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Utility of Amino-Terminal Pro-Brain Natriuretic Peptide, Galectin-3, and Apelin for the Evaluation of Patients With Acute Heart Failure

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OBJECTIVES This study sought to explore the role of new biomarkers in heart failure (HF).

BACKGROUND We investigated the utility of novel serum markers alone or together with natriuretic peptide testing for diagnosis and short-term prognosis estimation in subjects with acute HF.

METHODS Plasma levels of amino-terminal pro-brain natriuretic peptide (NT-proBNP), apelin, and galectin-3 were measured in 599 patients presenting with dyspnea at the emergency department, of which 209 (35%) had acute HF.

RESULTS The NT-proBNP was superior to either apelin or galectin-3 for diagnosis of acute HF, although galectin-3 levels were significantly higher in subjects with HF compared with those without. Receiver operating characteristic analysis for mortality prediction showed that, for 60-day prognosis, galectin-3 had the greatest area under the curve (AUC) at 0.74 ($p = 0.0001$), whereas NT-proBNP and apelin had an AUC of 0.67 ($p = 0.009$) and 0.54 ($p = 0.33$). In a multivariate logistic regression analysis, an elevated level of galectin-3 was the best independent predictor of 60-day mortality (odds ratio 10.3, $p < 0.01$) or the combination of death/recurrent HF within 60 days (odds ratio 14.3, $p < 0.001$). The Kaplan-Meier analyses showed that the combination of an elevated galectin-3 with NT-proBNP was a better predictor of mortality than either of the 2 markers alone.

CONCLUSIONS Our data show potential utility of galectin-3 as a useful marker for evaluation of patients with suspected or proven acute HF, whereas apelin measurement was not useful for these indications. Moreover, the combination of galectin-3 with NT-proBNP was the best predictor for prognosis in subjects with acute HF. (J Am Coll Cardiol 2006;48:1217–24)

It is estimated that the probability for an adult individual to develop heart failure (HF) during their lifetime is 20% to 30% (1,2), with attendant financial costs for modern society (3). Additionally, despite the introduction of therapies to reduce mortality, the 30-day cumulative survival rate after incident HF exacerbation is only 86% (1), leaving much room for improvement in the optimal evaluation and risk stratification for those so afflicted (3–5).

To improve the short-term and long-term prognosis of those with HF, it would be important not only to improve the identification of those with advanced HF, but to also be able to recognize those at highest risk for hazard. Unfortunately, even for experienced physicians, it is a complex task to properly diagnose HF and accurately assess prognosis. Accordingly, objective assessment using cardiac biomarkers may be one avenue in which patients may be routinely evaluated for the presence and severity of HF. To optimize such a strategy, the multiple mechanisms by which HF leads to higher morbidity and mortality must be addressed. Thus, a single biomarker is not likely to be sufficient for a comprehensive evaluation; a multimarker approach is therefore more likely to be of use (6).

Brain natriuretic peptide (BNP) and its cleavage equivalent amino-terminal proBNP (NT-proBNP) are established serum markers for diagnosis and prognosis in acute or chronic HF (7–10). Both peptides derive from the cleavage of proBNP, which production is rapidly upregulated when cardiomyocytes are stretched (11). These peptides identify increased wall stress, and have proven utility for confirming the diagnosis of acute HF in breathless subjects and predict adverse outcomes in these patients (7,8).

Aside from myocardial stretch, other mechanisms, such as inflammation (12) or pathways regulating cardiac contractility, might also play a role in HF, whereas these processes might not be reflected by natriuretic peptide levels. Previous data suggest that activated cardiac macro-

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Abbreviations and Acronyms

AUC	= area under the curve
BNP	= brain natriuretic peptide
CAD	= coronary artery disease
CI	= confidence interval
gal	= galectin
HF	= heart failure
IQR	= interquartile range
NT-proBNP	= amino-terminal pro-brain natriuretic peptide
NYHA	= New York Heart Association
OR	= odds ratio
PRIDE	= N-Terminal Pro-BNP Investigation of Dyspnea in the Emergency Department
ROC	= receiver-operating characteristic

phages produce galectin-3 (gal-3), a substance that impairs cardiac function (13). In addition, concentrations of apelin, a potent endogenous inotropic peptide, have also been suggested to identify the presence of HF and to parallel risk for death (14). Despite promise of both gal-3 and apelin for the evaluation of HF, little is known about the clinical utility of either marker. Accordingly, in an effort to better understand the individual and integrative role of these novel markers of HF, we tested the individual and combined diagnostic and prognostic utilities of gal-3 or apelin, together with NT-proBNP, for the evaluation of subjects with suspected or proven HF.

METHODS

Patients. The institutional review board approved all investigational procedures involved in this study. The details of the PRIDE (Pro-BNP Investigation of Dyspnea in the Emergency Department) study have been published previously (8). Briefly, this study examined 599 acutely dyspneic subjects presenting to the Emergency Department of the Massachusetts General Hospital, Boston. For each subject, a study physician assigned a final diagnosis of acute HF (from any cause) versus noncardiac dyspnea, using all hospital records pertaining to the subject, starting from the time of emergency department presentation through 60-day follow-up. These records included (when available) office notes, hospital discharge records, and results of all laboratory and imaging tests except for NT-proBNP levels. In addition, at 60 days an attempt was made to contact every patient and review subsequent medical course after presentation.

Laboratory measurements. At the time of presentation, a blood sample was collected into tubes containing ethylene diaminetetraacetic acid; the blood was immediately processed and frozen at −80°C for later measurement of NT-proBNP, gal-3, and apelin.

The NT-proBNP analysis was performed with a commercially available immunoassay (Elecsys proBNP, Roche Diagnostics, Indianapolis, Indiana) on an Elecsys 1010 analyzer according to established methods.

The gal-3 was analyzed using a commercially available enzyme-linked immunosorbent assay kit (Bender Medsystems, Vienna, Austria) and was measured on a Victor 2 plate reader (Perkin Elmer, Turku, Finland). Calibration of the assay was according to the manufacturer's protocol. Values were normalized to a standard curve. The intra-assay and interassay variances for gal-3 were 5.6% and 8.6%, respectively.

Plasma apelin levels were determined using a commercially available enzyme immunoassay without extraction (Phoenix Pharmaceuticals, Belmont, California) according to the manufacturer's instructions. This assay employs an immunoaffinity purified rabbit antibody specific for apelin 1-12. All apelin assays were performed in duplicate with intraexperimental standards using a Victor 3 plate reader (Perkin-Elmer, Wellesley, Massachusetts). Values were normalized to a standard curve. The intra-assay and interassay variances for apelin were 19% and 17%, respectively. This assay uses an immunoaffinity purified rabbit antibody specific for apelin 1-12. The antibody has 100% cross-reactivity to apelin 1-12, 1-13, and 1-36; there is no cross reactivity to adrenomedullin-52, BNP-32, CNP-22, ANP (25-56), ghrelin, endothelin-1, or bradykinin.

Statistical analyses. Medians of each marker in those with and without acute HF were compared using nonparametric testing, whereas comparisons of biomarker concentrations between groups categorized by the New York Heart Association (NYHA) functional classification were performed using Kruskal-Wallis testing. Otherwise, concentrations of NT-proBNP, gal-3, and apelin were log-transformed to achieve normality, especially for the multivariate analyses. Comparisons of clinical characteristics between those surviving to 60 days from presentation versus those who died were performed using chi-square tests for categorical data and the Wilcoxon rank-sum test for continuous data.

Receiver-operating characteristic (ROC) curves were utilized to evaluate the utility of gal-3 and apelin compared to NT-proBNP for the diagnosis of acute HF in breathless subjects, as well as for identifying risk of death by 60 days in those with a diagnosis of acute HF. For those markers with a significant area under the ROC curves, optimal cut-points for identifying or excluding risk of 60-day mortality in those with acute HF were identified. ROC analyses were performed using Analyse-It software (Analyse-It, Ltd, Leeds, United Kingdom).

After the identification of optimal cut points for identifying risk of mortality, Kaplan-Meier survival curves were constructed to compare 60-day mortality rates in groups divided as a function of cardiac marker categories (neither above the prognostic threshold, NT-proBNP alone above prognostic threshold, or both above the prognostic threshold) using the log-rank test to compare the rates of mortality.

Multivariate analyses. Variables considered for univariate analyses are listed in Tables 1 and 2, as well as several not listed, namely race, duration of symptoms, prior medication use (including beta blocker, angiotensin-converting enzyme inhibitor, and so on), body mass index, other laboratories at presentation, as well as hospital course for index hospital-

Table 1. Comparison of Demographic Characteristics of Heart Failure and Non-Heart Failure Patients

Characteristic	Acute HF (n = 209)	No Acute HF (n = 390)	p Value
Age, yrs (mean ± SD)	72.8 ± 13.6	56.9 ± 16.3	<0.001
Male gender	51%	51%	0.70
Past medical history			
Cardiomyopathy	20%	6%	0.001
Arrhythmia	32%	9%	<0.001
Hypertension	64%	41%	<0.001
Diabetes mellitus	42%	18%	<0.001
Coronary artery disease	42%	20%	0.001
Myocardial infarction	21%	9%	0.004
Congestive heart failure	54%	9%	<0.001
Obstructive lung disease	25%	42%	<0.001
Pulse rate, beats/minute (mean ± SD)	86.5 ± 23.5	88.2 ± 22.3	0.85
Glomerular filtration rate, ml/min/1.73 m^2	58.1 ± 25.3	82.6 ± 29.2	<0.001
Creatinine, mg/dl	1.32 ± 0.48	0.98 ± 0.33	0.005
Troponin T >0.03 ng/ml	26%	5%	<0.001
Gal-3, ng/ml (median, IQR)	9.2 (7.4–12.1)	6.9 (5.2–8.7)	<0.001
Apelin, pg/ml (median, IQR)	357 (277–480)	360 (290–495)	0.86

Gal = galectin; HF = heart failure; IQR = interquartile range.

ization, including index diagnosis, and laboratory testing (including NT-proBNP and gal-3 results). Multivariable stepwise logistic regression was used to identify independent clinical and biochemical predictors of death or death/readmission by 60 days in all subjects; 39 variables were considered before univariate analysis, and only those with retention parameters <0.05 were considered for multivariate analyses. These are listed in Table 3.

In each analysis, goodness of fit was verified using the Hosmer-Lemeshow test. For each independent predictor in multivariable analyses, odds ratios (OR) were calculated with 95% confidence intervals (CI). For all statis-

Table 2. Comparison of Characteristics Between Patients Dying During the 60 Days of Follow-Up With Those Surviving

Characteristic	Mortality (n = 17)	No Mortality (n = 192)	p Value
Age, yrs (mean ± SD)	80 ± 8.3	72 ± 13.7	0.06
Male gender	53%	51%	0.41
New York Heart Association functional class IV	53%	54%	0.74
Past medical history			
Cardiomyopathy	29%	19%	0.20
Atrial arrhythmia	41%	31%	0.55
Hypertension	59%	64%	0.74
Diabetes mellitus	22%	3.4%	0.002
Coronary artery disease	47%	42%	0.79
Myocardial infarction	24%	20%	0.71
Congestive heart failure	65%	53%	0.51
Medications at presentation			
Beta-blocker	53%	56%	0.98
Loop diuretic	59%	55%	0.78
Digoxin	29%	22%	0.88
Angiotensin-converting enzyme inhibitor	18%	34%	0.14
Angiotensin receptor blocker	12%	7%	0.20
Nitrate	15%	13%	0.45
Pulse rate, beats/minute (mean ± SD)	92 ± 19	86 ± 24	0.09
Systolic blood pressure, mm Hg (mean ± SD)	123 ± 29	141 ± 30	0.39
Body mass index, kg/m^2 (mean ± SD)	26 ± 4.9	27 ± 7.0	0.88
Glomerular filtration rate, ml/min/1.73 m^2 (mean ± SD)	46.3 ± 18.5	67.4 ± 26.0	<0.001
Creatinine, mg/dl (mean ± SD)	1.4 ± 0.52	1.1 ± 0.48	<0.001
Left ventricular ejection fraction, % (mean ± SD)	47.5 ± 20	46.3 ± 18	0.88
Troponin T, ng/ml (median)	0.03	<0.01	0.009
NT-proBNP, pg/ml (median, IQR)	9,332 (3,864–15,717)	3,511 (1,610–9,541)	0.02
Gal-3, ng/ml (median, IQR)	12.9 (9.3–16.5)	9.0 (7.3–11.6)	<0.001
Apelin, pg/ml (median, IQR)	339 (250–576)	361 (277–478)	0.66

NT-proBNP = amino-terminal pro-brain natriuretic peptide; other abbreviations as in Table 1.

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Table 3. Adjusted Multivariate Analysis for Death Within 60 Days and for the Composite of Death/Recurrent Heart Failure Within 60 Days

Predictor	Odds Ratio	95% Confidence Interval	p Value
Death within 60 days			
Log galectin-3	10.3	1.6–174.1	0.007
Log NT-proBNP	2.11	0.63–7.1	0.22
Age	1.05	1.00–1.10	0.08
Glomerular filtration rate	1.0	0.97–1.03	0.86
NYHA functional classification	1.5	0.67–3.57	0.31
Composite of death/recurrent HF within 60 days			
Log galectin-3	14.3	5.6–45.1	<0.001
Log NT-proBNP	2.92	0.53–9.11	0.42
Age	1.10	1.01–1.15	0.01
Glomerular filtration rate	0.98	0.96–0.99	0.05
NYHA functional classification	1.56	0.78–3.97	0.20

NYHA = New York Heart Association; other abbreviations as in Table 2.

tical analyses, all p values are 2-sided, with results <0.05 considered.

RESULTS

Diagnosis of HF in breathless subjects. As documented previously, of the 599 subjects in the PRIDE study, 209 (35%) had acute HF as a final diagnosis. In these 209 patients, 135 patients had ischemic HF as cause of their HF, whereas in 74 patients, there was a nonischemic or unknown origin. Among the 390 subjects without acute HF, 150 had obstructive airways disease exacerbation, 64 had pneumonia, 31 had acute coronary syndromes, 19 had pulmonary embolism, and in 116 subjects dyspnea was attributed in <10 cases each to allergic reactions, anxiety, ascites, atrial fibrillation, fever, fibrothorax, gram-negative sepsis, herpes zoster, hypertension, lung carcinoma, pericarditis, supraventricular tachycardia, or unknown.

As previously described for this population, median concentrations of NT-proBNP were higher in those with acute HF versus those without (4,054 vs. 131 pg/ml, $p < 0.001$) (8) (Table 1). Similarly, median concentrations of gal-3 were significantly higher in subjects with acute HF (9.2 pg/ml vs. 6.9 pg/ml, $p < 0.001$). Median concentrations of apelin were not different between those with and without acute HF (357 pg/ml vs. 360 pg/ml, $p = 0.86$).

With respect to HF etiology or type, there were no significant differences in median concentrations of each marker when considering ischemic versus nonischemic/unknown origin of HF (NT-proBNP: 4,549 pg/ml vs. 3,332 pg/ml, $p = 0.61$; gal-3: 9.41 ng/ml vs. 8.80 ng/ml, $p = 0.35$; apelin = 357.0 pg/ml vs. 361.5 pg/ml; $p = 0.61$). In contrast, considering type of HF, median concentrations of NT-proBNP were higher in patients with systolic HF versus those with nonsystolic HF (6,196 pg/ml vs. 3,134 pg/ml, $p < 0.001$). This pattern was not seen when considering gal-3 (9.42 ng/ml vs. 8.96 ng/ml, $p = 0.97$) or apelin (351 pg/ml vs 362 pg/ml, $p = 0.66$).

As previously described (8), ROC analysis examining NT-proBNP for diagnosis of acute HF yielded an area under the curve (AUC) for NT-proBNP of 0.94 ($p < 0.0001$). The ROC analysis for gal-3 for the diagnosis of HF showed an AUC of 0.72 ($p < 0.0001$), with an optimal cutoff of 6.88 ng/ml yielding a sensitivity of 80% and a specificity of 52%. In contrast, the AUC for apelin for diagnosis of acute HF was 0.52 ($p = 0.23$). A comparison of the 3 ROC curves is showed in Figure 1. The NT-proBNP had significantly greater AUC than either gal-3 or apelin for the diagnosis of acute HF ($p < 0.0001$); gal-3 had significantly greater AUC than apelin ($p < 0.001$). Neither gal-3 nor apelin concentrations correlated with NYHA functional classification.

When considering other important diagnoses in patients without acute HF, we found no significant differences in either gal-3 or apelin concentrations in subjects with exacerbation of obstructive airway disease (median gal-3 concentration 7.38 ng/ml in patients with obstructive airway

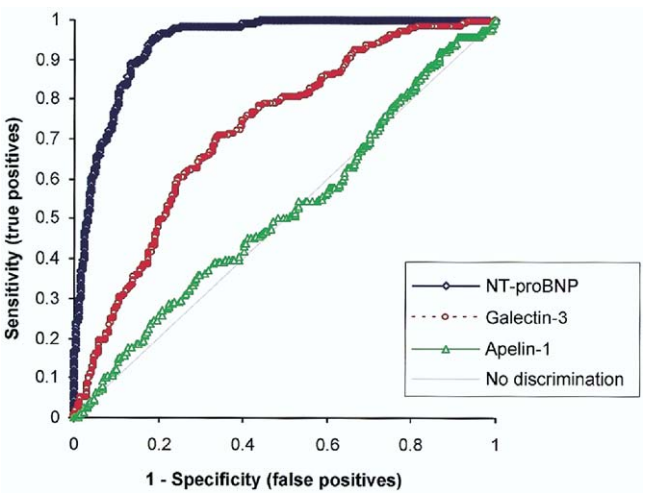

Figure 1. Combined receiver-operating characteristic (ROC) curves for amino-terminal pro-brain natriuretic peptide (NT-proBNP), galectin (gal)-3 and apelin for the diagnosis of heart failure in dyspneic patients. The ROC analysis for NT-proBNP showed an area under the curve (AUC) for NT-proBNP of 0.94 ($p < 0.0001$). The ROC analysis for gal-3 showed an AUC of 0.72 ($p < 0.0001$). The AUC for apelin for diagnosis of acute heart failure was 0.52 ($p = 0.23$).

disease vs. 8.15 ng/ml in the patients without obstructive airway disease, p = 0.08; median apelin concentrations 359 pg/ml vs. 360 pg/ml, p = 0.67 respectively) or pulmonary thromboembolism (median gal-3 concentration 7.37 ng/ml in patients with pulmonary thromboembolism vs. 7.51 ng/ml in the patients without pulmonary thromboembolism, p = 0.88; median apelin concentrations 356 pg/ml vs. 360 pg/ml respectively, p = 0.45) as a cause of their dyspnea.

Gal-3 and apelin concentrations and short-term prognosis in HF. Among the 209 subjects with acute HF, 60 (29%) had recurrent HF within 60 days, whereas 17 patients (8%) died by 60 days of follow-up. Demographic characteristics expressed as a function of 60-day survival are shown in Table 2. Median concentrations of gal-3 were significantly higher among those subjects dying by 60 days of follow-up (12.9 ng/ml, interquartile range [IQR] = 9.3 to 16.5 ng/ml) than in those surviving (9.0 ng/ml, IQR = 7.3 to 11.6, p < 0.001) (Fig. 2). In contrast, median apelin levels were not significantly different in decedents (339 pg/ml, IQR = 250 to 576 pg/ml) versus those surviving (361 pg/ml, IQR = 277 to 478 pg/ml; p = 0.66).

The ROC analysis for 60-day prognosis in acute HF showed an AUC for gal-3 of 0.74 (p = 0.0001), an AUC for NT-proBNP of 0.67 (p = 0.009), and an AUC for apelin of 0.54 (p = 0.33), as depicted in Figure 3. In contrast to diagnosis of acute HF, gal-3 showed a significantly greater AUC than either NT-proBNP (p = 0.05) or apelin (p < 0.0001). The optimal cut point for gal-3 for prediction of 60-day mortality was 9.42, which was 75% sensitive and 56% specific.

In adjusted multivariate analysis, log-transformed gal-3 serum levels were the best predictor of short-term mortality (OR 10.3, 95% CI 1.6 to 174.1, p < 0.01; Table 3), superior to NT-proBNP for this purpose. However, considering the

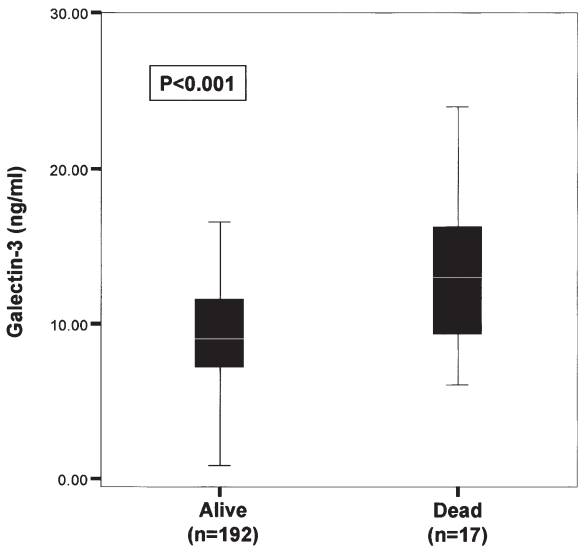

Figure 2. Median galectin-3 levels among heart failure patients who died (n = 17) within 60 days and those who survived (n = 192). **Boxes** = interquartile ranges; **whiskers** = 5th and 95th percentiles.

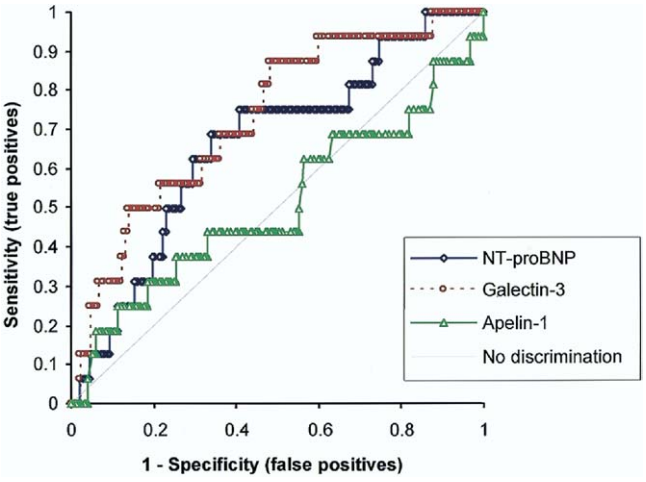

Figure 3. Combined receiver operating characteristic (ROC) curves for amino-terminal pro-brain natriuretic peptide (NT-proBNP), galectin (gal)-3 and apelin for 60-day mortality in heart failure. The ROC analysis showed an area under the curve (AUC) for gal-3 of 0.74 (p = 0.0001), an AUC for NT-proBNP of 0.67 (p = 0.009), and an AUC for apelin of 0.54 (p = 0.33).

combination of NT-proBNP and gal-3 for identifying those at highest risk for short-term mortality, Kaplan-Meier analyses showed that rates of death were highest in subjects with the combination of a gal-3 in excess of 9.42 ng/ml combined with NT-proBNP above 5,562 pg/ml (Fig. 4A).

A second adjusted multivariate analyses was performed, examining the combined end point of death or recurrent HF, and also showed that the log-transformed gal-3 serum levels were the best predictor of events (OR 14.3, 95% CI 5.6 to 45.1, p < 0.001) (Table 3). Notably, the rates of death/recurrent HF were highest in subjects with elevations of both NT-proBNP and gal-3 (Fig. 4B), with event rates somewhat differently affected by respective markers: elevated NT-proBNP concentrations paralleled higher rates of death (rather than recurrent HF), whereas gal-3 paralleled elevated rates for both death or recurrent HF.

DISCUSSION

We have shown that serum gal-3 levels are elevated in subjects with acute HF, and are prognostic of adverse outcomes over a 60-day period after presentation. In contrast, another novel marker with suggested utility in HF evaluation, apelin, did not seem to have similar utility for either diagnosis or short-term prognosis in our study. Notably, gal-3 was able to identify those HF patients at risk for short-term death or the combination of death or readmission within 60 days better than NT-proBNP; however, the combination of both markers seemed to further refine predictive utility. This finding may be of clinical importance because the list of possible mortality-reducing interventions in HF continues to grow (15–18), although risk stratification of HF populations continues to be imperfect. This leads to considerable burden for patients, health care workers, and health care policy makers. Therefore,

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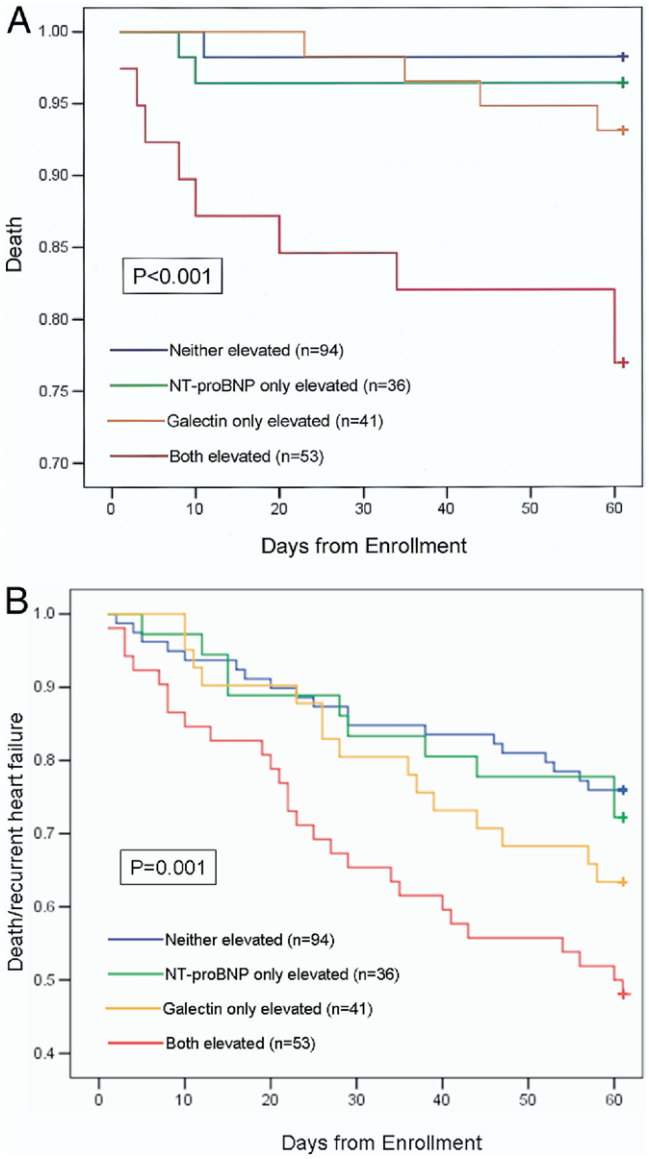

Figure 4. Kaplan-Meier analyses show that in patients with acute heart failure, at presentation the combination of a galectin-3 in excess of 9.42 ng/ml combined with amino-terminal pro-brain natriuretic peptide (NT-proBNP) above the optimal cut point (5,562 pg/ml) was associated with higher rates of death **(A)** or mortality/recurrent heart failure **(B)** than either of the 2 markers alone.

improving prognostication of HF patients by combined assessment of serum markers may help to tailor the most appropriate treatment strategy on a more individualized basis.

To date, various approaches have been proposed to prognosticate HF patients; recently the potential role of serum markers for this role has drawn considerable interest (6). Although in our study NT-proBNP was superior to gal-3 for diagnosing acute HF, gal-3 was the stronger predictor of short-term mortality. Interestingly, combining gal-3 measurement with NT-proBNP allowed identification of a high-risk subgroup of acute HF subjects with a considerably higher risk of mortality compared with those with low concentrations of both NT-proBNP and gal-3. In contrast to the findings with gal-3, in this study we could not confirm a role for apelin (14) because it was neither able to diagnose acute HF, nor to add prognostic information.

Because cardiomyocytes represent only one-third of the myocardium, it is not surprising that cells other than myocytes are also involved in the progression of acute HF (19). It is also well known that in the failing human heart, immunologic and inflammatory processes play an important role. Proinflammatory cytokines modulating several cardiovascular mechanisms and activated monocytes and macrophages are all involved in the pathogenesis of not only ischemic, but all forms of HF (12,20–22). We have previously shown that cardiac macrophages are activated at an early stage in failure-prone, hypertrophied hearts and that these macrophages express gal-3 (13). Intrapericardial infusion of gal-3 led to both cardiac structural changes and functional impairment in rats (13). Here we translate these experimental findings to a clinical application. Measurement of gal-3 is readily feasible and reliable in stored plasma, and we here show that gal-3 levels are elevated in a discriminatory fashion in those with acute HF, with useful information regarding both diagnosis and prognosis.

The gal-3 is a 26-kDa protein and a member of the galectin family, a group of carbohydrate-binding proteins with a specific amino-acid sequence able to recognize beta-galactose (23). Within this family, gal-3 is unique in structure; rather than consisting of merely one or two carbohydrate-recognition domains, gal-3 consists of tandem repeats of short amino-acid stretches fused onto the carbohydrate-recognition domain (23–25). Secondly, although galectins lack a signal sequence necessary for secretion, after macrophage activation, cytosolic gal-3 shifts to the plasma membrane and integrates in vesicles extruding from the plasma membrane (26). Therefore, although gal-3 is expressed in cancerous tissue, cancer-associated stromal cells, and even atherosclerotic lesions (23,27), it is not commonly significantly elevated in serum, other than in relatively rare clinical situations, such as in widely metastatic adenocarcinoma (28). Although theoretically this may limit the utility of gal-3 as a marker in HF, the differentiation between disseminated cancer and HF is usually easily done by history and physical examination.

The identification of biologically unique biomarkers offering complementary information for diagnosis (NT-proBNP), and prognosis (gal-3), as well as additive prognostic information when considered together, raises the possibility that our findings might be considered a step further toward a multimarker strategy for the evaluation of subjects with suspected or proven HF. Lee et al. (6) have already proposed a possible multimarker strategy and classified serum biomarkers in HF into four categories: neurohormonal markers, markers of myocyte injury, markers of matrix remodeling, and inflammation-related markers. In our analysis, we measured neurohormonal (NT-proBNP, apelin) and inflammatory markers (gal-3), showing the value of combining the two classes to improve prognostication. Interestingly, a marker of myocyte necrosis (cTnT) did

not add independently useful information in our analysis, despite previous reports about the added value of combining cTnT with (NT-pro)BNP in HF (29–32). This may merely reflect the limited power of our study.

Several other inflammation-related novel biomarkers have been previously investigated in smaller numbers of HF patients, including tumor necrosis factor-alpha receptor 1, C-C chemokines, soluble ST-2 receptor, and apelin (33–35). Although the exact role of apelin is still not completely elucidated, it is considered to be a potent endogenous inotrope, involved in neurohumoral pathways (36,37). Because the expression pattern of apelin and the APJ receptor is similar to expression pattern of angiotensinogen and the AT I receptor, combined with the fact that APJ-deficient mice show an increased vasopressor response to angiotensin II, this strongly suggests that apelin plays an important role in the counter-regulation of the effects of angiotensin II (14,38,39). Previous reports suggest apelin as a candidate marker in HF (14), although the relationship between apelin and HF remains poorly understood. Although Foldes et al. (40) showed that circulating levels of apelin indeed are elevated in the early stages of HF, such levels actually decreased in more advanced stages of HF. Our findings may represent a dynamic intermediate situation between these two steady states.

Limitations of our study include the fact that gal-3 did not correlate with severity of dyspnea as categorized by the NYHA functional classification. Such a lack of correlation makes, according to some investigators, a new diagnostic serum marker less suitable for clinical practice (41). However, we did not intend to investigate a new diagnostic marker, because it is already known that NT-proBNP is an excellent diagnostic marker in this population (8). A prognostic marker in HF actually does not necessarily correlate with NYHA functional classification, which is according to some investigators rather subjective (42) and merely a "crude estimation of a patient's functional capacity" (43). In addition, the relatively small number of subjects in our analysis, with relatively limited numbers of adverse events, may be a limitation as well. In addition, we were limited in the ability to adequately explore the additional utility of a third marker with potential prognostic value in HF such as cTnT (29–32).

In conclusion, in contrast to negative findings with apelin, we found that gal-3 may be a novel marker for the diagnosis and prognosis of acute HF. Combining clinical measurement of gal-3 and NT-proBNP may be a paradigm for the steps further toward a multimarker approach for diagnosis, prognosis, and potentially optimal, more focused therapeutic management of HF.

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