122/0.251	0.283/.438	0.058/0.265	0.043/0.676
Age	**20.9/<0.0001**	**85.7<.0001**	**6.00/<0.0001**	**35.8/<0.0001**
BMI	0.0014/0.900	0.501/.275	**0.501/0.005**	0.002/0.927
eGFR	**0.786/<0.005**	**3.55/<.01**	**0.457/0.005**	0.642/0.108
Acute HF				
Diabetes	0.003/0.819	0.006/.869	0.148/0.087	0.116/0.419
Gender	**0.523/<0.005**	**1.33/<.05**	0.005/0.743	**1.22/<0.01**
Age	0.224/0.0571	0.213/.347	0.040/0.373	**3.26/<0.0001**
BMI	**0.238/<0.05**	**2.26/<.005**	**0.590/<0.001**	**1.24/<0.01**
eGFR	**3.78/<0.0001**	**3.48/<.005**	**02.57/<0.0001**	**19.9/<0.0001**
History of HF				
Diabetes	0.022/0.636	0.192/.522	0.027/0.499	0.166/0.445
Gender	0.052/0.467	0.073/0.694	0.005/0.774	0.068/0.626
Age	**1.14/<0.001**	**3.26/<0.01**	**0.471/<0.005**	**1.98/<0.01**
BMI	0.014/0.707	1.02/0.142	**1.11/<0.0001**	0.586/0.153
eGFR	**1.07/<0.005**	1.78/0.053	**1.27/<0.0001**	0.127/0.505

[a]Bolded values indicate clinical significance (<0.05).
[b]Sum of squares and corresponding *p* value.

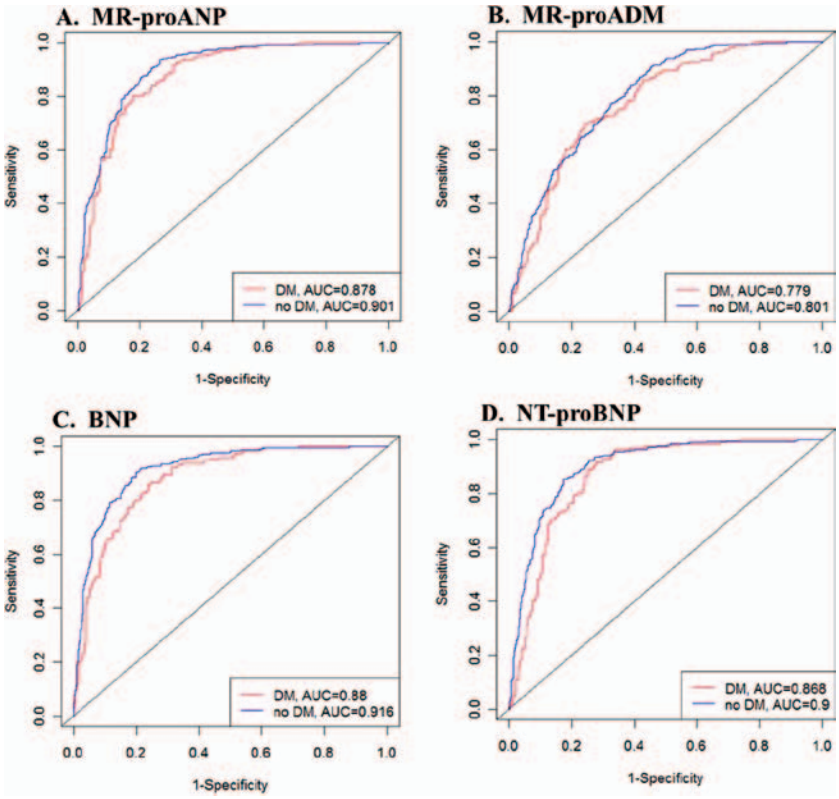

Figure 1. Receiver operating characteristic curve analysis for prediction of heart failure with and without diabetes. (A). MR-proANP. (B). MR-proADM. (C). BNP. (D). NT-proBNP. See inset for area under the ROC curve for diabetes vs. no diabetes.

biomarker in HF, and MR-pro-ANP has been shown to produce equivalent diagnostic results (Maisel et al. 2010). Increased concentrations of ANP have been observed in hypertension, glomerular hyperfiltration and microalbuminuria (McKenna et al. 2000). Among diabetics, the presence of these attributes are predictors of future overt nephropathy. There is, however, conflicting evidence as to whether or not ANP is increased in diabetic patients who are chronically hyperglycemic (Bell et al, 1989, McKnight et al. 1991).

Adrenomedullin originates from many tissues including the adrenal medulla, pancreatic islet cells,

and especially the vascular smooth muscle cells (Ichiki et al. 1994). Most investigators believe that circulating adrenomedullin originates predominately from the vasculature (Sugo et al. 1994). Adrenomedullin inhibits insulin secretion from the pancreas, and may therefore have a role in the pathophysiology of diabetes (Katsuki et al. 2002). Some investigators have shown that plasma ADM was higher in uncontrolled diabetics than healthy controls (Hayashi et al. 1997), while others have reported that ADM in diabetes was not correlated with glucose levels (Kinoshita et al. 2000). Increased expression of ADM mRNA was observed in rats with streptozotocin-induced hyperglycemia (Hayashi et al. 1999). Rats injected with ADM-developed hyperglycemia reversed by administration of anti-adrenomedullin antiserum (Martinez et al. 1999). Given the vascular complications of diabetes, adrenomedullin may be released in these patients as a compensatory mechanism, just as the natriuretic peptides are in AHF.

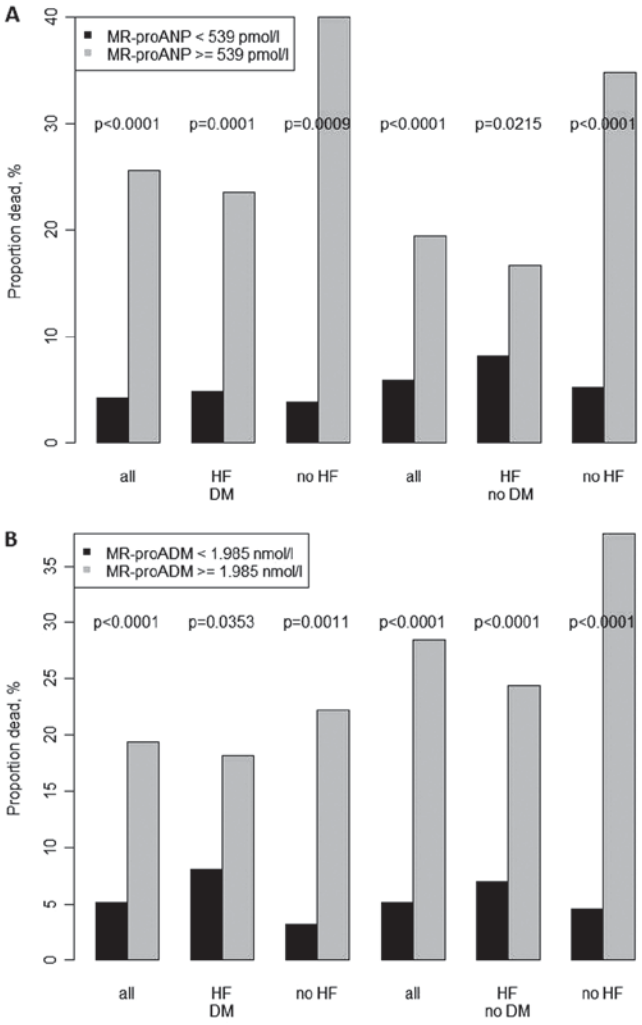

Figure 2. Prognosis of patient subgroups for death. 1st pair: all diabetic patients. 2nd pair: diabetics with, and 3rd pair: without heart failure. 4th pair: all non-diabetics, 4th pair: non-diabetics with, and 6th pair: without heart failure. (A). Results for MR-proANP. (B). Results for MR-proADM.

In the BACH study, the amino terminal mid-region of pro-peptides of both ANP and adrenomedullin were measured. The primary objective of this trial was to compare the performance of MR-proANP for the diagnosis of AHF, and MR-proADM for risk stratification against the more established biomarkers, BNP and NT-proBNP (Maisel et al. 2002). Since the patient's diabetic status was recorded in the case report forms of BACH, we were able to examine whether diabetes influenced test performance of MR-proANP and MR-proADM. MR-proADM but not MR-proANP levels were associated with diabetes in patients without heart failure. The results from this latter group are consistent with the earlier reports measuring adrenomedullin in diabetic patients.

The BACH Trial also examined clinical outcomes at 90 days. Published reports have suggested that ANP and adrenomedulnin participate in the pathophysiology of diabetic complications. Results form BACH suggest that both MR-ANP and MR-proADM may also be useful predictors of short-term mortality in acute heart failure. The correlation of ANP to diabetic complications may partly reflect the effect of the hormone on renal and vascular permeability (Zietse et al. 1995). Microalbuminuria is a surrogate for basement membrane damage and is an established marker for predicting diabetic nephropathy (Mogensen & Christensen, 1984). Investigators have

Table 3. Multivariate logistic regression models for outcome HF including biomarker, DM and biomarker*DM interaction.

Biomarker	N	Events	LR c^2	LR p	C Index	*p* (Wald-Test)	*p* value
MR-proANP	1615	561	860.42	<0.00001	0.9	ANP	<0.0001
						DM	0.6714
						Interaction	0.9895
MR-proADM	1615	561	426.19	<0.00001	0.807	ADM	<0.0001
						DM	0.0053
						Interaction	0.2837
BNP	1618	563	948.34	<0.00001	0.913	BNP	<0.0001
						DM	0.1822
						Interaction	0.4479
NT-proBNP	1603	559	848.75	<0.00001	0.901	proBNP	<0.0001
						DM	0.1754
						Interaction	0.4411

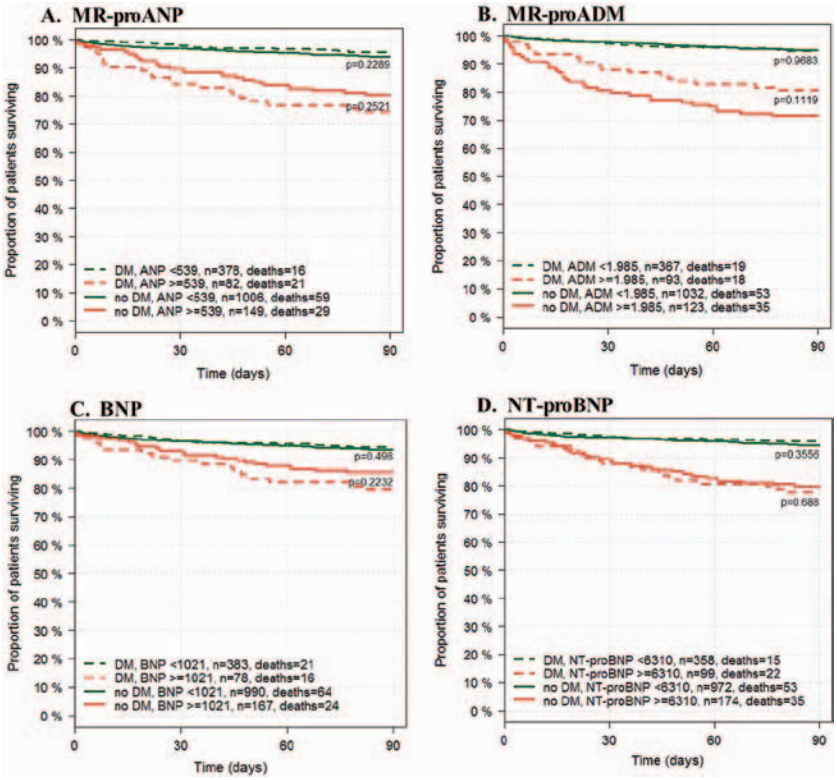

Figure 3. Aplan–Meier survival curves. (A). MR-proANP. (B). MR-proADM. (C). BNP. (D). NT-proBNP. See inset for cutoff concentrations and number of deaths. Biomarker concentrations exceeding the cutoff were associated with reduced survival ($p < 0.0001$ for all biomarkers). No difference in outcomes was observed for diabetes vs. non-diabetics (p listed are for all pairs).

demonstrated increased plasma levels of adrenomedullin in diabetes complicated by nephropathy and retinopathy (Nakamura et al. 1998). Others have shown that adrenomedullin correlated with a marker of oxidative stress (8-epi-prostaglandinF2α) among hypertensive diabetics (Katsaki et al. 2003). Our data showed that both MR-proANP and MR-proADM predicted mortality in diabetes and non-diabetics alike.

Earlier research studies on ANP and adrenomedullin centered about assays designed to detect the mature active circulating forms, with the correlation to pathophysiology established from the mid-1980s (for ANP) onward. The paucity of reports in more recent years may reflect the problems with the assay of adrenomedullin itself. The inability of producing an accurate measure of ANP and ADM may be responsible for the conflicting data reported in the literature (Bell et al., 1989, McKnight et al. 1991). Given the longer half-life in blood, improved *in vitro* stability in plasma, and availability of an automated commercial assays for MR-proANP and MR-proADM (Morgenthaler et al. 2004, Morgenthaler et al. 2005), testing for these biomarker may now be better suited for clinical studies on diabetes.

In conclusion, we determined from the BACH Trial that the presence of diabetes was not a variable in the diagnostic performance of MR-proANP and MR-proADM for acute heart failure. Results were similar to BNP and NT-proBNP, established AHF biomarkers. All markers including MR-proANP and MR-proADM were associated with death at 90 days. There was no influence of diabetes on risk stratification. Among non-AHF patients, increased MR-proADM was associated with diabetes.

Declaration of interest

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