

Prognostic Significance of a Multimarker Strategy of Biomarkers in Acute Heart Failure

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ABSTRACT

Background: Heart failure (HF) is a growing public health problem. Patients often present to emergency department (ED) with acute onset dyspnea where a rapid triage is required to avoid misdiagnosis and to institute appropriate therapy. An objective risk-stratification in the ED is warranted to identify patients at high risk of adverse outcomes, so that more intensive therapy and vigilant follow-up after discharge are instituted.

Methods and Results: Fifty two consecutive acute HF (AHF) patients in NYHA class III/IV were enrolled for the present study. N-terminal pro B-type natriuretic peptide (NT-proBNP), high-sensitivity cardiac troponin T (hsTropT), high-sensitivity C-reactive protein (hsCRP) and Uric acid (UA) were evaluated at admission; a second sample for NT-proBNP and hsTropT was obtained 48h later. The end-point of the study, a composite of cardiovascular death, rehospitalisation for worsening HF symptoms and refractory HF was reached in 32.7% of patients

during a median follow-up of 4.8mnth. Although, hsTropT (>0.014ng/ml), hsCRP (>0.5mg/dl) and UA (>5.6mg/dl for females and >7 mg/dl for males) were elevated in the vast majority of patients (92.3%, 75% and 63.5% respectively), baseline and changing patterns of NT-proBNP following treatment were the only predictors of adverse outcomes on follow-up. A significant correlation between hsTropT, hsCRP and UA was observed suggesting a link between inflammation, myocyte injury and oxidative stress in AHF.

Conclusion: Baseline and changing patterns of NT-proBNP predicted adverse outcomes on follow-up suggesting that a strategy of serial measurement of NT-proBNP could prove invaluable in early risk stratification. Further research is needed to understand the link between inflammation, myocyte injury and oxidative stress in AHF which could provide potential therapeutic targets.

Keywords: Acute heart failure, Biomarkers, Prognosis

INTRODUCTION

Heart failure (HF) is a growing public health problem [1]. Patients with acute decompensated heart failure (ADHF) often present to emergency department (ED) where rapid triage and institution of appropriate treatment is critical and a delay can increase the mortality associated with this condition [2]. Conversely, a misdiagnosis of HF can be hazardous, because treatments for HF can adversely affect mortality when instituted in patients with other conditions having similar presentation [2,3]. Serum biomarkers like N-terminal proB-type Natriuretic peptide (NT-proBNP), cardiac-specific troponin (cTn) and C-reactive protein (CRP), apart from being diagnostically useful can provide a simple means of objective risk-stratification in the ED [4]. NT-proBNP has been shown in several small and large studies to be a powerful and independent predictor of adverse outcomes in acute heart failure (AHF) [5-7]. cTns have revolutionised the management of patients with acute coronary syndromes (ACS) but their role in AHF is far from clear [8,9]. Limited data exists on the relation between high-sensitivity (hs)CRP and Uric acid (UA) and adverse outcomes in AHF. With these considerations, the present study was undertaken to describe the association between these biomarkers and adverse cardiovascular outcomes in patients hospitalised for AHF.

MATERIALS AND METHODS

Study design and patient population

Fifty two consecutive patients with AHF in New York Heart Association (NYHA) class III/IV were enrolled for the present study. AHF was defined as a sudden or recent onset of symptoms of HF that represented either a de novo presentation or decompensation of an established chronic heart failure. After admission to the intensive

care unit (ICU), all patients received optimal HF treatment as per current guidelines [10-13]. Ischemic aetiology of HF was classified based on the evidence of an old myocardial infarction (MI) on Electrocardiography (ECG), chronic stable angina or documented history of coronary artery disease (CAD) or revascularization. Patients with significant renal (serum creatinine>2mg/dl), hepatic, pulmonary or valvular heart disease, sepsis and those who presented with ACS within the past one month were excluded from the study. Informed consent was obtained from all patients before participation in the study and the protocol was approved by the Ethical Committee of our institution.

Biomarker evaluation

For biomarker analysis, 5ml venous blood was collected within 24h of admission and a second sample [for NT-proBNP and hs Troponin T (hsTropT)] was obtained 48h later. Serum for hsTropT, hsCRP and UA were analysed immediately whereas for NT-proBNP, aliquots of serum were stored at -20°C until all the 52 samples were collected for analysis. NT-proBNP was analysed using the electrochemiluminescence immunoassay (ECLIA) method on the Elecsys 2010 analyzer (Hitachi High-Technologies Corporation, Tokyo, Japan). The cut-off values of NT-proBNP for diagnosis and exclusion of AHF were those derived in the ICON study [14]. Briefly, a 'rule-out' cut-point of 300 pg/ml was used for all patients and, values of 450, 900 and 1800 pg/ml in the age groups <50, 50-75 and >75 yrs respectively were used for 'ruling-in' a diagnosis of AHF. hsTropT was analysed using the ECLIA sandwich principle on the same analyser that was used for NT-proBNP. The upper reference limit (99th percentile) for the current assay was 0.014ng/ml (95% CI, 0.012-0.024ng/ml). A level >0.014ng/ml was hence used as the definition of an 'elevated troponin'. hsCRP was analysed using the

Baseline Characteristics	Values*
Age (in years)	57.4±16.0
% of males	55.8
Duration of hospital stay (in days)	5.7±2.9
Diabetes mellitus (%)	40.4
Hypertension (%)	36.5
BMI (in kg/m ²)	23.1±2.5
Smoking History (%)	46.2
% of Ischemic heart failure	30.8
Blood Glucose (mg/dl)	165.0±126.9
Hemoglobin (gm/dl)	12.5±2.7
Total blood count (per cumm)	10480±4467
Blood Urea (mg/dl)	52.2±29.3
Serum Creatinine (mg/dl)	1.3±0.4
Uric Acid (mg/dl)	8.1±3.3
Sodium (mEq/L)	133.8±5.6
Potassium (mEq/L)	4.2±0.9
Admission NT-proBNP (pg/ml)	10194.6±10397.7
48-hour NT-proBNP (pg/ml)	8414.7±10551.8
hsTropT (ng/ml)	0.24±0.56
LDL (mg/dl)	84.8±40.7
hsCRP (mg/dl)	2.13±1.82
LVIDd (mm)	5.6±0.9
LVIDs (mm)	4.4±1.1
% fractional shortening (FS)	20.9±8.8
EF (%)	37.4±9.6
% of patients with EF≥50%	15.4
Diastolic Stage	2.0±1.6

[Table/Fig-1]: Baseline Characteristics of the Study Patients., * Values expressed as mean± standard deviation (SD) and percentages, (BMI=Body mass index; CRP=C-reactive protein; EF=Left ventricular ejection fraction; hs=high sensitivity; LDL=Low-density lipoprotein cholesterol; LVID= Left ventricular internal dimension in d (diastole) or s (systole); NT-proBNP=N-terminal pro B-type natriuretic peptide; TropT=cardiac Troponin T)

particle enhanced immunoturbidimetric assay on the MODULAR P800 analyzer (Hitachi High-Technologies Corporation, Tokyo, Japan). A value ≥ 0.5 mg/dl as per International Federation of Clinical Chemistry consensus reference interval was used to define an 'elevated hsCRP' level.

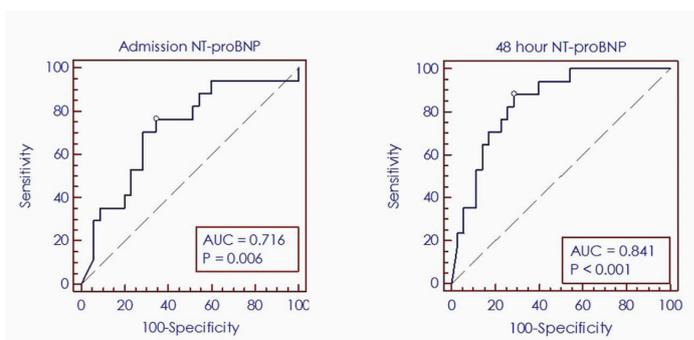
Echocardiography

A detailed Echocardiographic examination was performed on all patients on the PHILIPS En Visor C HD machine (PHILIPS ULTRASOUND, Bothell, WA, USA). Left ventricular (LV) and left atrial (LA) dimensions and LV ejection fraction (EF) were quantified as per American Society of Echocardiography (ASE) recommendations on chamber quantification (2005) [15]. LV diastolic function was assessed as per the recommendations of ASE (2009) [16]. Based on the LV EF, patients were divided into two groups: those with heart

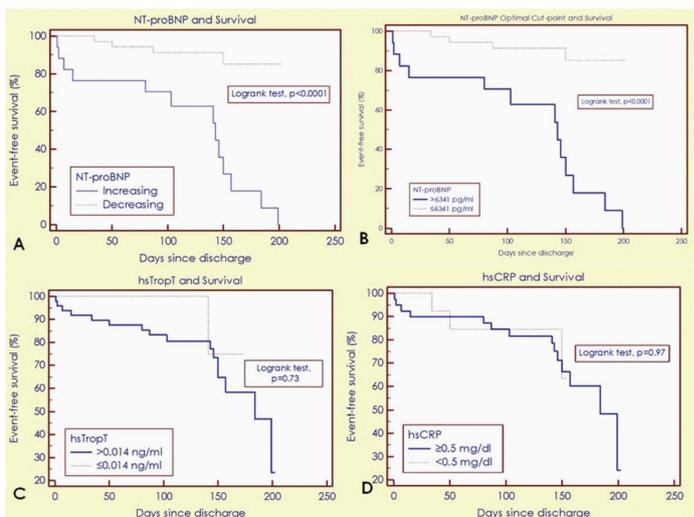
failure with reduced EF (HF_rEF) (EF<50%) and those with preserved EF (HF_pEF) (EF \geq 50%).

Discharge, Follow-up and study end-points

Patients were discharged at the discretion of the treating physician as and when clinical stability permitted. Patients were instructed to report worsening HF symptoms and advised revisit for any exacerbation of symptoms. Periodic follow-up on telephonic contact was also performed to assess the postdischarge functional



[Table/Fig-2]: Receiver Operating Characteristic (Roc) Curves for the Performances of Admission and 48 Hour NT-ProBNP (N-Terminal Pro B-Type Natriuretic Peptide) Assays



[Table/Fig-3]: RKaplan-Meier Survival Curves for a) Changing Patterns of NT-ProBNP after Admission B) Optimal NT-proBNP Value >6341pg/ml c) hsTropT and D) hsCRP (CRP=C-reactive protein; hs=high sensitivity; NT-proBNP=N-terminal pro B-type natriuretic peptide; TropT=cardiac Troponin T)

status and mortality. The end-point of the study was a composite of adverse events of cardiovascular death, rehospitalisation for worsening HF symptoms and refractory HF (NYHA functional class III or IV despite optimal medical treatment).

STATISTICAL ANALYSIS

Continuous variables are expressed as mean± standard deviation (SD). Categorical variables are expressed as percentages. The baseline characteristics were compared using Student's t-test for continuous variables and χ^2 (Chi-square) test for categorical variables. Correlation co-efficients between variables was calculated using the Spearman's rank correlation co-efficient test. The prognostic performance of the assays was assessed using Receiver operating-characteristic (Roc) curves. Optimal cut-points for the assays were determined using the

ROC curves. Kaplan-Meier analysis was performed on the cumulative rates of event-free survival. Log-rank test was used as a test of significance between the two curves. All reported p-values are two-sided; tests were considered statistically significant if p-value was <0.05.

RESULTS

Baseline Characteristics

The baseline characteristics of the 52 study patients are presented in [Table/Fig-1]. The mean age of patients was 57.4±16.0 yrs. The mean admission blood glucose was 165±126.9 mg/dl and nearly a

Baseline Characteristics	HFREF (n=44)	HFpEF (n=8)	p-value
Age (in years)	55.0±15.4	70.9±13.1	0.009
% of males	54.5	62.5	0.677
Diabetes Mellitus (%)	36.4	62.5	0.166
Hypertension (%)	29.5	75	0.014
BMI (in kg/m ²)	23.1±2.5	23±2.8	0.919
% of Ischemic heart failure	27.3	50	0.200
Uric Acid (mg/dl)	8.4±3.2	6.7±3.6	0.18
Sodium (mEq/L)	134.2±5.7	131.5±4.3	0.209
Admission NT-proBNP (pg/ml)	11327±10743.4	3966.4±5126.5	0.06
48-hour NT-proBNP (pg/ml)	9386.9±11146.5	3068±3059	0.12
hsTropT (ng/ml)	0.28±0.6	0.05±0.05	0.288
hsCRP (mg/dl)	1.96±1.75	3.0±2.0	0.136
LVIDd (mm)	5.8±0.8	4.1±0.3	<0.0001
LVIDs (mm)	4.8±0.7	2.6±0.4	<0.0001
LV ejection fraction (%)	33.9±4.9	56.8±3.9	<0.0001
Diastolic Stage	2.2±1.7	1±0.0	0.05
Adverse Events (%)	31.8	37.5	0.753
Mortality (%)	13.6	0	-

[Table/Fig-4]: A Comparison of the two Groups of Heart Failure Patients with Reduced or Preserved Ejection Fraction., (BMI=Body mass index; CRP=C-reactive protein; HFREF and HFpEF=Heart failure with reduced (r) and preserved (p) ejection fraction; hs=high sensitivity; LVID= Left ventricular internal dimension in d (diastole) or s(systole); NT-proBNP=N-terminal pro B-type natriuretic peptide, TropT=cardiac Troponin T).

third had history of hypertension. The average duration of stay in the hospital was 5.7±2.9 days and modestly correlated with hsTropT (Spearman $\rho=0.27$, $p=0.04$).

Aetiology of heart failure

Nearly half of the patients had idiopathic dilated cardiomyopathy (46.2%), followed by ischemic heart failure (30.7%), hypertensive heart disease (17.3%), peripartum cardiomyopathy (3.9%) and isolated LV noncompaction (1.9%). Thus, more than two-thirds (69.2%) of cases were of non-ischemic aetiology ($p=0.004$), of which idiopathic dilated cardiomyopathy was the dominant form.

NT-proBNP

The mean admission and 48h NT-proBNP values were 10194.6±10397.7 and 8414.7±10551.8 pg/ml respectively; thus, an 18% fall in the initial NT-proBNP was noted following initiation of treatment. A comparison of the admission and 48h NT-proBNP thus yielded two groups of patients. Group 1 consisted of patients with a rising pattern of NT-proBNP at 48h (32.7%) whereas in Group 2, the 48h value was showing a declining trend compared with the admission value (67.3%). A statistically significant increase in adverse events and a trend towards increasing mortality was noted in group 1 (76.5% vs 11.4%, $p<0.0001$ and 23.5% vs 5.7%, $p=0.059$ respectively); conversely, a fall in NT-proBNP value of nearly 37% was seen in patients who did not experience adverse outcomes on follow-up. A Receiver operating characteristic (ROC) curve analysis of admission and 48h NT-proBNP was performed [Table/Fig-2]. At admission, an optimal cut-point of >6341 pg/ml predicted adverse outcomes with a sensitivity and specificity of 76.5% and 65.7% respectively (Area under the curve [AUC]=0.72, $p=0.0056$) with a relative risk (RR) of adverse events of 3.51 (95% CI=1.32-9.35, $p=0.012$). A 48 Hour NT-ProBNP >4988 pg/ml similarly predicted adverse outcomes with a sensitivity and specificity of 88.2% and 71.4% respectively (AUC=0.84, $p<0.0001$) with a RR of 8.1 (95% CI=2.05-31.92, $p=0.0028$). The Kaplan-Meier survival curves for NT-proBNP [Table/Fig-3a&b] for the parameters of changing NT-proBNP status and optimal cut-point of 6341 pg/ml showed a statistically

Baseline Characteristic	Adverse Event	No Adverse Event	p-value
Age (in years)	57.6±10.8	57.3±18.2	0.95
% of males	52.9	57.1	0.78
Duration of hospital stay (days)	5.9±2.9	5.6±3.1	0.74
Diabetes Mellitus (%)	52.9	34.3	0.19
Hypertension (%)	35.3	37.1	0.89
Body mass index (kg/m ²)	23.3±2.4	23.1±2.6	0.79
Blood Glucose (mg/dl)	169.4±117.8	162.9±132.8	0.86
Hemoglobin (gm/dl)	11.9±2.4	12.8±2.9	0.27
Total blood count (per cumm)	11741±6285	9868±3189	0.15
Serum Creatinine (mg/dl)	1.3±0.4	1.2±0.4	0.615
Uric Acid (mg/dl)	8.8±3.9	7.8±2.8	0.295
Sodium (mEq/L)	132.7±7.1	134.3±4.8	0.341
Admission NT-proBNP (pg/ml)	14800.4±11637.4	7957.6±9092.9	0.0245
48hour NT-proBNP (pg/ml)	15547.7±12348.2	4950.0±7580.1	0.0004
Group 1 (%)	76.5	11.4	<0.0001
Group 2 (%)	23.5	88.6	<0.0001
hsTropT (ng/ml)	0.25±0.47	0.23±0.6	0.905
hsCRP (mg/dl)	2.2±1.9	2.0±1.7	0.703
LVIDd (mm)	5.4±0.9	5.6±0.9	0.456
LVIDs (mm)	4.3±1.0	4.5±1.1	0.529
EF (%)	36.9±9.9	37.6±9.5	0.807
Follow-up duration (in days)	96.9±67.8	136.8±34.3	0.007

[Table/Fig-5]: Comparison Between Patients with or without Adverse Outcomes on Follow-Up., (CRP=C-reactive protein; EF=Left ventricular ejection fraction; Groups 1 and 2=Groups with rising or declining NT-proBNP levels respectively after admission; hs=high sensitivity; LVID= Left ventricular internal dimension in d (diastole) or s (systole); NT-proBNP=N-terminal pro B-type natriuretic peptide; TropT=cardiac Troponin T).

significant divergence (log-rank test, $p<0.0001$) suggesting that a rising NT-proBNP level after admission and a baseline NT-proBNP value >6341 pg/ml are associated with adverse cardiovascular outcomes on follow-up.

hsTropT

Elevated Troponin (>0.014ng/ml) was observed in the majority of patients (48/52) (92.3%). Specifically, patients with heart failure of ischemic aetiology had higher troponin values compared with patients of non-ischemic aetiology (0.47±0.68 versus 0.14±0.47 ng/ml, $p<0.05$). hsTropT correlated with serum creatinine ($p=0.46$, $p=0.007$), hsCRP ($\rho=0.34$, $p=0.01$) and a modest correlation was found with UA ($\rho=0.25$, $p=0.07$). When hsTropT was analysed as a dichotomous variable on Kaplan-Meier survival curves [Table/Fig-3c], no significant difference in survival was observed at a discriminatory cut-off of 0.014ng/ml (log-rank test, $p=0.73$).

hsCRP

Elevated hsCRP (≥ 0.5 mg/dl) was seen in 39 out of 52 patients (75%). A Kaplan-Meier survival curve analysis [Table/Fig-3d] with a discriminatory value of 0.5 mg/dl did not reveal statistical significance between the two curves (log-rank test, $p=0.97$).

Uric Acid

An elevated UA (>5.6mg/dl for females and >7 mg/dl for males) was found in 33 out of 52 patients (63.5%) with a mean value of 8.1±3.3 mg/dl. A significant inverse relation was noted between UA and LV EF (Spearman $\rho=-0.29$, $p=0.03$). A higher value was also observed in patients with adverse outcomes (8.8±3.9 vs 7.8±2.8 mg/dl) although this was not statistically significant ($p=0.29$).

Variable	Odds Ratio (95% CI)	p-value
Age>65 years	0.4 (0.09-1.7)	0.22
Serum Creatinine>1.5mg/dl	0.77 (0.2-2.94)	0.70
Admission NT-proBNP>6341pg/ml	6.23 (1.66-23.32)	0.007
48-hour NT-proBNP>4988pg/ml	18.75 (3.6-97.4)	0.0005
Rising NT-proBNP	18.6 (4.26-81.22)	0.0001
Declining NT-proBNP	0.05 (0.01-0.23)	0.0001
hsTropT	1.5 (0.14-15.59)	0.73
hsCRP	1.87 (0.44-7.93)	0.39
Uric Acid>7mg/dl	1.54 (0.47-5.11)	0.48
LVIDd	0.83 (0.23-3.02)	0.78
EF<40%	0.71 (0.19-2.63)	0.61
Sodium<135mEq/L	1.73 (0.53-5.72)	0.37

[Table/Fig-6]: Univariate Analysis for Predicting Adverse Events on Follow-Up., (CRP=C-reactive protein; EF=Left ventricular ejection fraction; hs=high sensitivity; LVID= Left ventricular internal dimension in d (diastole) or s (systole); NT-proBNP=N-terminal pro B-type natriuretic peptide; TropT=cardiac Troponin T).

Echocardiography: The average EF was 37.4±9.6%; majority of the patients thus had HF_rEF (84.6%), the remainder (15.4%) having HF_pEF. A comparison of these two groups with respect to baseline characteristics can be seen in [Table/Fig-4].

Follow-up duration: Median follow-up of 4.8 months (range 1 day-6.6 months)

Adverse Events: A total of 17 patients (32.7%) had an end point on follow-up which included 6 deaths (11.5% overall mortality), 3 rehospitalisations for worsening HF symptoms and 8 patients in refractory HF (NYHA class III or IV). [Table/Fig-5] shows the comparison between the patients with or without an adverse event on follow-up. It can be observed from [Table/Fig-5] and the Kaplan-Meier survival curve in [Table/Fig-3a] that even a modest increase in the mean admission NT-proBNP level of 5% (absolute value of +747 pg/ml) resulted in a statistically significant increase in adverse outcomes. On the other hand, a 37.8% reduction in the mean admission NT-proBNP level was observed in patients who remained free of adverse events at the end of follow-up, suggesting that both the absolute values and the relative change in the NT-proBNP values following initiation of treatment can predict adverse outcomes. The other three biomarkers (hsTropT, hsCRP and UA) were not significantly different between the two groups. On univariate analysis [Table/Fig-6], the only predictors of adverse outcomes were the admission and 48h NT-proBNP levels together with the change in the NT-proBNP status.

Effect of renal dysfunction on biomarkers: The biomarkers showed significant correlation with serum creatinine (NT-proBNP $\rho=0.38$, $p=0.005$; hsTropT $\rho=0.46$, $p=0.007$; hsCRP $\rho=0.34$, $p=0.01$ and UA $\rho=0.48$, $p=0.003$).

DISCUSSION

NT-proBNP was determined at admission and 48h later with an objective of evaluating the predictive power of both the absolute and the change in NT-proBNP values following initiation of treatment. A 48h value would also reflect the kinetics of circulating NT-proBNP, given that half life of NT-proBNP is only 70-120 min [17,18]. Increased ventricular wall stress due to cardiomyocyte stretch secondary to volume or pressure overload is the primary stimulus for the synthesis and secretion on BNP [5,18,19]. In a prospective study of 1586 patients in the Breathing Not Properly (BNP) multinational study, Maisel et al found BNP to be the single most accurate predictor of the presence or absence of congestive heart failure [3]. Similar outcomes of better diagnostic accuracy over conventional diagnostic strategy was established in the

BASEL[20] (BNP) and PRIDE[5] (NT-proBNP) studies. We found a statistically significant increase in adverse outcomes with a trend towards increasing mortality in patients showing a rising pattern of NT-proBNP following admission; conversely, a fall in NT-proBNP value of nearly 37% was seen in patients who did not experience adverse outcomes on follow-up. On univariate analysis, we found that an admission NT-proBNP level >6341 pg/ml and 48h NT-proBNP level >4988 pg/ml predicted adverse outcomes on follow-up (Odds Ratio, OR-6.23, 95%CI-1.66-23.32, $p=0.007$ and OR-18.75, 95%CI-3.6-97.4, $p=0.0005$ respectively). BNP/NT-proBNP have proven to be independent predictors of adverse cardiovascular outcomes in several multicentre prospective studies[3,6,14,21]. Several studies and reviews on the predictive power of serial measurement of BNP/NT-proBNP have been published [7,21-23]. Both rising [7] and declining [21,23,24] patterns have been shown to be associated with clinical outcomes in prospective studies. In a prospective study of 241 adult patients aged >25y, Michtalik et al reported that a <50% decrease of admission BNP at discharge was found to increase the risk of readmission or death within one year by 40% [7]. Conversely, in one small study of 37 ADHF patients with serial measurements of NT-proBNP at admission, 4,12,24 hours and predischage, progressive reduction to a complete relief of HF symptoms was accompanied by a reduction of 58% in the level of NT-proBNP [23]. Given the robust data on the serial changes in NT-proBNP and association with adverse outcomes, a 'BNP guided therapy' may soon be accepted as a standard of care in AHF. Current guidelines [12] however, do not support a routine use of serial measurements to guide therapy, although most standard HF guidelines [11-13,25] recommend the use of NT-proBNP/BNP in patients presenting to ED with acute dyspnea to improve diagnostic accuracy, especially when the diagnosis is uncertain [12,25]. We found an inverse correlation of NT-proBNP with body mass index (BMI) ($r= -0.35$, $p=0.01$) similar to that observed in the BNP study [26]. Although, increased clearance from adipocytes has been postulated as a cause of lower BNP values in obese people [22], a greater degree of cardiac cachexia and increased BNP was thought to simply reflect the severity of disease in the BNP study [26].

In our study, patients with HF_pEF had nearly three times lower values of NT-proBNP compared to those with HF_rEF ($p=0.06$). A lower BNP in patients with HF_pEF was also observed in studies where echocardiographic data was available [8,14]. In the DOSE trial[8], the variability was attributed to less severe HF, high prevalence of obesity in patients with HF_pEF and increased clearance by adipocytes.

cTn is a heterotrimeric complex consisting of TnC, TnI and TnT; all being present in a 1:1:1 stoichiometric ratio [1,27]. Troponin was almost universally elevated in our patients (92.3%) with a mean value of 0.24±0.56 ng/ml. Such large scale elevations upto 98% in AHF have been described in several studies using sensitive cTn assays [28,29]. Conventional older generation assays have yielded lower prevalence of cTn elevation in several prospective studies (34.5-80%) [30,31]. hsTropT correlated with serum creatinine ($\rho=0.46$, $p=0.007$) similar to the observation in the EFFECT study [30]. An important observation was the significant difference in hsTropT levels in ischemic versus non-ischemic heart failure (0.47±0.68 vs 0.14±0.47 ng/ml, $p<0.05$). Data on the differing incidence of cTn elevation in ischemic vs nonischemic HF are conflicting [9,29,32,33]. While some studies[9,29] including the ASCEND-HF [29] did not find significant difference, other studies [32,33] have found a significant increase in the Tn levels in patients with ischemic HF which was shown to be related to increased mortality in the ischemic group [33]. It has been speculated that increased cTn in the ischemic group reflects a higher degree of myocyte injury in HF [33]. Pathophysiological factors thought to be responsible for ongoing

myocyte injury include excessive adrenergic stimulation through renin, angiotensin, aldosterone or endothelin signalling pathways, abnormalities in calcium handling, inflammatory cytokines, nitric oxide and oxidative and mechanical stress [3,30,34]. Transient increase in cell membrane permeability secondary to myocardial strain or subendocardial ischemia can cause leakage of cytosolic troponin [1,35]. However, most of the cTn in cells is not free (5-8% of TnT and 3.5% of TnI is the free fraction in cell)[27,36] but bound to myofibrils, suggesting a more severe injury in AHF. Apoptosis has been suggested as another mechanism of cTn release [1,35,37]. Troponin has been shown to be independent predictor of in-hospital mortality and adverse outcomes on follow-up in several prospective studies [9,28,30]. Both positive cTn at baseline and conversion to positive levels were associated with worse outcomes at 60d in an analysis from the PROTECT Pilot Study [37]. cTn was found to predict both short term and one year mortality in the BASEL V study[38]. However, not all studies are consistent with these results. In the ASCEND-HF biomarker substudy, elevated cTn was associated with worsened in-hospital outcomes like length of stay and worsening HF, but was not associated with worsened postdischarge outcomes at 30 or 180 days [29].

hsCRP is a hepatocyte-derived inflammatory cytokine shown to be increased in the setting of AHF, with high plasma hsCRP levels portending poor long-term outcomes (ADEPT Neurohormonal substudy) [39]. hsCRP correlated with Troponin ($p=0.34$, $p=0.01$) and UA ($p=0.25$, $p=0.07$) suggesting a link between inflammation, myocyte injury and increased oxidative stress in this cohort of AHF patients.

An elevated UA (>5.6 mg/dl for females and >7 mg/dl for males) was found in 33 out of 52 patients (63.5%) with a mean value of 8.1 ± 3.3 mg/dl. A significant inverse relation was noted between UA and LV EF (Spearman $\rho = -0.29$, $p=0.03$) suggesting a more prevalent state of oxidative stress in patients with more severe LV systolic dysfunction. UA correlated with admission NT-proBNP ($p = 0.34$, $p=0.01$) and hsTropT ($p = 0.27$, $p=0.05$) and creatinine ($p=0.48$, $p=0.0003$). UA is a marker of impaired oxidative metabolism and increased oxidative stress [40]. Activation of xanthine oxidase by tissue hypoxia in HF has been proposed as the mechanism of elevated UA in HF [40] although increased oxidative stress can result from an imbalance between reactive oxygen species and endogenous oxidative defence mechanisms [4]. A higher value was also observed in patients with adverse outcomes (8.8 ± 3.9 vs 7.8 ± 2.8 mg/dl) although this was not statistically significant ($p=0.29$).

Hyponatremia is the most common electrolyte abnormality in HF. In an analysis from OPTIMIZE-HF registry, Gheorghiadu et al analysed the predictive value of hyponatremia [41]. Both in-hospital and follow-up mortality was increased in patients with lower sodium values. In the ESCAPE study [42], hyponatremia was shown to be an independent predictor of mortality. Hyponatremia was seen in more than half (55.8%) of our patients. A trend towards lower sodium levels was noted in both the groups with rising NT-proBNP levels and increased adverse events.

CONCLUSIONS

We have prospectively evaluated the prognostic significance of four serum biomarkers in this small cohort of acute heart failure patients. Although hsTropT, hsCRP and UA were elevated in the vast majority of patients, only NT-proBNP predicted adverse outcomes on follow-up. Several important relations of biomarkers with various outcomes were derived in this study. For instance, the significant excess increase in troponin values in patients with ischemic heart failure, the correlations between hsCRP, hsTropT and UA suggesting a link between inflammation, myocyte injury and oxidative stress, as well as the linear relation between all the biomarkers and worsening renal function. NT-proBNP levels were inversely related to BMI and

LV function. A change in the admission NT-proBNP level during the course of treatment was significantly associated with adverse outcomes. Even a modest increase of 5% above the baseline value was associated with increase in adverse outcomes; conversely, a fall in NT-proBNP value of nearly 37% was seen in patients who did not experience adverse outcomes on follow-up. We, therefore suggest a serial measurement of NT-proBNP, with at least one sample 48h after admission in patients admitted with AHF. It is hoped that more intensive and frequent follow-up of these patients would translate into early referral for rehospitalisation and reduced mortality. Large scale prospective studies to test a strategy of 'BNP-guided therapy' are warranted. Likewise, more research is needed to understand the link between inflammation, myocyte injury and oxidative stress in AHF which could provide therapeutic targets in treating this group of patients with an often dismal prognosis.

REFERENCES

- [1] Robb D. Kociol, Peter S. Pang, Mihai Gheorghiadu, Gregg C. Fonarow, Christopher M. O'Connor, G. Michael Felker. Troponin Elevation in Heart Failure: Prevalence, Mechanisms, and Clinical Implications. *J Am Coll Cardiol.* 2010;56:1071-78.
- [2] Alan S. Maisel, William F. Peacock, N. McMullin, Robert Jessie, Gregg C. Fonarow, Janet Wynne, et al. Timing of Immunoreactive B-Type Natriuretic Peptide Levels and Treatment Delay in Acute Decompensated Heart Failure: An ADHERE (Acute Decompensated Heart Failure National Registry) Analysis. *J Am Coll Cardiol.* 2008;52:534-40.
- [3] Alan S Maisel, Padma Krishnaswamy, Richard M Nowak, James McCord, Judd E Hollander, Philippe Duc, et al. Rapid measurement of B-type natriuretic peptide in the emergency diagnosis of heart failure (BNP Multinational Study). *N Engl J Med* 2002;347:161-67.
- [4] Eugene Braunwald. Biomarkers in Heart Failure. *N Engl J Med.* 2008;358:2148-59.
- [5] James L. Januzzi, Carlos A. Camargo, Saif Anwaruddin, Aaron L. Baggish, Annabel A. Chen, Daniel G. Krauser, et al. The N-Terminal Pro-BNP Investigation of Dyspnea in the Emergency Department (PRIDE) Study. *Am J Cardiol.* 2005; 95:948-54.
- [6] T.A. McDonagh, S. Holmer, I. Raymond, A. Luchner, P. Hildebrandt, H.J. Dargie. NT-proBNP and the diagnosis of heart failure: a pooled analysis of three European epidemiological studies. *Eur J Heart Fail.* 2004; 6:269-73.
- [7] Henry J. Michtalik, Hsin-Chieh Yeh, Catherine Y. Campbell, Nowreen Haq, Haeseong Park, William Clarke, et al. Acute Changes in N-Terminal Pro-B-Type Natriuretic Peptide During Hospitalization and Risk of Readmission and Mortality in Patients With Heart Failure. *Am J Cardiol.* 2011;107:1191-95.
- [8] Domingo A. Pascual-Figal, Teresa Casas, Jordi Ordóñez-Llanos, Sergio Manzano-Fernández, Juan C. Bonaque, Miguel Boronat, et al. Highly sensitive troponin T for risk stratification of acutely destabilized heart failure. *Am Heart J.* 2012;163:1002-10.
- [9] W. Frank Peacock IV, Teresa De Marco, Gregg C. Fonarow, Deborah Diercks, Janet Wynne, Fred S. Apple, et al (ADHERE Investigators). Cardiac Troponin and Outcome in Acute Heart Failure. *N Engl J Med.* 2008;358:2117-26.
- [10] Markku S. Nieminen, Michael Böhm, Martin R. Cowie, Helmut Drexler, Gerasimos S. Filippatos, Guillaume Jondeau, et al. Executive summary of the guidelines on the diagnosis and treatment of acute heart failure: The Task Force on Acute Heart Failure of the European Society of Cardiology. *Eur Heart J.* 2005; 26: 384-416.
- [11] Scott M. Silvers, John M. Howell, Joshua M. Kosowsky, Ivan C. Rokos, Andy S. Jagoda. Critical Issues in the Evaluation and Management of Adult Patients Presenting to the Emergency Department with Acute Heart Failure Syndromes. *Ann Emerg Med.* 2007;49:627-69.
- [12] Sharon Ann Hunt, William T. Abraham, Marshall H. Chin, Arthur M. Feldman, Gary S. Francis, Theodore G. Ganiats, et al. 2009 Focused Update Incorporated Into the ACC/AHA 2005 Guidelines for the Diagnosis and Management of Heart Failure in Adults: A Report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *Circulation.* 2009;119:e391-479.
- [13] John J.V. McMurray, Stamatis Adamopoulos, Stefan D. Anker, Angelo Auricchio, Michael Böhm, Kenneth Dickstein, et al. ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure 2012. *Eur Heart J.* 2012; 33:1787-847.
- [14] James L. Januzzi, Roland van Kimmenade, John Lainchbury, Antoni Bayes-Genis, Jordi Ordóñez-Llanos, Miguel Santalo-Bel, et al. NT-proBNP testing for diagnosis and short-term prognosis in acute destabilized heart failure: an international pooled analysis of 1256 patients: The International Collaborative of NT-proBNP (ICON) Study. *Eur Heart J.* 2006; 27: 330-37.
- [15] Roberto M. Lang, Michelle Bierig, Richard B. Devereux, Frank A. Flachskampf, Elyse Foster, Patricia A. Pellikka, et al. Recommendations for Chamber Quantification: A Report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, Developed in Conjunction with the European Association of Echocardiography, a Branch of the European Society of Cardiology. *J Am Soc Echocardiogr.* 2005;18:1440-63.

- [16] Sherif F. Nagueh, Christopher P. Appleton, Thierry C. Gillebert, Paolo N. Marino, Jae K. Oh, Otto A. Smiseth, et al. Recommendations for the Evaluation of Left Ventricular Diastolic Function by Echocardiography. *J Am Soc Echocardiogr.* 2009; 22 (2): 107-33
- [17] Han-Na Kim, James L. Januzzi. Natriuretic Peptide Testing in Heart Failure. *Circulation.* 2011;123:2015-19.
- [18] Richard W. Troughton, A. Mark Richards. B-Type Natriuretic Peptides and Echocardiographic Measures of Cardiac Structure and Function. *J Am Coll Cardiol Img.* 2009;2:216–25.
- [19] James L. Januzzi, Alan S. Maisel, Marc Silver, Yang Xue, Christopher DeFilippi. Natriuretic Peptide Testing for Predicting Adverse Events Following Heart Failure Hospitalization. *Congest Heart Fail.* 2012;18(5 Suppl. 1):S9–S13.
- [20] Christian Mueller, Andre Scholer, Kirsten Laule-Kilian, Benedict Martina, Christian Schindler, Peter Buser, et al. Use of B-Type Natriuretic Peptide in the Evaluation and Management of Acute Dyspnea. *N Engl J Med.* 2004; 350:647-54.
- [21] Van Cheng, Radmila Kazanagra, Alex Garcia, Leslie Lenert, Padma Krishnaswamy, Nancy Gardetto, et al. A Rapid Bedside Test for B-Type Peptide Predicts Treatment Outcomes in Patients Admitted for Decompensated Heart Failure: A Pilot Study. *J Am Coll Cardiol.* 2001; 37:386–91.
- [22] Alan Maisel, Christian Mueller, Kirkwood Adams, Stefan D. Anker, Nadia Aspromonte, John G.F. Cleland, et al. State of the art: *Using natriuretic peptide levels in clinical practice European Journal of Heart Failure.* 2008; 10:824–39.
- [23] Salvatore Di Somma, Laura Magrini, Marinella Mazzone, Raffaella De Leva, Fabio Tabacco, Rossella Marino, et al. Decrease in NTproBNP plasma levels indicates clinical improvement of acute decompensated heart failure. *Am J Emerg Med.* 2007;25(3):335–39.
- [24] T Goto, H Takase, T Toriyama, T Sugiura, K Sato, R Ueda, et al. Circulating concentrations of cardiac proteins indicate the severity of congestive heart failure. *Heart.* 2003;89:1303–07.
- [25] Lindenfeld J, Albert NM, Boehmer JP, Collins SP, Ezekowitz JA, Givertz MM, et al. Executive Summary: HFSA 2010 Comprehensive Heart Failure Practice Guideline. *J Card Fail.* 2010;16:475-539.
- [26] James McCord, Brian J. Mundy, Michael P. Hudson, Alan S. Maisel, Judd E. Hollander, William T. Abraham, et al. Sandberg, Peter A. McCullough. Relationship between Obesity and B-Type Natriuretic Peptide Levels. *Arch Intern Med.* 2004;164:2247-52.
- [27] Kalkidan Bishu, Anita Deswal, Horng H. Chen, Martin M. LeWinter, Gregory D. Lewis, Marc J. Semigran, et al. Biomarkers in acutely decompensated heart failure with preserved or reduced ejection fraction. *Am Heart J.* 2012; 164:763-70.e3.
- [28] Harvey D. White. Pathobiology of Troponin Elevations: Do Elevations Occur With Myocardial Ischemia as Well as Necrosis? *J Am Coll Cardiol.* 2011; 57(24):2406–08.
- [29] G. Michael Felker, Vic Hasselblad, W.H. Wilson Tang, Adrian F. Hernandez, Paul W. Armstrong, Gregg C. Fonarow, et al. Troponin I in acute decompensated heart failure: *insights from the ASCEND-HF study Eur J Heart Fail.* 2012;14 (11):1257-64.
- [30] John J. You, Peter C. Austin, David A. Alter, Dennis T. Ko, Jack V. Tu. Relation between cardiac troponin I and mortality in acute decompensated heart failure. *Am Heart J.* 2007; 153:462-70.
- [31] Junnichi Ishii, Masanori Nomura, Yuu Nakamura, Hiroyuki Naruse, Yoshihisa Mori, Takashi Ishikawa, et al. Risk Stratification Using a Combination of Cardiac Troponin T and Brain Natriuretic Peptide in Patients Hospitalized for Worsening Chronic Heart Failure. *Am J Cardiol.* 2002; 89:691–95.
- [32] Eduardo R. Pernaa, Stella M. Macin, Juan P. Cimbaro Canellaa, Pablo M. Alvarengaa, Nelson G. Rios, Rolando Pantich, et al. Minor myocardial damage detected by troponin T is a powerful predictor of long-term prognosis in patients with acute decompensated heart failure. *Int J Cardiol.* 2005;99:253– 61.
- [33] Wayne L. Miller, Karen A. Hartman, Mary F. Burritt, John C. Burnett, Allan S. Jaffe. Troponin, B-Type Natriuretic Peptides and Outcomes in Severe Heart Failure: Differences Between Ischemic and Dilated Cardiomyopathies. *Clin Cardiol.* 2007; 30:245–50.
- [34] Damien Logeart, Pascale Beyne, Christine Cusson, Maria Tokmakova, Monique Leban, Chabnam Guiti, et al. Evidence of cardiac myolysis in severe nonischemic heart failure and the potential role of increased wall strain. *Am Heart J.* 2001;141:247-53.
- [35] Thomas J. Wang. Significance of Circulating Troponins in Heart Failure: If These Walls Could Talk. *Circulation.* 2007; 116:1217-20.
- [36] Tuomo Ilva, Johan Lassus, Krista Siirila-Waris, John Melin, Keijo Peuhkurinen, Kari Pulkki, et al. Clinical significance of cardiac troponins I and T in acute heart failure. *Eur J Heart Fail.* 2008;10:772–79.
- [37] Christopher M. O'Connor, Mona Fiuzat, Carlo Lombardi, Kenji Fujita, Gang Jia, Beth A. Davison, et al. Impact of Serial Troponin Release on Outcomes in Patients With Acute Heart Failure Analysis From the PROTECT Pilot Study. *Circ Heart Fail.* 2011;4:724-32.
- [38] N. Arenja, T. Reichlin, B. Drexler, S.Oshima, K. Denhaerynck, P. Haaf, et al. *Sensitive cardiac troponin in the diagnosis and risk stratification of acute heart failure J Intern Med.* 2012;271:598–607.
- [39] W.H. Wilson Tang, Kevin Shrestha, Frederick Van Lente, Richard W. Troughton, Maureen G. Martin, Allen G. Borowski, et al. Usefulness of C-Reactive Protein and Left Ventricular Diastolic Performance for Prognosis in Patients With Left Ventricular Systolic Heart Failure. *Am J Cardiol.* 2008;101:370–73.
- [40] Hyoung-Seob Park, Hyungseop Kim, Ji-Hyun Sohn, Hong-Won Shin, Yun-Kyeong Cho, Hyuck-Jun Yoon, et al. Combination of Uric Acid and NT-ProBNP: A More Useful Prognostic Marker for Short-Term Clinical Outcomes in Patients with Acute Heart Failure. *Korean J Intern Med.* 2010;25:253-59.
- [41] Mihai Gheorghide, William T. Abraham, Nancy M. Albert, Wendy Gattis Stough, Barry H. Greenberg, Christopher M. O'Connor, et al (OPTIMIZE-HF Investigators and Coordinators). Relationship between admission serum sodium concentration and clinical outcomes in patients hospitalized for heart failure: an analysis from the OPTIMIZE-HF registry. *Eur Heart J.* 2007;28: 980–8
- [42] Mihai Gheorghide, Joseph S. Rossi, William Cotts, David D. Shin, Anne S. Hellkamp, Ileana L. Pina, et al. Characterization and Prognostic Value of Persistent Hyponatremia in Patients With Severe Heart Failure in the ESCAPE Trial. *Arch Intern Med.* 2007;167(18):1998-2005.

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