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"VALOR PRONOSTICO DE NUEVOS BIOMARCADORES EN INSUFICIENCIA CARDIACA AGUDA"

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El contenido de la presente tesis constituye un compendio de trabajos previamente publicados o aceptados para publicación:

1. Complementary prognostic value of Cystatin C, NT-proBNP and cardiac Troponin T in acute heart failure patients. American Journal of Cardiology; 2009 Jun 15; 103 (12): 1753-9. S. Manzano-Fernández, M. Boronat-García, M.D Albaladejo-Otón, Patricia Pastor, Francisco J. Pastor-Pérez, I.P. Garrido, Pedro Martínez-Hernández, M. Valdés-Chavarri, D.A. Pascual-Figal.

2. β-trace Protein and Cystatin C as Predictors of Long Term Outcomes in Patients with Acute Heart Failure. Journal of the American College of Cardiology 2011. Sergio Manzano-Fernández, James L. Januzzi, Miguel Boronat-Garcia, Juan Carlos Bonaque-González, Quynh A. Truong, Francisco J. Pastor-Pérez, Carmen Muñoz-Esparza, Patricia Pastor,María D. Albaladejo-Otón, Teresa Casas, Mariano Valdés, and Domingo A. Pascual-Figal.

3. Usefulness of Soluble Concentrations of the Interleukin Family Member ST2 as Predictor of Mortality in Patients with Acutely Decompensated Heart Failure Relative to Left Ventricular Ejection Fraction. American Journal of Cardiology 2011. Sergio Manzano-Fernández, MD, Thomas Mueller, MD, Domingo Pascual-Figal, MD, PhD, Quynh A. Truong, MD and James Louis Januzzi, MD.

A mi familia y amigos.

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Complementary Prognostic Value of Cystatin C, N-terminal pro-B-type Natriuretic Peptide and Cardiac Troponin T in Patients with Acute Heart Failure

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1.2 Abstract

We sought to compare the prognostic value of cystatin C over creatinine and Modified of Diet and Renal Disease (MDRD) equation, and to evaluate whether it provides complementary information to cardiac biomarkers in the risk stratification of an unselected cohort of patients with acute heart failure (AHF). We prospectively studied consecutive hospitalized patients with an established AHF diagnosis. Blood samples were collected on hospital arrival to determine cystatin C, cTnT and NT-proBNP. Clinical follow-up was obtained and the occurrence of mortality and/or heart failure (HF) readmission was registered. One-hundred thirty-eight patients (74 [67-80] years, 54% male) were studied. During a median follow up of 261 [161-449] days, 60 patients (43.5%) presented adverse events. After multivariable adjustment cystatin C, NT-proBNP, cTnT, New York Heart Association Functional Classification class III/IV and diabetes mellitus were identified as independent predictors of mortality and/or HF readmission. In contrast to creatinine and MDRD, the highest cystatin C tertile (>1.50 mg/L) was a significant independent risk factor for adverse events (Hazard Ratio (HR) 3.08 95%CI 1.54-6.14;p=0.004). A multimarker approach combining cTnT, NT-proBNP and cystatin C improved risk stratification further, showing that patients with two (HR 2.37, 95%CI 1.10-5.71) or three (HR 3.64, 95%CI 1.55-8.56) elevated biomarkers had a higher risk of adverse events than patients with no elevated biomarkers (p for trend=0.015). In this unselected cohort, cystatin C was a stronger predictor of adverse events than conventional measures of kidney function. In addition, cystatin C offered complementary prognostic information to cardiac biomarkers and could help clinicians to perform a more accurate risk stratification of patients with AHF.

Key words: prognosis, cystatin C, heart failure

1.3 Introduction

Since patients with acute heart failure (AHF) represent a group at high risk for cardiovascular events and death, risk stratification may be particularly useful in this population[1]. N-terminal pro-B-type natriuretic peptide (NT-proBNP) and cardiac troponins (cTn) have emerged as consistent prognostic biomarkers in heart failure (HF)[2-7]. Several recent multimarker approaches combining NT pro-BNP and cTn, have been reported to improve the risk stratification of patients with AHF[8, 9]. Nevertheless, to the best of our knowledge, the prognostic value of the simultaneous assessment of cystatin C, NT-proBNP and cTnT has not been previously evaluated in this important clinical scenario. Moreover, it has not been clarified whether cystatin C is more useful than creatinine and Modified of Diet and Renal Disease equation (MDRD) in the risk stratification of hospitalized patients with AHF. We designed a prospective study to compare the prognostic value of cystatin C over conventional measures of kidney function, and to evaluate whether it provides complementary information to cTnT and NT-proBNP in the risk stratification of an unselected cohort of patients with AHF.

1.4 Methods

Between September 2006 and February 2008, we prospectively included a total of 145 consecutive patients admitted with an *initial diagnosis* of AHF to the Cardiology Department at the Virgen de la Arrixaca University Hospital. Blood samples were collected for all patients on arrival at the emergency department. After hospital admission, an established *final diagnosis* of AHF was made in 138 of these cases, which represented the population included in this study analysis. The 7 remaining patients had other established diagnoses. AHF diagnosis was established on the basis of current criteria guidelines and defined as the rapid or gradual onset of signs and symptoms of HF, resulting in unplanned hospitalization, and included new onset AHF and acute decompensation of chronic HF[1].

The following baseline clinical characteristics were prospectively recorded in detail on admission: age, sex, hypertension, diabetes mellitus, smoking, hypercholesterolemia, anemia, chronic obstructive pulmonary disease, previous HF, previous stroke, New York Heart Association Functional Classification, etiology of cardiomyopathy, heart rhythm, branch block, need for inotropic support and medication at discharge. Hypertension was defined as elevated systolic blood pressure of >140 mm Hg, diastolic blood pressure >90mm Hg, or when patients had taken antihypertensive drugs. Hyperlipidemia was defined as augmented total cholesterol level of >220 mg/dL, triglyceride >150 mg/dL, decreased high-density lipoprotein level of <40 mg/dL, or when patients had taken drugs of antihyperlipidemia. Anemia was defined using the World Health Organization definition (Hb<12.0 g/dl in women and <13.0 g/dl in men)[10]. During the entire hospitalization period, clinical management decisions about each patient were decided by the cardiologist responsible, who was unaware of the patient's cystatin C levels. An echocardiogram was also performed on all patients before hospital discharge, using a Sonos 5500 (Philips, Andover, Massachusetts, USA). The left ventricular ejection fraction (LVEF) was measured using the Simpson biplane method. All patients received standard management as recommended by contemporary guidelines[1].

Determination of cystatin C was performed using a BN ProSpec analyzer (Dade Behring). The intra-assay coefficient of variation was 2.5% for 0.9 mg/L and 2.3% for 1.8 mg/L. The inter-assay coefficient of variation was 2.0% for 0.9 mg/L and 2.2% for 1.8 mg/L. Kidney function was also assessed through serum creatinine levels and creatinine-based estimating equation. Estimated glomerular filtration rate was calculated using the simplified MDRD (mL/min/1.73 m^2, 186.3× [plasma creatinine]–1.154 × [age]–0.203) (the correction factor for women was ×0.742)[11]. NT-proBNP and cTnT levels were measured by electrochemiluminescence immunoassay using a Modular Analytics E170 analyser (Roche Diagnostics GmbH, Mannheim, Germany). The intra-assay coefficient of variation for NT-proBNP was 1.8% for 221 pg/mL and

3.1% for 4.250 pg/mL. Regarding cTnT, the detection limit provided by the supplier was <0.01 ng/mL. The intra-assay coefficient of variation was 1.2% for 0.141 ng/mL and 0.8% for 3.00 ng/mL. All patients were clinically followed up for at least 6 months (median 261 days [IQR 161-449]) and the occurrence of clinical events was registered. In patients requiring hospitalization, medical records were carefully reviewed to further characterize the cause of hospitalization. The study endpoint was defined as the combination of mortality and/or HF readmission. The study was approved by the Local Ethics Committee, and informed consent was obtained from each patient at inclusion.

Continuous variables were tested for a normal distribution by the Kolmogorov-Smirnov test. Normally distributed data are presented as the mean ± standard deviation and non-normally distributed data as the median (IQR, interquartile data). Categorical variables are expressed as percentages. Since age-, sex-, and race-specific normal ranges for serum cystatin C have not been established, we categorized cystatin C into tertile groups. Patients were grouped according to cystatin C tertiles. Differences in baseline characteristics were compared using ANOVA or the Kruskal-Wallis test for continuous variables and the Chi-Square test for categorical variables. We calculated hazard ratios (HR) derived from the Cox regression analysis to identify predictors of mortality and/or HF readmission during follow-up. The independent effect of variables on prognosis was calculated using a Cox multivariable regression analysis, incorporating covariates with p values <0.10 in the univariable analysis. Cystatin C, creatinine, and MDRD were first entered individually in the multivariable model. Subsequently, creatinine and MDRD were adjusted for cystatin C in a separate multivariable analysis. To compare measures of kidney function as predictors of adverse clinical outcomes cystatin C, creatinine and MDRD were divided by tertiles. Creatinine tertiles were gender-specific to ensure an adequate representation of men and women within each. We determined the unadjusted and multivariable-adjusted risk for the 3rd and 2nd tertiles compared with the 1st tertile for each measure of kidney function. To test the hypothesis that simultaneous assessment of cystatin C, cTnT and

NT-proBNP would improve risk stratification, patients were categorized on the basis of the number of *"elevated biomarkers".* Cystatin C, cTnT and NT-proBNP were defined as elevated if their levels were above the median (>1.21mg/L for cystatin C, >0.011ng/ml for cTnT and >3.345pg/ml for NT-proBNP). The cumulative incidence of mortality or HF readmission was estimated according to the Kaplan–Meier method and the log-rank statistic was used for comparisons. All p values <0.05 were accepted as statistically significant. Statistical analysis was performed using SPSS 15.0 for Windows (SPSS, Inc., Chicago, IL, USA).

1.5 Results

A total of 138 patients were included in the study analysis. The median cystatin C concentration was 1.21 [0.97-1.67] mg/L, median serum creatinine was 1.15 [0.87-1.45] mg/dl and mean estimated MDRD was 63±25 ml/min per 1.73m^2. The distribution of baseline characteristics and laboratory parameters by cystatin C tertiles are shown in Tables 1 and 2. Patients with higher cystatin C levels were older, had higher prevalence of anemia and needed more frequent in-hospital inotropic support. Serum creatinine, urea nitrogen, uric acid and NT-proBNP were higher among patients with higher cystatin C levels; whereas serum albumin, hemoglobin and MDRD were lower among these patients. Serum cystatin C concentrations were significantly correlated with serum creatinine and estimated glomerular filtration rate [Spearman rank correlation r=0.77 and -0.67 (p<0.001), respectively].

Over the study period, a total of 60 patients (43.5%) presented adverse clinical events: 27 patients died and 40 patients were readmitted to hospital owing to HF decompensation. In the univariable Cox regression analysis, all measures of kidney function were associated with a higher risk of adverse clinical events (Table 3). After adjusting for other univariable predictors in the multivariable Cox regression models, cystatin C levels (mg/L, HR 1.46 95%CI 1.02-2.09; p=0.037) remained as a significant predictor of adverse events, while serum creatinine and MDRD were no longer

significant. When cystatin C, creatinine and MDRD were combined in a single adjusted model, cystatin-C was also the only measure of kidney function that remained as an independent predictor of adverse clinical events (mg/dl, HR 1.46 95%CI 1.02-2.09; p=0.037). Other independent predictors of mortality and HF readmission were New York Heart Association Functional Classification class III/IV, diabetes mellitus, NT-proBNP and cTnT (Table 3). As shown in the Kaplan-Meier survival analysis (Figure 1), cystatin C tertiles were associated with an incremental rate of adverse clinical events. Mortality and/or HF readmission at 1 year was 65.0% for 3rd cystatin C tertile, 44.1% for 2nd cystatin C tertile and 30.1% for 1st cystatin C tertile (log rank test p=0.001). In the univariable Cox regression analysis and after multivariable adjustment, the highest tertile of cystatin C (>1.50 mg/L) was significantly associated with a higher risk of mortality and/or HF readmission (HR 3.08 95%CI 1.54-6.14; p=0.004). In contrast, the highest tertile of creatinine and MDRD only achieved significance in the univariable analysis (Table 4).

In a multivariable regression analysis incorporating all 3 biomarkers [*elevated* Cystatin C (>1.21mg/L; HR 1.92, 95%IC 1.12-3.28; p=0.017), NT-proBNP (>3.345pg/ml; HR 1.79, 95%IC 1.06-3.03; p=0.029) and cTnT (>0.011ng/ml; HR 2.01, 95%IC 1.24-3.41; p=0.003)] each was an independent predictor of mortality and/or HF readmission. By categorizing patients on the basis of the number of elevated biomarkers we found that 23% had elevations in none of the biomarkers, 25% had an elevation in one, 28% had elevations in 2, and 24% had elevations in all 3. There was a significant gradual increase in risk of mortality and/or HF readmission as the number of elevated biomarkers increased (25.8%, 37.1%, 43.6% and 66.7% of patients with 0, 1, 2 and 3 elevated biomarkers respectively reached the study end point; p for trend=0.015). Relative risk of mortality and/or HF readmission, according to the number of the elevated study biomarkers are presented in Fig. 2.

1.6 Discussion

In this study, we noted a striking relationship between a high cystatin C level and the incidence of mortality and/or HF readmission in hospitalized patients with AHF. In this unselected cohort, we also found cystatin C to be a stronger predictor of adverse clinical events than creatinine and MDRD. Importantly, simultaneous assessment of cystatin C, cTnT and NT-proBNP provided complementary prognostic information and could enable clinicians to perform a more accurate risk stratification of this population.

In clinical practice, serum creatinine levels and creatinine based estimating equations are routinely used for the evaluation of kidney function. However, recent data has suggested that cystatin C has a stronger prognosis value than creatinine and MDRD in patients with HF. Shlipak et al[12], first reported cystatin C as a better predictor of mortality than creatinine in selected elderly ambulatory patients with chronic HF. Arimoto et al[13] suggested cystatin C may represent a more useful marker for detecting early kidney dysfunction and recommended discrimination between patients with and without cardiac events in a cohort of patients with mild to moderate HF. These authors showed that patients with elevated cystatin C had a higher cardiac event rate than those with normal cystatin C levels even in patients with normal serum creatinine. Our report mainly differs from these studies in its prospective design and targeted focus on hospitalized patients. Furthermore, it includes an unselected cohort of AHF patients who were treated more according to contemporary recommendations, with a higher rate of beta-blockers and ACE inhibitors/ARB prescription[1]. In a selected population with chronic systolic HF (LVEF≤35%), Tang et al.[14] have also recently demonstrated cystatin C was associated with poor long-term prognosis. Unlike to this recent study, we studied patients with AHF, including those with preserved LVEF and with a higher rate of co-morbidities, such as hypertension, diabetes mellitus and kidney dysfunction.

In the present cohort, we found high cystatin C levels were associated with a higher risk of mortality and/or HF readmission; whereas neither creatinine nor MDRD were significant predictors of adverse events after multivariable adjustment. The

highest cystatin C tertile (>1.50 mg/L) was associated with a 3-fold higher risk of adverse events than the lowest cystatin C tertile (<1.04 mg/L). Kaplan-Meier survival analysis showed widely diverging outcomes according to cystatin C tertiles throughout the follow-up period. Lassus et al[15], recently published similar results in a prospective, observational, multicentre study conducted in hospitalized patients with AHF. These authors showed that the prognostic value of cystatin C remained significant after adjustment for established biochemical predictors of adverse events such as creatinine, estimated glomerular filtration rate (Cockcroft–Gault), hemoglobin, sodium and NT-proBNP. According to Lassus et al[15], higher cystatin C levels were found in hospitalized patients with AHF than in ambulatory chronic HF patients[12-14].These findings probably reflect a more advanced kidney dysfunction in patients with AHF resulting in a poor prognosis in this population. In our opinion, since the presence of chronic kidney disease or impaired kidney function during hospitalization have been related to worse outcomes in patients with HF[16-18], the evaluation of kidney function should have a pivotal role in the risk stratification of these patients. Furthermore, we also suggest that cystatin C represents the more useful measure of kidney function for predicting adverse clinical events in this population group.

Lassus et al[15], suggested the use of simultaneous cystatin C and NT-proBNP assessment to improve the risk stratification of patients with AHF. Our results not only confirm these earlier findings but also expand on them given that we have also included cTnT in our multimarker risk stratification approach. In this study, we found cTnT level was an important independent predictor of adverse clinical events among hospitalized patients with AHF. Thus, we believe that cTnT should be taken into account in multimarker approaches for risk stratification of these patients. The prognostic value of NT-proBNP is well established, and our results were in accordance with it[5-7]. As expected in hospitalized patients with AHF, we found high NT-proBNP concentrations (median 3345 [1900-7205] ng/l) which could also be explained by the older age of the patients and a higher rate of kidney failure in this population. In the

present study, we found that each biomarker (cystatin C, NT-proBNP and cTnT) provided independent and complementary prognostic information. By categorizing patients by the number of elevated biomarkers, simultaneous assessment of these 3 biomarkers enabled a powerful prediction of risk of adverse clinical events. Even after adjustment for traditional clinical predictors of adverse events, the prognostic value of the multimarker approach remained significant and was more powerful than the single marker approach. Therefore, our results suggest that a multimarker strategy, with biomarkers combinations targeting various stages of HF development, may allow physicians to identify patients at high risk, who may benefit from closer follow-up and the intensification of proven therapies.

The limitations of our study are similar to those of any single centre prospective observational study. The small sample size and relatively small number of patients included in each group also makes it difficult to detect differences across the lower tertiles of cystatin C and to draw firm conclusions. It has been reported that factors other than kidney function, such as corticosteroid use, inflammation, and thyroid function, might influence cystatin C levels[19, 20]. However, other studies and meta-analyses[21, 22] have not identified such associations in our population. For the prognosis of outcomes, the number of covariates included in multivariable models was >1 for each 10 outcome events. Therefore, it remains possible that the models were over adjusted. A potential limitation to the multimarker approach is the loss of quantitative information. It should also be acknowledged that elevations in each biomarker may confer different relative risks for individual components of the study endpoint.

1.7.1 Table 1. Baseline clinical characteristics according to tertiles of cystatin C.

Variable	Tertiles 1st (n=48)	2nd (n=45)	3rd (n=45)	*p*
Age (years)	72 [56-78]	76 [68-78]	78 [71-84]	0.002
Men	24 (50%)	27 (60%)	23 (51%)	0.576
Body mass index, Kg/m^2	28 [25-33]	29 [26-32]	28 [26-31]	0.522
Systolic blood pressure (mmHg)	149±40	151±32	153±38	0.937
Diastolic blood pressure (mmHg)	86±21	86±20	82±19	0.828
Heart rate (beat/min)	112±30	100±35	101±23	0.371
LVEF	47 [37-60]	49 [30-63]	52 [33-65]	0.946
NYHA III-IV	11 (23%)	14 (31%)	19 (42%)	0.135
Chronic heart failure	26 (54%)	29 (64%)	31 (69%)	0.321
Coronary heart failure	16 (33%)	14 (31%)	18 (40%)	0.534
Diabetes mellitus	23 (48%)	26 (58%)	21 (47%)	0.511
Hypertension	37 (79%)	39 (87%)	38 (84%)	0.574
Hyperlipidemia	16 (33%)	17 (38%)	21 (47%)	0.410
Current smoking	11 (23%)	7 (16%)	5 (11%)	0.303
Atrial fibrillation/flutter	27 (59%)	33 (73%)	26 (58%)	0.228
Branch block	13 (27%)	16 (36%)	13 (29%)	0.650
Previous STEMI	10 (21%)	8 (18%)	18 (40%)	0.033
Previous stroke	7 (15%)	9 (20%)	6 (13%)	0.655
Anemia	15 (31%)	18 (40%)	31 (69%)	0.001
Chronic Obstructive Pulmonary Disease	3 (6%)	7 (16%)	8 (18%)	0.213
In-hospital inotropic use	3 (6%)	4 (9%)	12 (27%)	0.009
Treatment at discharge				
Beta-blockers	31 (66%)	26 (59%)	17 (44%)	0.107
ACE inhibitors/ARB	40 (84%)	41 (91%)	35 (85%)	0.951
Statins	25 (53%)	24 (54%)	22 (56%)	0.956
Spironolactone/Eplerenone	18 (38%)	18 (41%)	11 (28%)	0.451
Loop diuretics	43 (91%)	41 (93%)	38 (97%)	0.261
Follow up (days)	289 [211-542]	261 [160-452]	198 [175-393]	0.240

Data are expressed as mean±SD or median (quartiles), and number (%). LVEF denotes left ventricular ejection fraction, STEMI denotes ST segment elevation myocardial infarction, NYHA denotes New York Heart Association, ACE denotes angiotensin-converting enzyme and ARB denotes angiotensin-receptor blocker.

1.7.2 Table 2. Laboratory parameters according to tertiles of cystatin C.

Variable	Tertiles 1st (n=48)	2nd (n=45)	3rd (n=45)	*p*
Hemoglobin (g/dl)	13.4±2.0	12.8±1.9	11.3±1.8	<0.001
Glucose (mg/dl)	181±97	166±79	158±61	0.792
Cystatin (mg/L)	0.86 [0.77-0.97]	1.21 [1.10-1.39]	1.85 [1.65-2.44]	<0.001
Creatinine (mg/dl)	0.82 [0.77-1.00]	1.14 [0.94-1.29]	1.61 [1.29-2.08]	<0.001
eGFR (ml/min/1.73^2)	83.6±19.9	63.9±19.2	41.1±16.8	<0.001
Urea nitrogen (mg/dl)	38 [33-48]	51 [42-64]	79 [54-115]	<0.001
Albumin (g/dl)	4.2±0.4	4.1±0.5	3.9±0.4	0.006
Sodium (mEq/l)	139±4.5	139±6.8	138±5.4	0.754
Uric acid (mg/dl)	6.4±2.0	7.8±2.7	9.1±2.5	<0.001
C-reactive protein (mg/dl)	0.9 [0.4-2.1]	0.9 [0.5-5.0]	1.5 [0.7-4.3]	0.270
Troponin T (ng/ml)	0.01 [0.01-0.03]	0.01 [0.01-0.58]	0.02 [0.01-0.08]	0.094
NT-proBNP (pg/ml)	2358 [1359-3853]	3571 [1680-7597]	5255 [2968-14543]	<0.001

Data are expressed as mean±SD or median (quartiles), and number (%).eGFR denotes estimated glomerular filtration rate and NT-proBNP denotes amino-terminal pro-brain natriuretic peptide.

1.7.3 Table 3. Cox regression risk analysis for predictor of mortality and/or HF readmission.

	Univariable		Multivariable*	
Variable	Hazard Ratio	*P*	Hazard Ratio	*p*
Age (per year)	1.03 (1.01-1.05)	0.012	-	0.379
Hyperlipidemia	1.62 (0.97-2.69)	0.062	-	0.432
NYHA III-IV	3.17 (1.89-5.32)	<0.001	3.13 (1.85-5.29)	<0.001
Diabetes mellitus	1.78 (1.06-3.00)	0.030	1.81 (1.07-3.07)	0.028
Previous STEMI.	1.75 (1.01-3.05)	0.046	-	0.208
Anemia	1.69 (1.01-2.82)	0.046	-	0.757
Inotropic	2.07 (1.10-3.90)	0.024	-	0.460
NT-proBNP (per 100 pg/dl)	1.003 (1.001-1.006)	0.013	1.004 (1.001-1.007)	0.032
Troponin T (per ng/ml)	2.48 (1.38-4.45)	0.002	2.46 (1.33-4.54)	0.004
Cystatin C (per mg/L)	1.64 (1.19-2.27)	0.003	1.46 (1.02-2.09)	0.037
Creatinine (per mg/dl)	1.55 (1.07-2.24)	0.019	-	0.114
eGFR (per ml/min/1.73m^2)	0.96 (0.97-0.99)	0.011	-	0.104

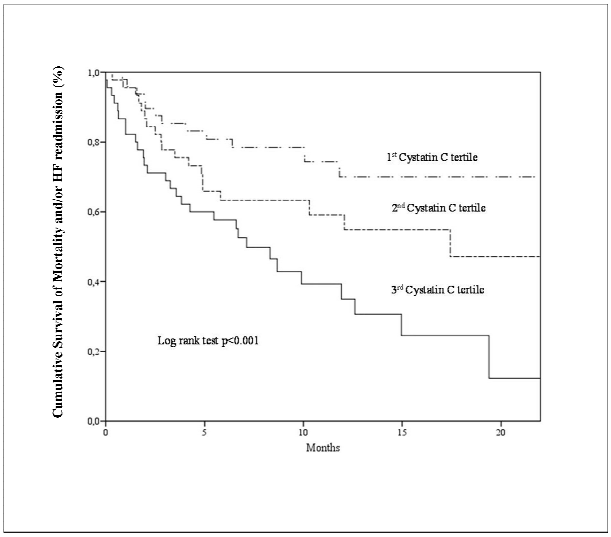
Data are expressed as mean±SD or median (quartiles), and number (%). *Cystatin C (mg/L), creatinine (mg/dl) and eGFR (ml/min/1.73m^2) were all tested separately and multivariable "HR" and "p" for other variables shown from the cystatin C model. Abbreviations as in Tables 1 and 2.

1.7.4 Table 4. Kidney function parameters and risk of mortality and/or HF readmission.

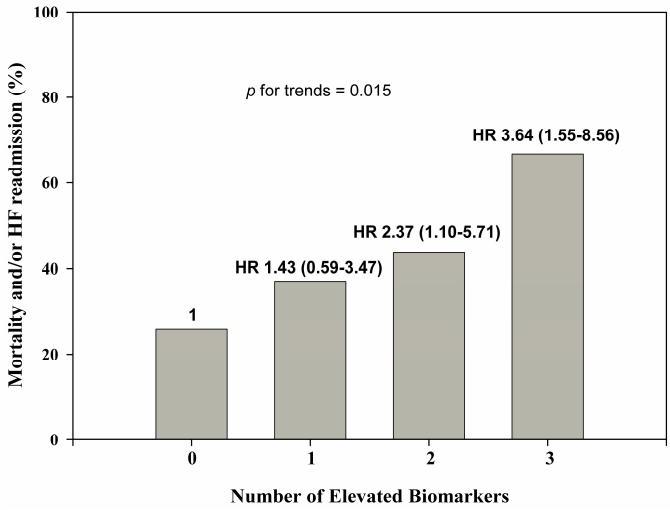
	Tertiles			
Variable	1st	2nd	3rd	*p*
Cystatin C (per mg/l)				
Range	0.63-1.04	1.04-1.50	1.50-3.97	
n	48	45	45	
Unadjusted Hazard Ratio	1	1.89 (0.91-3.88)	3.43 (1.75-6.75)	0.001
Adjusted Hazard Ratio *	1	1.61 (0.78-3.33)	3.08 (1.54-6.14)	0.004
Creatinine (per mg/dl)				
Range in women	0.52-0.82	0.82-1.20	1.20-2.48	
Range in men	0.60-1.13	1.13-1.41	1.41-3.69	
n	47	46	45	
Unadjusted Hazard Ratio	1	1.38 (0.69-2.74)	2.28 (1.19-4.37)	0.034
Adjusted Hazard Ratio *	1	1.62 (0.78-3.36)	1.76 (0.85-3.63)	0.280
eGFR (per ml/min/1.73^2)				
Range	17-48	48-71	72-142	
n	46	46	46	
Unadjusted Hazard Ratio	1	0.64 (0.36-1.14)	0.35 (0.18-0.69)	0.009
Adjusted Hazard Ratio *	1	0.86 (0.47-1.58)	0.52 (0.25-1.08)	0.207

*Adjusted for age, New York Heart Association Functional Classification, diabetes mellitus, hyperlipidemia, previous ST segment elevation myocardial infarction, anemia, in-hospital inotropic use, troponin T and NT-proBNP. Abbreviations as in Table 1 and 2.

1.8.1 Figure 1. Kaplan-Meier survival analysis of adverse clinical events related to cystatin C tertiles.



1.8.2 Figure 2. Adverse clinical events according to the number of elevated biomarkers. *Adjusted for age, New York Heart Association Functional Classification, diabetes mellitus, hyperlipidemia, previous ST segment elevation myocardial infarction, anemia and in-hospital inotropic use.



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β-trace Protein and Cystatin C as Predictors of Long Term Outcomes in Patients with Acute Heart Failure

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Short title: β-trace Protein and Cystatin C Acute Heart Failure

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2.2 Abstract

Objective: To evaluate the prognostic importance of novel markers of renal dysfunction among patients with acutely destabilized heart failure (ADHF). Background: β-trace protein (BTP) and cystatin C are newer biomarkers for renal dysfunction; the prognostic importance of these tests, particularly BTP, relative to standard measures of renal function remains unclear. Methods: Two hundred and twenty consecutive hospitalized patients with ADHF were prospectively studied. Blood samples were collected on presentation. In-hospital worsening renal function, as well as mortality and/or HF hospitalization over a median follow-up period of 500 days was examined as a function of BTP or cystatin C concentrations; results were compared to creatinine, estimated glomerular filtration rate (eGFR) and blood urea nitrogen (BUN). Results: Neither BTP nor cystatin C were associated with worsening renal function during index hospitalization. 116 subjects (53%) suffered either death/HF hospitalization during follow up. Those with adverse outcomes had higher BTP (1.04 [0.80-1.49] vs 0.88 mg/L [0.68-1.17], p=0.003) and cystatin C (1.29 [1.00-1.71] vs 1.03 mg/L [0.86-1.43], p=0.001). After multivariable adjustment, both BTP (HR 1.41 95%CI 1.06-1.88; p=0.018) and cystatin C (HR 1.50 95%CI 1.13-2.01; p=0.006) were significant predictors of death/HF hospitalization, whereas serum creatinine, eGFR, and BUN were no longer significant. In patients with eGFR>60mL/min, elevated concentrations of BTP and cystatin C were still associated with significantly higher risk of adverse clinical events ($p < 0.05$). Net reclassification index analysis suggested cystatin C and BTP to deliver comparable information regarding prognosis. Conclusion: Among patients hospitalized with ADHF, BTP and cystatin C predict risk for death and/or HF hospitalization, and are superior to standard measures of renal function for this indication.

Key words: β-trace Protein, cystatin C, prognosis and acute heart failure.

2.3 Introduction

Kidney dysfunction is an exceptionally important adverse prognostic factor in patients with acutely destabilized heart failure (ADHF) (1). Accordingly, the identification of laboratory parameters capable of more accurately assesses renal function than conventional measures of renal function (e.g. creatinine, estimated glomerular filtration rate [eGFR] or blood urea nitrogen [BUN]) may be particularly relevant for this population. Although prognostically meaningful, conventional measures of renal function have limitations: creatinine and BUN levels are affected by several non-renal factors including age, body weight, nutritional status and sex; as well, creatinine, eGFR, and BUN are generally insensitive for detecting mild chronic kidney disease (2-4).

Cystatin C, a 13 kDa cysteine protease inhibitor, has recently emerged as a novel marker of renal function with a high prognostic value in cardiovascular disease, including ADHF (5-13). β-trace protein (BTP) is a low molecular mass protein belonging to the lipocalin protein family that has been established as an accurate marker of cerebrospinal fluid leakage (14). Furthermore, it has been also recently described as a more sensitive marker than serum creatinine in detecting impaired renal function, with comparable performance to cystatin C (15-17). The prognostic role of plasma BTP in hospitalized patients with ADHF has not been previously studied and its comparative value to cystatin C or conventional measures of renal function in these patients is not known. Therefore, in this prospective study of hospitalized patients with ADHF, we aimed to assess the prognostic value of BTP and cystatin C, relative to more conventional measures of renal function.

2.4 Methods

Study population and design.

From September 2006 to March 2009, we prospectively enrolled 220 consecutive patients admitted with established final diagnoses of ADHF (diagnosed clinically using current guidelines (1)) to the Department of Cardiology at Virgen de la Arrixaca University Hospital (Murcia, Spain). Blood samples were collected for all patients on arrival at the emergency department. Baseline clinical characteristics and hospital events were prospectively recorded. Echocardiography was also performed on all patients before hospital discharge. Left ventricular ejection fraction was measured using Simpson's biplane method. All patients received standard HF management as recommended by contemporary guidelines (1). During the entire hospitalization period, clinical management decisions about each patient were decided by the cardiologist responsible, who was unaware of the patient's BTP and cystatin C concentrations.

The determination of both BTP and cystatin C were performed using a BN ProSpec analyzer (Dade Behring GmbH, Liederbach, Germany). The intra-assay and inter-assay coefficients of variation for BTP were 2.8% and 4.7% respectively. The intra-assay and interassay coefficients of variation for cystatin C were 2.5% and 2.0% respectively. Conventional measures of renal function included serum creatinine, eGFR (calculated using the simplified Modification of Diet in Renal Disease equation: 186.3 × plasma creatinine − 1.154 × age − 0.203; the correction factor for women was 0.742) (18) and BUN.

Follow up and clinical endpoint.

We examined worsening renal function (defined as a maximum rise in serum creatinine during hospitalization of ≥0.3 mg/dL (19)) as a function of BTP or cystatin C concentrations; results were compared to creatinine, eGFR and BUN. For the primary outcome measure as the combination of mortality and/or HF hospitalization, all patients were clinically followed during a median of 500 days (interquartile range 231 to 796). Death was ascertained from available medical records and death certificates. If hospital records were ambiguous or non-available National

Death Records were consulted. In patients requiring hospitalization, medical records were carefully reviewed to further characterize the cause of hospitalization. The study was approved by the local ethics committee, and informed consent was obtained from each patient at inclusion.

Statistical analysis

Continuous variables were tested for a normal distribution by the Kolmogorov-Smirnov test. Normally distributed data are presented as the mean ± standard deviation and non-normally distributed data as the median (IQR, interquartile data). Categorical variables are expressed as percentages. Categorized analyses were performed according to the presence of adverse clinical events during the follow up. Differences in baseline characteristics were compared using student t-test or the Mann-Whitney U-test for continuous variables and the χ^2 test for categorical variables. Relationships between BTP, cystatin C and other clinical and analytical parameters were assessed by the Spearman rank correlation. Univariable and multivariable logistic regression analysis was used to examine associations between variables and worsening renal function. To contrast prognostic accuracy, statistical comparison of receiver-operating characteristic (ROC) curves was performed. To compare different predictive values, we constructed areas under the ROC for sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). The best prognostic cut-off for survival status was defined as the highest product of sensitivity and specificity. Net reclassification improvement (NRI) and integrated discrimination improvement (IDI) were performed with biomarkers kept as dichotomous variables as described by Pencina et al(20), where the categories of probabability for events are defined based on prognostication scheme of the Heart Failure Survival Score [1]. We calculated hazard ratios (HR) derived from the Cox regression analysis to identify predictors of mortality and/or HF hospitalization during follow-up. The independent effect of variables on prognosis was calculated using a Cox multivariable regression analysis, incorporating covariates with p values <0.10 in the univariable analysis. To

avoid colinearity effects, due to their extremely high correlation (r >0.80), serum creatinine and eGFR, as well as BTP and cystatin C, were not entered together in multivariate models. The cumulative incidence of all-cause death or HF hospitalization was estimated according to the Kaplan–Meier method and the log-rank statistic was used for comparisons. All p values <0.05 were accepted as statistically significant. Statistical analysis was performed using SPSS 15.0 (SPSS, Inc., Chicago, IL, USA) and SAS software (version 9.2, SAS Institute Inc, Cary, NC).

2.5 Results

Among the 220 subjects with ADHF, the median plasma BTP concentration was 0.97 [0.74-1.37] mg/L, median plasma cystatin C concentration was 1.15 [0.90-1.59] mg/L, median serum creatinine was 1.14 [0.84-1.45] mg/dL, median eGFR was 64 [45-78] mL/min per 1.73m^2, and the median BUN was 24 [18-34] mg/dL.

Plasma BTP concentration was positively correlated with cystatin C concentration (r = 0.86; p <0.001). Both, plasma BTP and cystatin C concentrations were positively correlated with serum creatinine, BUN, age, New York Heart Association functional class, NT-proBNP, uric acid, troponin T and C-reactive protein, while both were negatively correlated with eGFR, serum albumin and haemoglobin. BTP and cystatin C concentrations were not correlated with body mass index or left ventricular ejection fraction (Table 1).

Worsening renal function during hospital admission occurred in 66 patients (30%). Patients with worsening renal function and those without worsening renal function presented similar plasma BTP (1.03 [0.75-1.44] vs 0.96 mg/L [0.71-1.35]; p=0.35), cystatin C (1.12 [0.87-1.71] vs 1.19 mg/L [0.90-1.54], p=0.73), creatinine (1.11 [0.84-1.49] vs 1.15 mg/L [0.85-1.44]; p=0.83), eGFR (64 [44-77] vs 64 mL/min per 1.73m^2 [45-79]; p=0.77) and BUN (24 [21-34] vs 24 mg/L [17-34]; p=0.32). In a multivariable logistical regression model, only a previous history of coronary artery

bypass surgery (OR 3.87 95%CI 1.120-12.5; p=0.023) and female (OR 2.07 95%CI 1.13-3.78; p=0.019) were significantly associated with worsening of renal function.

Over the study period (median 500 days, interquartile range 231 to 796), a total of 116 patients (53%) presented adverse clinical events: 62 patients died and 76 patients were readmitted to hospital owing to HF decompensation. The distribution of characteristics and laboratory parameters in accordance to the occurrence of adverse clinical events are shown in Tables 2 and 3. Patients who presented adverse clinical events during the follow up were more likely to be older, were more likely to have prior HF, poorer functional status, needed more frequent in-hospital inotropic support and suffered a higher rate of worsening renal function during the hospital admission.

Those with events had higher plasma BTP (1.04 [0.80-1.49] vs 0.88 mg/L [0.68-1.17], p=0.003; Figure 1A) and cystatin C concentrations (1.29 [1.00-1.71] vs 1.03 mg/L [0.86-1.43], p=0.001; Figure 1B) compared to those who did not have events. Serum creatinine, BUN, NT-proBNP, and Troponin T were also higher among patients who presented adverse clinical events; whereas glucose and eGFR were lower among these patients.

Tertile analyses of BTP and cystatin C concentrations examined as a function of adverse clinical event rates revealed that there was a graded increase in mortality and/or HF hospitalization with rising concentrations of these markers (Figure 2).

To define optimal prognostic accuracy of BTP values regarding adverse clinical events survival, we performed ROC curve analyses. For comparison, the same ROC analyses were performed with cystatin C, as well as creatinine, eGFR, and BUN. As detailed in Table 4, BTP and cystatin C had comparable AUC, with overall performance characteristics that appeared similar, if not slightly superior to the more conventional measures of renal function. The NRI from the addition of cystatin C to eGFR was 0.28 (95% CI 0.13 to 0.43, p =0.002), whereas the IDI was 0.05 (95% CI 0.02 to 0.08, p =0.001). The probability of correctly predicting death and/or HF readmission when cystatin C was added to eGFR was reflected particulary in the percentage of non-

events correctly reclassified (44%), while the % of events reclassified was -16%. When compared to cystatin C, BTP had an NRI of 0, with an IDI of 0.00008; compared to cystatin C, BTP had a 0% reclassification of either events or non-events, indicating BTP offered comparable information to cystatin C.

In univariable Cox regression analysis, all measures of kidney function were associated with a higher risk of adverse clinical events (Table 5). However, after adjusting for confounding factors in the multivariable Cox regression models, only BTP and cystatin C concentrations remained as significant predictors of adverse events, while creatinine, eGFR, and BUN were no longer significant (Table 5). This remained the case when measurements of renal function were evaluated as dichotomous variables in multivariate Cox regression models: BTP and cystatin C were significant predictors for mortality and/or HF readmission (HR 1.54, 95%CI 1.10 to 2.38; p = 0.02 for BTP >0.96 mg/L and HR 1.73 95%CI 1.15 to 2.62; p = 0.009 for cystatin C >1.05 mg/L), but creatinine , eGFR and BUN were not (p >0.2). Furthermore, when BTP and cystatin C, were adjusted for others conventional measures of kidney function, both remained associated with a higher risk of adverse events (Log_{10} BTP, HR 3.01, 95% CI 1.08 to 7.87, p = 0.033 and Log_{10} cystatin C, HR 3.56, 95% CI 1.22 to 9.35, p = 0.018).

As shown in the Kaplan-Meier survival analyses, elevated BTP concentration (>0.96 mg/L) as well as elevated cystatin C concentration (>1.05 mg/L) were associated with an increased risk of adverse clinical events (Figure 3; log rank test p <0.05). In stratified analyses of patients with eGFR >60 mL/min/1.73m^2 (n = 146), elevated BTP and cystatin C concentrations were also found to be associated with a higher mortality and/or HF hospitalization risk (Fig. 4; log rank test p <0.05)

2.6 Discussion

The importance of parameters of renal function in ADHF is considerable. Indeed, kidney dysfunction represents one of the most dominant variables for predicting adverse outcome in patients with ADHF (22, 23). Although powerfully

predictive of adverse outcome, conventional tests for kidney dysfunction such as creatinine, eGFR and BUN all have potential limitations (2-4); thus, characterization of newer markers of renal dysfunction for application in patients with ADHF is of considerable significance.

In this study, we examined novel markers of renal dysfunction, BTP and cystatin C, and compared them to conventional measures of renal function for their ability to predict adverse outcome. Neither BTP nor cystatin C at presentation (or other measures of renal function) predicted the onset of renal dysfunction after presentation with ADHF. This suggests that baseline renal function may be less important for predicting subsequent worsening in GFR than are the various clinical and therapeutic insults that occur in patients with ADHF that may lead to such decline. On the other hand, consistent with prior reports (5-13), we found cystatin C to independently predict death/HF hospitalization with greater accuracy than creatinine, eGFR, or BUN; moreover, BTP performed comparably to cystatin C for this indication. Of special note, among patients with eGFR>60 mL/min (an area of weakness for serum creatinine and eGFR), we found that elevated concentrations of both BTP and cystatin C were still associated with significantly higher risk of adverse clinical events. To the best of our knowledge, this is the first study that describes the prognostic usefulness of BTP in ADHF.

Over the last years, kidney dysfunction, including mild and moderate chronic kidney disease, has become increasingly recognized as an independent risk factor for morbidity and mortality in patients with HF (22-28). A recent meta-analysis (29) showed that the majority of patients with HF had some degree of renal impairment, and these patients represent a high-risk group with an approximately 50% increased relative mortality risk, compared with patients of normal kidney function. Because serum creatinine, eGFR equations, and BUN are insensitive to mild decrements in renal function (2-4), the detection of mild kidney dysfunction in routine clinical practice remains challenging. It has been suggested that BTP and cystatin C concentrations are

more sensitive for the detection of mild decrements of GFR (15-17, 30, 31). Consistent with this suggestion, we found both BTP and cystatin C to be independently superior to standard measures of renal function for predicting death and/or HF hospitalization. Given the reported value of conventional measures of renal function for prognostication in patients with ADHF (22, 23), our findings are of significance.

BTP is a low-molecular weight glycoprotein, belongs to the lipocalin protein family, with a molecular weight of 22-29 kDa depending on the degree of glycosylation (32, 33). BTP is synthesized in the central nervous system, male genital organs and heart; and is secreted into the cerebrospinal fluid, seminal plasma and plasma, respectively (34-36). Hoffmann et al. (33) showed that “brain type” BTP is absent in serum and urine (as it is cleared by the liver via specific hepatic glycoprotein receptors), whereas sialyzed glycoforms are protected against hepatic metabolism, and are eliminated via glomerular filtration, allowing for sensitive estimation of renal function.

As mentioned above, plasma BTP concentration appears superior to standard means of renal function estimation for the detection of mild decrements of GFR. Thus, our results might indicate that the risk of adverse events attributable to kidney disease is not completely captured by estimates of kidney function routinely used in clinical practice. On the other hand, BTP has also been implicated in other numerous physiologic and pathologic processes including inflammatory responses (37), endothelial cell function (38), atherogenesis (36, 39), insulin sensitivity (40) and systemic arterial hypertension (41). We therefore cannot exclude the possibility that circulating BTP concentrations reflect (directly or indirectly) pathophysiologic processes pivotal for HF progression.

Cystatin C is a low molecular weight protein (13 kDa), which is released at a constant rate and is expressed in all nucleated cells (42). It has multiple biological functions, including controlling extracellular proteolysis via inhibition of cysteine peptidases (especially cathepsins B, H, L and S) (43), modulation of the immune

system (44), exertion of antibacterial and antiviral activities and modification of the body's response to brain injury. Cystatin C is freely filtrated in the glomerulus, and subsequently absorbed in the renal tubules where it is fully degraded locally, without re-entering the bloodstream. No active tubular secretion occurs, nor significant extrarenal elimination (45, 46). Therefore, plasma cystatin C concentration are mainly dependent on GFR. Although several previous studies have shown that plasma cystatin C concentration predicts adverse clinical outcomes across a wide spectrum of patients including those with HF (5-13), it also remains unclear, however, if the association with adverse outcomes is due to cystatin C being a more precise measure of kidney function or if cystatin C in a reflection of other pathologic processes independent of GFR. Importantly, cystatin C has not been previously compared to BTP as a prognostic risk factor, so the present study adds to the existing literature by demonstrating that BTP is at least comparable to cystatin C for predicting adverse clinical events in hospitalized patients with ADHF.

The limitations of our study are similar to those of any single center prospective observational study. The small sample size and relatively small number of patients included in each group also makes it difficult to draw firm conclusions. In this study we included unselected hospitalized patients with ADHF due to both systolic and non-systolic mechanisms, so the validity of our findings in selected HF populations remains to be established. In addition, the presence of unmeasured variables such as activity of coronary ischemia, diastolic abnormalities, or severity of valvular heart disease were not be factored into our multivariable Cox regression analyses for prediction of poor outcomes. For the prediction of outcomes, the number of covariates included in multivariable models was >1 for each 10 events. Therefore, it remains possible that the models were over-adjusted, and consequently our results could fail to be replicated in future samples. Among our study cohort, we found that elevated plasma NT-proBNP concentration did not predict adverse clinical events beyond cystatin C and BTP. However, since several previous studies have demonstrated that NT-proBNP and

cystatin C concentrations offered complementary prognostic value in this clinical setting (5, 11, 12), we can not exclude that our results may represent a false negative (type II) error due to over-adjustment of co-variaties in the context of a relatively small number of subjects with ADHF. Furthermore, we also found that WRF was not associated with adverse clinical events after adjustment for baseline clinical and biochemical risk factors. This likely reflects the dominance of baseline novel renal function parameters over a WRF definition based on absolute rise of serum creatinine during hospitalization. Lastly, we do not have follow-up (post-treatment) values for measures of renal function; such follow-up values would be expected to provide further prognostic information about our patients.

In conclusion, among patients with ADHF, BTP and cystatin C appear to add comparable and significant prognostic value, and were superior to creatinine, eGFR and BUN, each well-established prognostic variables in this context.

2.7.1 Table 1: Correlations between β-trace protein, cystatin C and clinical variables.

	β-trace protein		Cystatin C	
Variables	r	*p*	r	*p*
Age (year)	0.32	<0.001	0.29	<0.001
Albumin (g/dL)	-0.13	0.05	- 0.19	0.004
Body Mass Index (Kg/m^2)	- 0.06	0.42	0.09	0.23
C-reactive protein (mg/dL)	0.16	0.026	0.21	0.003
Creatinine (mg/dL)	0.70	<0.001	0.73	<0.001
Cystatin C (mg/L)	0.86	<0.001	-	-
eGFR ($mL/min/1.73^2$)	- 0.71	<0.001	- 0.76	<0.001
Hemoglobin (g/dL)	- 0.38	<0.001	- 0.33	<0.001
LVEF	0.07	0.28	0.04	0.56
NYHA fucntional class (I-IV)	0.22	0.001	0.25	<0.001
Plasma NT-proBNP (pg/mL)	0.29	<0.001	0.37	<0.001
Troponin T (ng/dL)	0.13	0.06	0.16	0.02
BUN (mg/dL)	0.67	<0.001	0.72	<0.001
Uric acid (mg/dL)	0.40	<0.001	0.45	<0.001

eGFR denotes estimated glomerular filtration rate, BUN denotes blood urea nitrogen, NT-proBNP denotes amino-terminal pro-brain natriuretic peptide, LVEF denotes left ventricular ejection fraction and NYHA denotes New York Heart Association.

2.7.2 Table 2: Study population clinical characteristics.

Variables	Events (n=116)	No events (n=104)	*p*
Age (years)	75±10	69±13	0.002
Male	67 (57.3)	49 (47.2)	0.15
Body mass index (Kg/m^2)	29±5	29±4	0.65
Systolic blood pressure (mmHg)	150±37	153±34	0.69
Heart rate (beat/min)	100±28	100±34	0.96
LVEF	45±17	48±16	0.26
NYHA functional class III-IV	53 (45.3)	12 (11.7)	<0.001
Prior Heart Failure	85 (72.6)	50 (48.5)	<0.001
Previous coronary artery bypass graft	6 (5.1)	7 (6.8)	0.60
Previous percutaneous coraonary intervention	32 (27.4)	20 (19.4)	0.17
Ischemic cause to Heart Failure	40 (34.2)	32 (31.1)	0.62
Diabetes mellitus	66 (56.4)	48 (46.6)	0.15
Hypertensión	95 (81.2)	85 (82.5)	0.80
Hyperlipidemia	50 (42.7)	43 (41.7)	0.88
Current smoking	14 (12)	17 (16.5)	0.33
Peripheral artery disease	11 (9.4)	6 (5.8)	0.32
Atrial fibrillation/flutter	72 (61.5)	54 (52.4)	0.17
Branch block	39 (34.2)	30 (29.1)	0.42
Previous STEMI	36 (30.8)	21 (20.4)	0.08
Previous stroke	15 (12.8)	13 (12.6)	0.96
Anemia	60 (51.3)	43 (41.7)	0.16
Chronic obstructive pulmonary disease	31 (26.5)	22 (21.4)	0.37
In-hospital inotropic use	20 (17.1)	3 (2.9)	0.001
Worsening of renal function	43 (37)	23 (22)	0.016
Treatment at discharge			
β-blocker	58 (55.2)	74 (72.5)	0.01
ACE inhibitors/ARB	88 (83.8)	87 (85.3)	0.77
Statin	58 (55.2)	55 (53.9)	0.85
Aldosterone antagonist	34 (32.4)	39 (38.2)	0.38
Loop diuretic	100 (95.2)	91 (89.2)	0.11

Data are expressed as mean+SD and number (%). ACE denotes angiotensin-converting enzyme, ARB denotes angiotensin-receptor blocker, STEMI denotes ST segment elevation myocardial infarction, other abbreviations as in Table 1.

2.7.3 Table 3. Baseline laboratory parameters.

Variable	Events (n=116)	No events (n=104)	*p*
Hemoglobin (g/dL)	12.1 [10.9-13.6]	12.3 [11.4-14.6]	0.09
Leucocytes	8350 [6735-11125]	8050 [6370-9590]	0.08
Glucose (mg/dL)	163 [114-214]	131 [109-188]	0.03
Creatinine (mg/dL)	1.18 [0.92-1.59]	1.02 [0.82-1.37]	0.02
Estimated GFR (mL/min/1.73^2)	62 [43-74]	66 [48-80]	0.03
Blood urea nitrogen (mg/dL)	27 [20-37]	22 [16-29]	0.001
Albumin (g/dL)	3.9±0.4	4.0±0.4	0.57
Sodium (mEq/L)	137±5	138±4	0.08
Uric acid (mg/dL)	7.7±2.9	7.4±2.2	0.29
C-reactive protein (mg/dL)	1.30 [0.60-4.02]	0.90 [0.40-2.20]	0.11
Troponin T (ng/mL)	0.018 [0.010-0.057]	0.010 [0.010-0.030]	0.008
Plasma NT-proBNP (pg/mL)	4010 [2021-7768]	2502 [1291-5357]	0.006

Data are expressed as mean+SD or median (quartiles). Abbreviations as in Table 1.

2.7.4 Table 4: Performance of measures of renal function and NT-proBNP for prediction of 1 year mortality and/or HF hospitalization among patients with ADHF.

Variables	AUC	95% CI	Cut-off	Sensitivity	Specificity	PPV	NPV	*p*
Btrace protein (mg/dL)	0.62	0.55-0.68	0.96	0.61	0.60	0.66	0.55	
Cystatin C (mg/dL)	0.63	0.56-0.69	1.05	0.72	0.55	0.64	0.63	0.73
Creatinine (mg/dL)	0.58	0.52-0.65	1.07	0.64	0.54	0.61	0.57	0.34
eGFR (mL/min/1.73^2)	0.57	0.50-0.64	72	0.65	0.44	0.57	0.53	0.26
BUN (mg/dL)	0.60	0.53-0.66	25	0.53	0.65	0.63	0.55	0.71
NT-proBNP (pg/mL)	0.60	0.53-0.67	3041	0.62	0.57	0.62	0.57	0.70

AUC denotes area under the curve, CI denotes confidence intervals, PPV denotes positive predictive value, NPV denotes negative predictive value, and other abbreviations as in Table 1. The “p values” shown for comparation between BTP and other variables.

2.7.5 Table 5. Cox regression analysis for prediction of mortality and/or HF hospitalization.

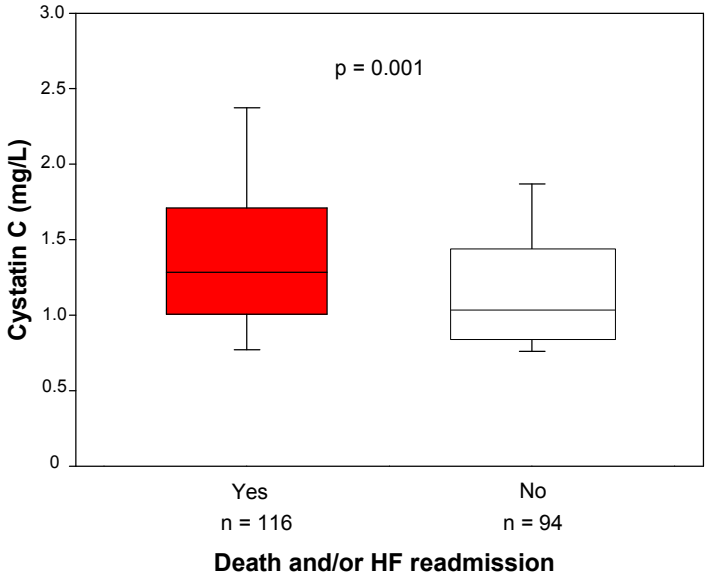
	Univariate		Multivariate	
Variables	Hazard Ratio	p	Hazard Ratio	p
Age (per year)	1.03 (1.01-1.05)	0.001	1.04 (1.01-1.06)	0.001
LVEF	0.99 (0.98-1.01)	0.06	0.98 (0.97-0.99)	0.023
NYHA Class III-IV	2.91 (2.01-4.22)	<0.001	2.11 (1.48-3.01)	<0.001
Diabetes mellitus	1.39 (0.96-2.00)	0.08	-	0.51
Prior Heart Failure	1.94 (1.29-2.91)	0.001	-	0.14
Previous STEMI	1.56 (1.05-2.32)	0.03	-	0.19
In-hospital inotropic use	2.43 (1.50-4.01)	<0.001	2.16 (1.26-3.72)	0.005
Log_{10} glucose	2.71 (1.05-7.02)	0.04	-	0.33
Sodium (per mEq/L)	0.96 (0.93-1.01)	0.09	-	0.11
Log_{10} leucocytes	5.26 (1.40-19.8)	0.014	8.58 (2.21-33.3)	0.002
Log_{10} plasma NT-proBNP	1.75 (1.18-2.59)	0.006	-	0.47
Log_{10} troponin T	1.51 (1.09-2.11)	0.014	2.66 (1.51-4.71)	0.001
Worsening renal function	1.52 (1.04-2.21)	0.031	-	0.14
Log_{10} β-trace protein	3.30 (1.39-7.84)	0.007	3.19 (1.15-8.92)	0.026
Log_{10} cystatin C	4.57 (1.66-12.6)	0.003	4.20 (1.31-13.3)	0.015
Log_{10} creatinine	4.21 (1.34-11.8)	0.006	-	0.14
Log_{10} eGFR	0.47 (0.34-0.85)	0.007	-	0.30
Log_{10} blood urea nitrogen	2.58 (1.05-8.8)	0.009	-	0.26

Abbreviations as in Table 1. *Log_{10} β-trace protein, Log_{10} cystatin C, Log_{10} creatinine and Log_{10} eGFR were all tested separately and multivariable “Hazard Ratio” and “p” for other variables shown from the β-trace protein model.

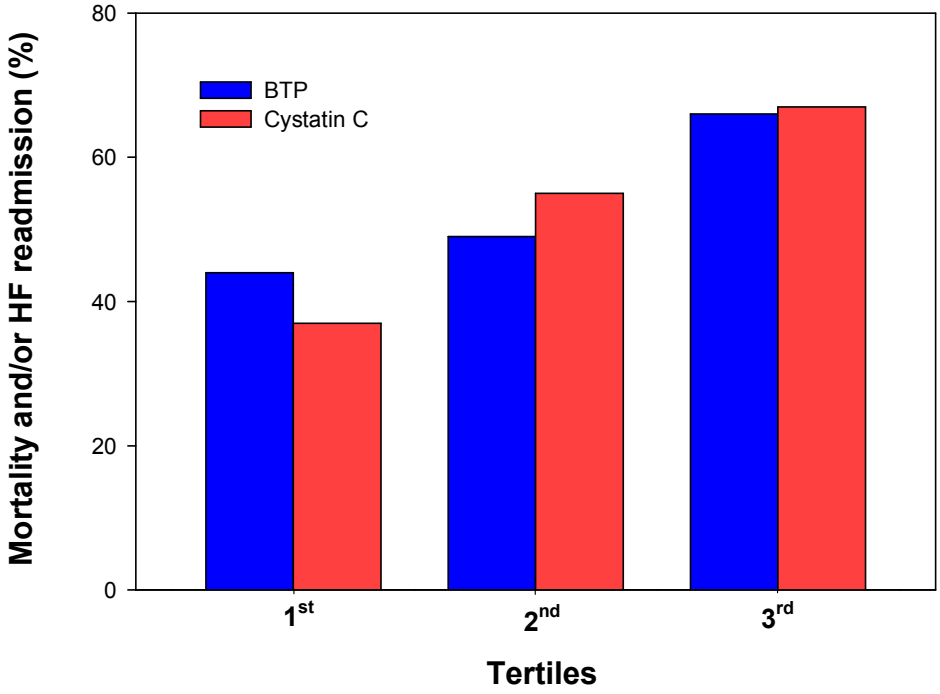
2.8.1 Figure 1. Box plots showing the concentrations of A) β-trace protein and B) cystatin C in patients experiencing mortality and/or HF hospitalization and those who did not have events. The bottom and top whiskers indicate the 5th and 95th percentile levels; the lower and upper boundaries of the boxes, the 25th and 75th percentile levels; and the horizontal line within the box, the median level.

A)

B)

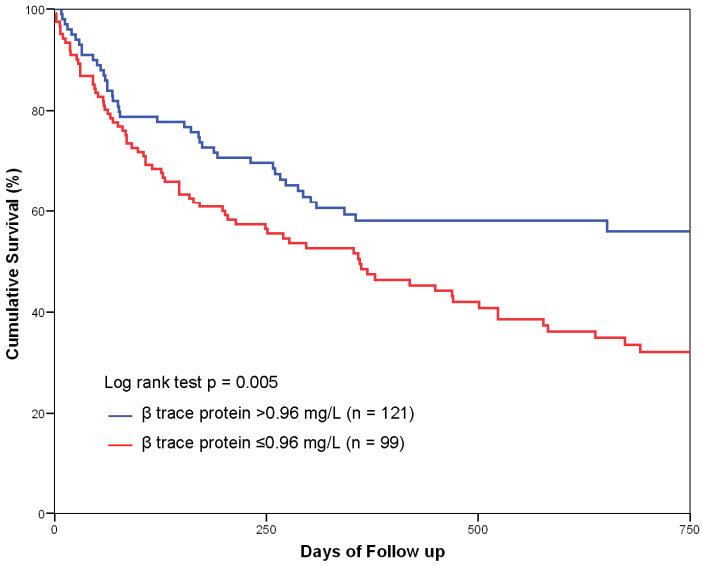


2.8.2 Figure 2. Mortality and/or HF hospitalization as a function of β-trace protein and cystatin C tertiles. Rates of mortality and/or HF were significantly higher with rising concentrations of both β-trace protein (p =.008) and cystatin C (p <.001).



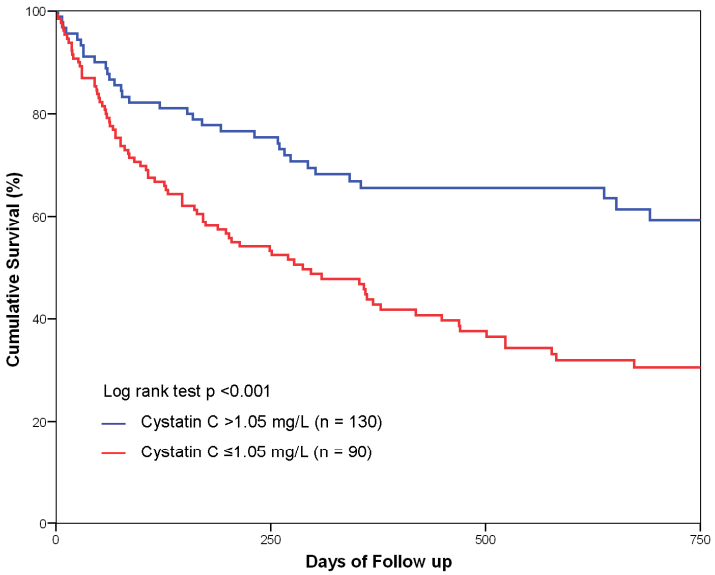
2.8.4 Figure 3. Kaplan–Meier survival curves for mortality and/or HF hospitalization according to A) β-trace protein or B) cystatin C concentrations.

A)



No. at risk				
β trace protein >0.96 mg/L	121	65	40	21
β trace protein ≤0.96 mg/L	99	61	37	20

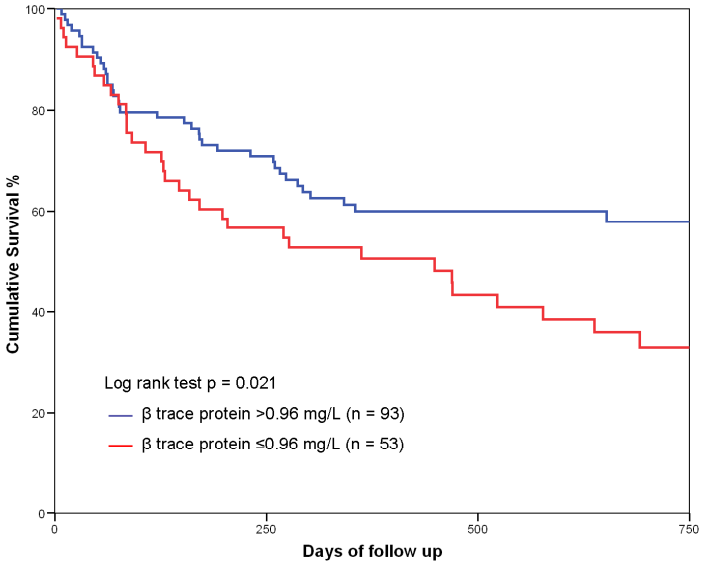
B)



No. at risk				
Cystatin C >1.05 mg/L	130	64	42	22
Cystatin C ≤1.05 mg/L	90	62	35	19

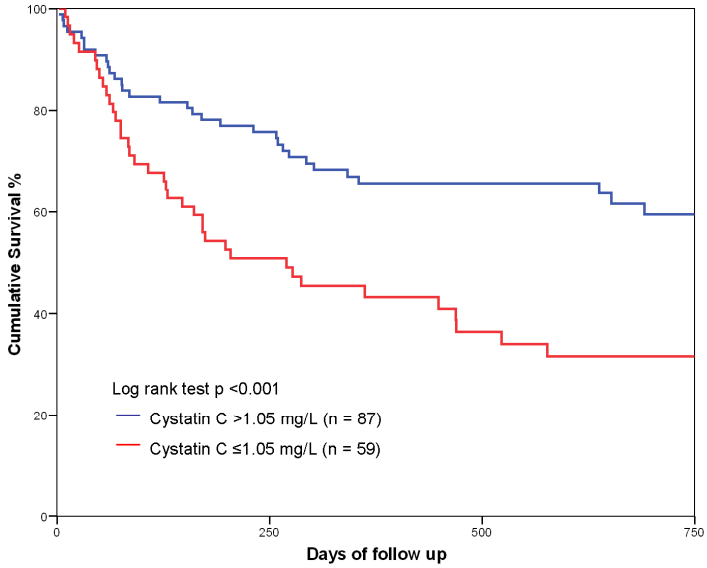
2.8.5 Figure 4. Kaplan–Meier survival curves for mortality and/or HF hospitalization, according to A) β-trace protein or B) cystatin C concentrations in patients with eGFR >60mL/min/1.73m^2.

A)



No. at risk				
β trace protein >0.96 mg/L	93	62	40	21
β trace protein ≤0.96 mg/L	53	24	18	9

B)



No. at risk				
Cystatin C >1.05 mg/L	87	62	42	22
Cystatin C ≤1.05 mg/L	59	29	16	8

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Usefulness of Soluble Concentrations of the Interleukin Family Member ST2 as Predictor of Mortality in Patients with Acutely Decompensated Heart Failure Relative to Left Ventricular Ejection Fraction

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3.2 Abstract

The aim of this study was to determine whether the risk of mortality associated with concentrations of soluble ST2 differs in patients with acutely decompensated heart failure with preserved ejection fraction (HFpEF) versus systolic heart failure. We prospectively enrolled 447 patients with acutely decompensated heart failure. Blood samples were collected at presentation to determine soluble ST2 concentrations. HFpEF was defined as symptoms or signs of acutely decompensated heart failure and left ventricular ejection fraction ≥50% on echocardiography. Patients were followed for 1 year, and vital status was obtained in all. The soluble ST2 concentrations were higher in patients with sistolic heart failure (n = 250) than in those with HFpEF (n = 197): 0.55 vs. 0.38 ng/mL, p <0.001. Receiver operator curve analyses showed different cut-off point values of soluble ST2 for the prediction of 1 year mortality in patients with HFpEF (>0.35 ng/mL) and sistolic heart failure (>0.56 mg//mL), which had similar prognostic accuracy (area under the curve 0.69 vs. 0.73; p >0.05). In adjusted analyses that included NT-proBNP concentrations, elevated soluble ST2 concentrations were associated with a higher mortality risk in both populations (HFpEF, per ng/mL, HR 1.41 95%CI 1.14-1.76, p = 0.002; Sistolic heart failure, per ng/mL, HR 1.20 95% CI 1.10-1.32, p <0.001). Soluble ST2 concentration improved clinical risk prediction over NT-proBNP, as assessed by both improved C-statistic as well as improvement in net reclassification index and integrated discrimination improvement analyses. In this multicenter study, soluble ST2 concetrations were lower in patients with HFpEF, but soluble ST2 remained an independent predictor of mortality regardless of left ventricular ejection fraction.

Key words: ST2, prognosis, preserved ejection fraction, heart failure.

3.3 Introduction

Beyond natriuretic peptides, other biochemical markers have been examined for prognostication in heart failure. Among these is the soluble form of the interleukin-1 receptor member, ST2. A biomarker suggested to potentially reflect ventricular remodeling and fibrosis[2-4], soluble ST2 represents an attractive candidate marker for understanding heart failure biology; moreover, concentrations of the marker represent a powerful prognostic variable in those with acutely decompensated heart failure[5-9]. However, the value of soluble ST2 as a biomarker of risk as a function of HFpEF versus sistolic heart failure has not been specifically examined. With this in mind, among a cohort of patients with acutely decompensated heart failure, we evaluated the effects of left ventricular function on concentrations of soluble ST2, associations between cardiac structure and function and soluble ST2, and prognostic meaning of soluble ST2 in those with HFpEF versus sistolic heart failure.

3.4 Methods

The study population consisted of subjects from 3 previously reported prospective clinical trials of acutely decompensated heart failure patients from Boston, Massachusetts, Linz, Austria, and Murcia, Spain[10-12]. These trials had compatible inclusion/exclusion criteria and obtained similar clinical and laboratory testing, including soluble ST2, troponin T, amino terminal B-type natriuretic peptide (NT-proBNP), and C-reactive protein. For the purposes of this study, a total of 447 patients with acutely decompensated heart failure had available data and were considered: 209 subjects from Boston, Massachusetts, 131 from Linz, Austria, and 107 from Murcia, Spain. The ProBNP Investigation of Dyspnea in the Emergency Department study was a prospective, blinded study of NT-proBNP testing performed in Boston, MA, USA, which examined 599 dyspneic subjects in the emergency department. All patients with acutely decompensated heart failure from Boston were eligible for the present analysis. The Linz study comprised 137 dyspneic patients presenting to the emergency

department with the final diagnosis of acutely decompensated heart failure; of these, 131 had complete data and were included in this study. The final source of data for the present analysis was a prospectively gathered group of subjects from a Spanish cohort study of patients with diagnosis of acutely decompensated heart failure consecutively admitted to the University Hospital of Virgen de la Arrixaca, between 1 September 2006 and 28 Febraury 2008. During this period, 107 subjects with soluble ST2 data on admission were available for analysis, and were included. Patients from each study group were followed for 1 year, and vital status was obtained in all.

Patients were characterized as having HFpEF if their left ventricular ejection fraction was ≥50%, as estimated by echocardiography using Simpson's biplane method[13, 14]. Concentrations of soluble ST2 were measured using an enzyme-linked immunosorbent assay (Medical & Biological Laboratories, Woburn, Massachusetts), on blood specimens frozen at -80°C. In addition, NT-proBNP was measured using a validated, commercially available immunoassay (Elecsys ProBNP, Roche Diagnostics, Indianapolis, IN, USA) using established methodology.

Normally distributed data are presented as the mean ± standard deviation and non-normally distributed data as the median and interquartile range. Differences in baseline characteristics were compared using t-student test for continuous variables and the χ^2 test for categorical variables. Mann-Whitney U-test was used to compare continuous variables in states of non-normality. The Kruskal-Wallis test was performed to assess and compare the soluble ST2 concentrations across New York Heart Association (NYHA) functional class. Soluble ST2 results were log-transformed to establish normality, and univariate Spearman correlation was used to evaluate the magnitude and significance of relationships among continuous variables. To evaluate the characteristics of soluble ST2 concentrations as a predictor of death in patients with either HFpEF or sistolic heart failure, several methods were employed. Patients were grouped into tertiles, and the frequency of mortality relative to increasing soluble ST2 concentrations was calculated as a function of HFpEF and sistolic heart failure.

Receiver operator characteristic analyses with death at 1 year were also performed, and area under the curve estimated. The added predictive ability of soluble ST2 over NT-proBNP for detection of events was evaluated using C-statistic, net reclassification improvement and integrated discrimination improvement analyses. Net reclassification improvement and integrated discrimination improvement were performed with biomarkers kept as dichotomous variables as described by Pencina et al[15], where the categories of probability for events are defined based on prognostication scheme of the Hear Failure Survival Score[16].To identify the independent predictors of death at 1 year we performed a multivariable Cox proportional hazards analyses using forward stepping. Variables were retained if their univariable p value was <0.05, and entered into a multivariable model; only those variables with significant p values were retained in the final multivariable model. The cumulative incidence of death was estimated according to the Kaplan–Meier method and the log-rank statistic was used for comparisons. All p values <0.05 were accepted as statistically significant. Statistical analysis was performed using SPSS 15.0 for Windows (SPSS, Inc., Chicago, IL, USA). Receiver operator characteristic curve analysis was performed with MedCalc statistical software 10.4 for Windows (MedCalc Software, Broekstraat 52, Mariakerke, Belgium).

3.5 Results

A total of 447 subjects were included for analysis. The distribution of clinical characteristics and laboratory parameters as a function of left ventricular ejection fraction are listed in Table 1. As shown in Table 2, soluble ST2 concentrations were correlated with several clinical characteristics and laboratory parameters; moderate significant positive correlations were observed between soluble ST2 and C-reactive protein, troponin T, and NT-proBNP (all p <0.001). The soluble ST2 concentrations were also weakly correlated with left ventricular ejection fraction (r = -0.12; p = 0.01); accordingly, patients with sistolic heart failure had higher soluble ST2 concentrations (0.55 [0.30-1.03] ng/mL vs. 0.38 [0.26-0.79] ng/mL, p <0.001). When patients were

categorized as a function of NYHA functional class, median soluble ST2 concentrations were noted to be higher in those with worse symptoms regardless of their left ventricular ejection fraction (≥50% (n = 197): NYHA II 0.28 [0.17-0.36] ng/mL, NYHA III 0.43 [0.30-0.84] ng/mL, NYHA IV 0.49 [0.29-0.96] ng/mL; p = 0.001 and <50% (n = 250): NYHA II 0.33 [0.19-0.72] ng/mL, NYHA III 0.59 [0.31-1.01] ng/mL, NYHA IV 0.63 [0.35-1.38] ng/mL; p <0.001).

Over 1 year of follow up, a total of 117 patients (26%) died. Median concentrations of soluble ST2 were significantly higher among decedents than survivors (0.80 [0.42 to 1.83] ng/mL vs. 0.38 [0.24 to 0.72] ng/mL; p <0.001). This pattern of higher ST2 concentrations in decedents remained in patients with HFpEF (0.57 [0.26-1.28] ng/mL vs. 0.35 [0.22-0.66] ng/mL; p <0.001) and in those with sistolic heart failure (0.98 [0.57-2.48] ng/mL vs. 0.42 [0.26-0.78] ng/mL; p <0.001) (Fig. 1). To evaluate the optimal prognostic accuracy of soluble ST2 concentrations for the prediction of 1 year mortality, we performed receiver operator characteristic analyses as a function of left ventricular ejection fraction. As detailed in Table 3, soluble ST2 had comparable area under the curve in patients with HFpEF and sistolic heart failure. In multivariable Cox regression analysis we found that elevated soluble ST2 levels, as a quantitative variable, were associated with a higher risk for 1 year mortality either in patients with HFpEF (per ng/mL, HR 1.41 95%CI 1.14-1.76, p = 0.002) and sistolic heart failure (per ng/mL, HR 1.20 95%CI 1.10-1.32, p <0.001) (Table 4). In addition, tertile analyses of soluble ST2 concentrations also revealed that there was a graded increase in 1 year mortality with rising concentrations of soluble ST2 in both groups of acutely decompensated heart failure (Fig. 2). Kaplan-Meier survival analysis showed early diverging rate of mortality according to soluble ST2 cut-off point values throughout the 1 year follow up in the entire cohort (sST2 ≥0.53 ng/mL (n = 201): 35% vs. <0.53 ng/mL (n = 246): 12%; log rank test p <0.001), as well as after the stratification by left ventricular ejection fraction (HFPEF, sST2 ≥0.35 ng/mL (n = 114): 31% vs. sST2 <0.35 ng/mL (n = 83): 9.6%; log rank test p <0.001; and sistolic heart

failure, sST2 ≥.0.56 ng/mL (n = 121): 37% vs. sST2<0.56 ng/mL (n = 129): 14%; log rank test p <0.001).

The added predictive ability of soluble ST2 over NT-proBNP for detection of events was evaluated using C-statistic, net reclassification improvement and integrated discrimination improvement analyses. Addition of soluble ST2 concentration to NT-proBNP improved the C-statistic and both net reclassification improvement and integrated discrimination improvement, regardless of left ventricular ejection fraction status (Table 5).

3.6 Discussion

In the present study, we provided novel data on the relationship between soluble ST2 concentrations and the clinical and biochemical characteristics of hospitalized patients with acutely decompensated heart failure considered as a function of preserved versus impaired left ventricular ejection fraction. The rationale for such an analysis is based on the biological and clinical significance of soluble ST2 as a potential marker of ventricular remodelling and prognosis in acutely decompensated heart failure[17-25]. Basic science studies suggest a pivotal biological role of soluble ST2 in the process of ventricular remodelling in the context of ventricular pressure or volume overload; in addition, concentrations of soluble ST2 appear to predict a clinical phenotype vulnerable to remodelling, and are prognostically meaningful in the context of acutely decompensated heart failure[26-34]. As remodelling is a meaningful process in most forms of heart failure[35], our hypothesis was that if soluble ST2 values reflect risk for remodelling, they would be–as a consequence—prognostically important across the wide spectrum of left ventricular function in our cohort.

Consistent with our primary hypothesis, in this multinational pooled analysis, regardless of the left ventricular ejection fraction status, we found that soluble ST2 concentrations were significantly correlated with several prognostically and biologically meaningful biomarkers involved in deleterious remodelling; clinically, soluble ST2

values were associated with heart failure symptom severity. Furthermore, we found soluble ST2 concentration to be an independent predictor for 1 year mortality in patients suffering acutely decompensated heart failure from either HFpEF or sistolic heart failure, irrespective to the presence of natriuretic peptides in the analysis. In fact, it is of note that in the presence of soluble ST2 in the model, NT-proBNP was not a predictor of death in those with HFpEF.

Cardiac remodelling is a common mechanism for the progression of heart failure, and involves multiple deleterious changes in the myocardium, including cardiomyocyte loss (by necrosis or apoptosis), left ventricular dilatation, cardiomyocyte hypertrophy, fibroblast proliferation and collagen accumulation[36]. Remodelling is associated with a higher rate of adverse outcomes among patients with heart failure, and a prime target for therapeutic strategies to reduce risk in affected patients[37-41]. In addition to those with sistolic heart failure, it is well established that cardiac remodelling plays a crucial role on the pathophysiology and complications of HFpEF, including an important effect on myocardial relaxation abnormalities [42, 43].

It is in this context that measurement of soluble ST2 is relevant. Several experimental and clinical studies have demonstrated soluble ST2 to be a biomarker of mechanical stress with a pivotal role in myocardial fibrosis. soluble ST2 has a broad role in the body, including inflammatory responses, atherosclerosis, autoimmunity and cardiac remodeling[44-49]. Recently, interlekin-33 has been identified as the ligand for soluble ST2; interleukin-33/ST2 signalling protects the myocardium under mechanical strain, and acts as a biomechanically activated fibroblast-cardiomyocyte paracrine system to prevents cardiac hypertrophy and fibrosis. Sanada et al[50] recently suggested that soluble ST2 abrogates this adaptative response in a dose-dependent manner by binding interleukin-33 and preventing signaling through ST2L. Moreover, Weinberg et al[51] showed that derangement of soluble ST2 signaling leads to a phenotype quite consistent with myocardial remodeling. More recently, Weir et al [52] showed a relationship between soluble ST2 levels and cardiac remodelling parameters including

left ventricular ejection fraction, left ventricular end diastolic volume, and myocardial infarct size, as well as with plasma aldosterone levels (which itself has strong profibrotic effects on the heart).

In a prior study[53], we observed the important prognostic associations between soluble ST2 and heart failure. However, as HFpEF may fundamentally differ from SHF, we wished to extend our initial observations, examining soluble ST2 as a function of LVEF. We found—similar to findings reported with the natriuretic peptides—that concentrations of soluble ST2 were lower in those with HFpEF, when compared to those with sistolic heart failure. This may be related to differences in wall stress—the trigger for both natriuretic peptide and soluble ST2. release. Nonetheless, much like natriuretic peptides, we found soluble ST2 to be prognostically meaningful in those with HFpEF; given the universally deleterious nature of remodelling in heart failure, our results lend internal consistency to the potential value of soluble ST2 for directed anti-remodelling therapies in those with heart failure, as has been suggested [54].

Limitations of our study include the fact as a pooled multinational analysis it lacks pre-defined endpoints, despite the similar designs and goals of the respective data sources. Another consideration is the timing of sample collection: a pre-discharge soluble ST2 value might have added stronger prognostic information; nonetheless our results remain significantly. As well, we lack complete echocardiography data on each subject; while not easily feasible in such a large analysis, such data would have provided important correlates of cardiac structure and function with respect to soluble ST2 concentrations. We recently published that soluble ST2 values correlate with important echocardiographic measures of remodelling including myocardial relaxation abnormalities[55]. Importantly, it also remains unclear if the described association between plasma soluble ST2 concentrations, cardiac function parameters and prognosis of patients with heart failure reflects what is occurring at cardiac level, or if soluble ST2 concentrations reflect other pathologic processes independent of cardiac function, such as pulmonary diseases, as we have shown[56]. Mechanistic studies of

myocardial expression and secretion of soluble ST2 are needed. We did not simultaneously measure interleukin-33 and therefore cannot comment on the IL-33/soluble ST2 ratio, but this is a clear focus for future research in both heart falilure and myocardial infarction.

3.7.1 Table 1: Characteristics of the study patients as a function of LVEF.

Variables	Overall (n = 447)	LVEF ≥50% (n = 197)	LVEF <50% (n = 250)	*p*
Age (year)	73±13	74±12	72±13	0.035
Man	290 (65%)	83 (42%)	207 (83%)	<0.001
Body-mass index (Kg/m^2)	27 [24-31]	28 [25-32]	26 [23-30]	<0.001
Systolic blood pressure (mmHg)	142±32	149±32	136±31	<0.001
Diastolic blood pressure (mmHg)	80±18	79±17	80±19	0.46
Heart rate (beat/min)	92±27	88±27	96±26	0.002
Hypertensión	306 (69%)	148 (75%)	158 (63%)	0.007
Diabetes mellitus	183 (41%)	79 (40%)	104 (42%)	0.75
Coronary artery disease	202 (45%)	64 (33%)	138 (55%)	<0.001
Prior Heart Failure	239 (54%)	81 (41%)	158 (63%)	<0.001
Obstructive airway disease	103 (23%)	49 (25%)	54 (22%)	0.42
Current smoking	63 (14%)	22 (11%)	41 (16%)	0.11
LVEF %	46 [32-60]	60 [55-65]	34 [25-42]	<0.001
NYHA functional class				
II	102 (23%)	45 (23%)	57 (23%)	0.46
II	156 (35%)	63 (32%)	93 (37%)	
IV	189 (42%)	89 (45%)	100 (40%)	
Atrial fibrillation/flutter	189 (42%)	82 (42%)	107 (43%)	0.80
Medication				
β-blocker	233 (52%)	105 (53%)	128 (51%)	0.66
ACE inhibitor	208 (47%)	71 (36%)	137 (55%)	<0.001
ARB	57 (13%)	34 (17%)	23 (9%)	0.011
Digoxin	104 (23%)	31 (16%)	73 (29%)	0.01
Loop diuretic	309 (69%)	124 (63%)	185 (74%)	0.012
Hemoglobin (g/dL)	12.7±2.2	12.1±2.3	13.1±2.1	<0.001
Leukocytes (per10^3)	8.7 [7.0-10.9]	8.6 [7.1-10.6]	8.7 [6.7-11.1]	0.79
Creatinine (mg//dL)	1.10 [0.83-1.50]	1.10 [0.82-1.49]	1.14 [0.88-1.56]	0.53
eGFR (mL/min/1.73m^2)	63 [43-86]	61 [40-83]	65 [45-90]	0.029
BUN (mg/dL)	25 [18-34]	24 [18-33]	25 [18-35]	0.36
C-reactive protein (mg/dL)	3.5 [0.9-16.3]	5.2 [1-22]	2.65 [0.80-9.95]	0.013
Troponin T (ng/mL)	0.01 [0.01-0.04]	0.01 [0.01-0.037]	0.016 [0.01-0.062]	0.004
Plasma NT-proBNP (pg/mL)	3558 [1646-9250]	2749 [1344-6634]	4709 [2099-11159]	<0.001
Soluble ST2 (ng/mL)	0.47 [0.28-0.94]	0.38 [0.26-0.79]	0.55 [0.30-1.03]	<0.001

Data are expressed as mean+SD or median (quartiles), and number (%). LVEF denotes left ventricular ejection fraction, NYHA denotes New York Heart Association, ACE denotes angiotensin-converting enzyme, ARB denotes angiotensin-receptor blocker, eGFR denotes estimated glomerular filtration rate, BUN denotes blood urea nitrogen and NT-proBNP denotes amino-terminal pro-brain natriuretic peptide.

3.7.2 Table 2. Correlations between sST2 and continuous covariates.

	Overall (n = 447)		LVEF ≥50% (n = 197)		LVEF <50% (n = 250)	
Variables	r	*p*	r	*p*	r	*p*
Age (year)	-	0.69	-	0.65	0.10	0.12
Body Mass Index (Kg/m^2)	-	0.23	-	0.94	-	0.42
Systolic blood pressure (mmHg)	-0.10	0.040	-	0.47	-0.10	0.11
Diastolic blood pressure (mmHg)	-	0.68	-0.11	0.13	-	0.59
Heart rate (beat/min)	0.20	<0.001	-	0.19	0.25	<0.001
Hemoglobin (g/dL)	-0.11	0.018	-0.12	0.09	-0.26	0.006
Leucocytes	0.23	<0.001	0.19	0.01	0.26	<0.001
Creatinine (mg/dL)	0.25	<0.001	0.18	0.015	0.30	<0.001
eGFR (mL/min/1.73m^2)	-0.23	<0.001	-0.18	0.01	-0.30	<0.001
BUN (mg/dL)	0.26	<0.001	0.13	0.08	0.36	<0.001
C-reactive protein (mg/dL)	0.40	<0.001	0.36	<0.001	0.47	<0.001
Plasma NT-proBNP (pg/mL)	0.41	<0.001	0.35	<0.001	0.43	<0.001
Troponin T (ng/mL)	0.31	<0.001	0.25	0.001	0.34	<0.001
LVESD (mm)	0.15	0.033	-	0.l6	0.23	0.034
LVEDD (mm)	-	0.382	-	0.49	-	0.39
RVSP (mmHg)	0.22	<0.001	0.16	0.08	0.27	<0.001

LVESD denotes left ventricular end-systolic diameter, LVEDD denotes left ventricular end-diastolic diameter and RVSP right ventricular sysstolic pressure. Other abbreviations as in Table 1.

3.7.3 Table 3: Performance of ST2 (ng/mL) values for prediction of mortality at 1 year.

Variables	AUC	95% CI	Cut-point	Sensitivity	Specificity	PPV	NPV
Overall (n = 447)	0.71	0.67-0.76	0.53	0.69	0.64	0.41	0.85
LVEF ≥50% (n = 197)	0.69	0.62-0.75	0.35	0.82	0.49	0.37	0.88
LVEF <50% (n = 250)	0.73	0.67-0.79	0.56	0.76	0.62	0.42	0.88

AUC = area under curve, PPV = positive predictive value and NPV = negative predictive value

3.7.4 Table 4: Univariate and multivariate Cox proportional hazards for soluble ST2 as a predictor of 1 year mortality.

	Overall (n=447)				LVEF ≥50% (n=197)		LVEF <50% (n=250)	
	Univariate		Multivariate		Multivariate		Multivariate	
Variables	Hazard Ratio	*p*	Hazard Ratio	*p*	Hazard Ratio	*p*	Hazard Ratio	*p*
Age (per year)	1.006 (1.004-1.008)	<0.001	1.05 (1.03-1.07)	<0.001	1.07 (1.03-1.11)	<0.001	1.03 (1.008-1.06)	0.008
Body mass index (per Kg/m2)	0.94 (0.90-0.97)	0.001						
Systolic blood presure (per mmHg)	0.985 (0.979-0.992)	<0.001	0.988 (0.982-0.994)	<0.001			0.986 (0.977-0.995)	0.002
Diastolic blood presure (per mmHg)	0.981 (0.971-0.992)	0.001						
Prior Heart Failure	1.72 (1.18-2.50)	0.005						
NYHA (per functional class)	1.43 (1.12-1.82)	0.004						
B-blocker	0.68 (0.48-0.98)	0.038	0.66 (0.45-0.97)	0.035			0.56 (0.34-0.91)	0.019
ACE inhibitor	0.64 (0.44-0.93)	0.018						
Hemoglobin (per g/dL)	0.87 (0.80-0.94)	<0.001						
Leukocyte (per U)	1.07 (1.02-1.03)	0.003			1.13 (1.03-1.24)	0.008		
Creatinine (per mg//dL)	1.74 (1.43-2.12)	<0.001						
eGFR (per mL/min/1.73^2)	0.985 (0.979-0.992)	<0.001			0.98 (0.97-0.99)	0.012		
BUN (per mg/dL)	1.02 (1.01-1.03)	<0.001	1.02 (1.01-1.03)	<0.001			1.03 (1.01-1.04)	<0.001
C-reactive protein (per mg/dL)	1.006 (1.004-1.008)	<0.001						
Plasma NTproBNP (per 100pg/mL)	1.003 (1.002-1.004)	<0.001	1.002 (1.001-1.003)	<0.001			1.002 (1.001-1.003)	<0.001
Soluble ST2 (per ng/mL)	1.74 (1.43-2.12)	<0.001	1.23 (1.13-1.34)	<0.001	1.37 (1.11-1.68)	0.003	1.20 (1.10-1.32)	<0.001
Soluble ST2 above cut-off points								
All patients (>0.53 mg/mL)	3.31 (2.23-4.89)	<0.001	2.43 (1.60-3.69)	<0.001				
LVEF ≥50% (>0.35 mg/mL)					3.26 (1.50-7.05)	0.003		
LVEF <50% (>0.56 mg/mL)							2.94 (1.66-5.19)	<0.001

Abbreviations as in Table 1 and 2.

3.7.5.1 Table 5A. Incremental Value of Biomarkers for the detection of 1 year mortality.

	C-statistic (model 1)	C-statistic (model 2)	Δ C-statistic (95% CI)	*p*
Overall (n=447)	0.64	0.71	0.07 (0.02, 0.11)	0.002
LVEF <50% (n = 250)	0.65	0.74	0.08 (0.03, 0.14)	0.08
LVEF ≥50% (n =197)	0.60	0.68	0.08 (0.02, 0.15)	0.01

3.7.5.2 Table 5B. Evaluating added predictive ability of Model 2 beyond Model 1 for detection of events using NRI.

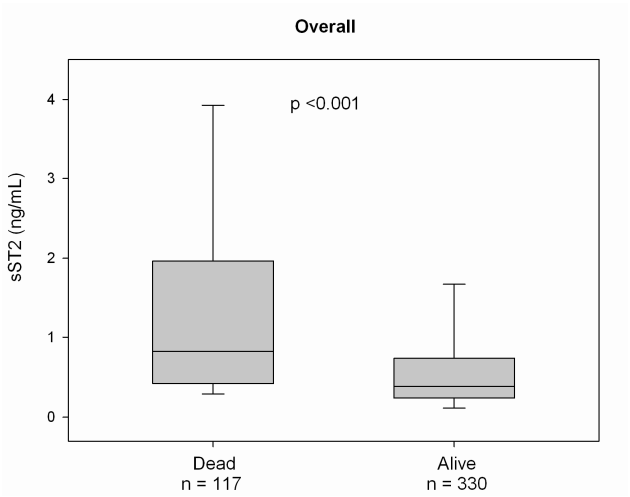
	NRI (95% CI)	Events % correctly reclassified	Non events % correctly reclassified	*p*
Overall (n=447)	0.56 (0.39, 0.73)	9%	48%	<0.0001
LVEF <50% (n = 250)	0.71 (0.49, 0.92)	26%	45%	<0.0001
LVEF ≥50% (n =197)	0.47 (0.22, 0.71)	16%	31%	0.0002

3.7.5.3 Table 5C. Evaluating added predictive ability of Model 2 beyond Model 1 for detection of events using IDI.

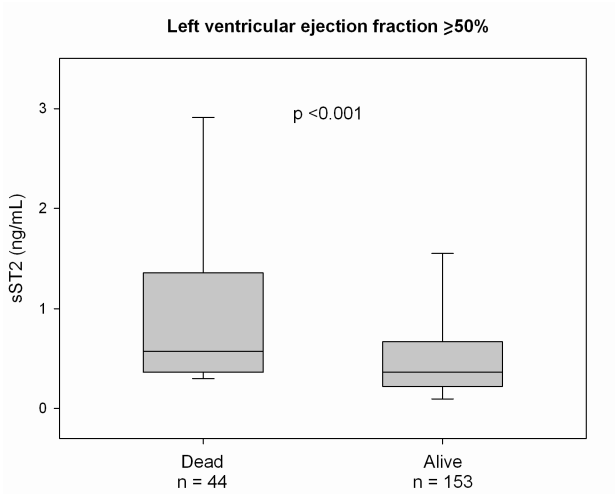
	IDI (95% CI)	Events change probability	Non events change probability	*p*
Overall (n=447)	0.04 (0.02, 0.06)	3%	-1.1%	<0.0001
LVEF <50% (n = 250)	0.07 (0.04, 0.10)	5%	-2.1%	<0.0001
LVEF ≥50% (n =197)	0.04 (0.01, 0.07)	3%	-0.9%	0.004

3.8.1 Figure 1: Soluble ST2 values as a function of 1 year mortality in A) all patients, and in patients with B) preserved and C) reduced left ventricular ejection fraction.

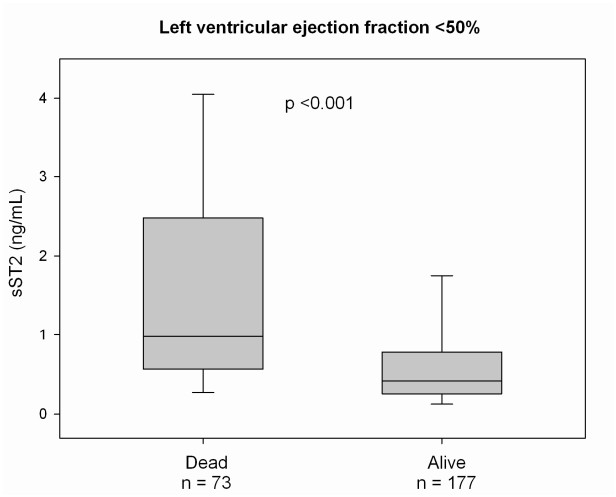
A



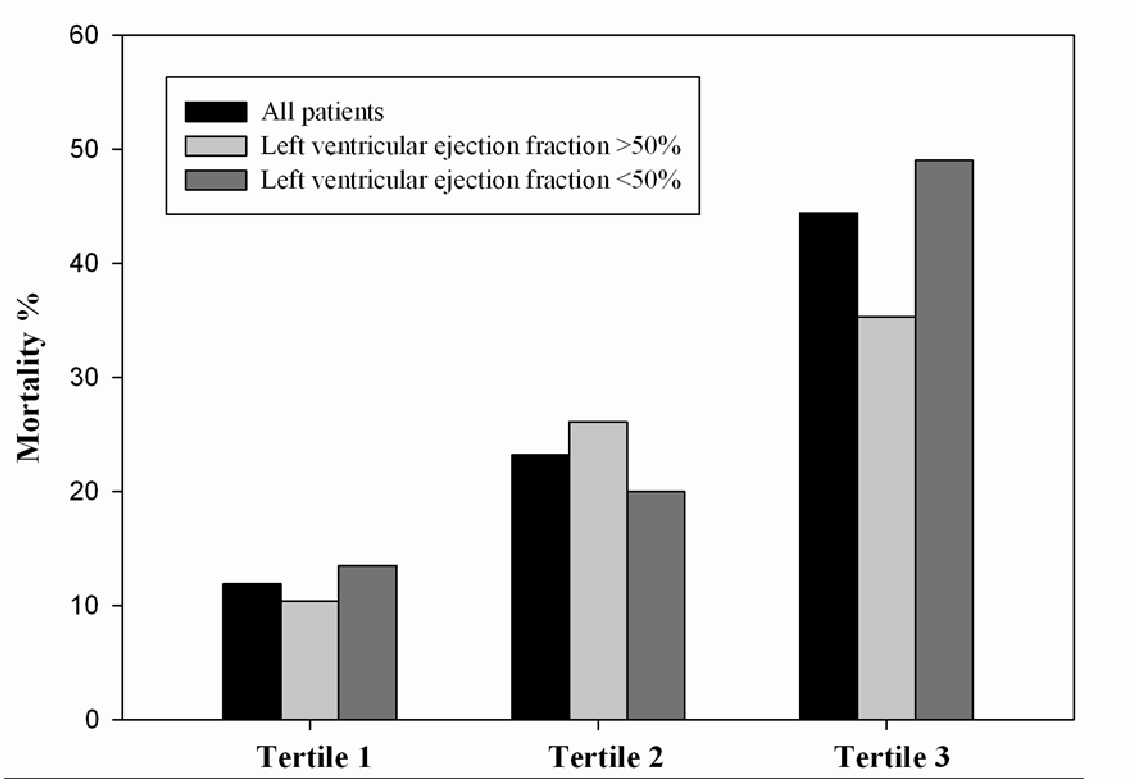
B



C



3.8.2 Figure 2: Tertile analysis, comparing concentrations of soluble ST2 relative to outcome in all subjects, as well as those with preserved or impaired left ventricular ejection fraction. Univariate and multivariate Cox proportional hazards for soluble ST2 tertiles as predictors of 1 year moratality are depicted.



	Tertile			P value
	1st ≤0.32 ng/mL	2nd 0.33-0.71 ng/mL	3rd ≥0.72 ng/mL	
All patients (n = 447)				
Mortality %	12	23	44	
*Unadjusted Hazard Ratio**	1	2.04 (1.15-3.60)	4.77 (2.83-8.03)	<0.001
Adjusted Hazard Ratio	1	1.68 (0.94-3.00)	3.08 (1.78-5.34)	<0.001
Left ventricular ejection fraction ≥50% (n = 197)				
Mortality %	10	26	35	
*Unadjusted Hazard Ratio**	1	2.67 (1.16-6.15)	4.07 (1.77-9.35)	0.004
Adjusted Hazard Ratio	1	2.63 (1.13-6.12)	4.18 (1.79-9.76)	0.004
Left ventricular ejection fraction <50% (n = 250)				
Mortality %	13	20	49	
*Unadjusted Hazard Ratio**	1	1.38 (0.69-2.74)	2.28 (1.19-4.37)	<0.001
Adjusted Hazard Ratio	1	0.90 (0.40-2.04)	2.64 (1.29-5.41)	<0.001

Adjusted for age, body mass index, systolic blood pressure, diastolic blood pressure, prior heart failure, left ventricular ejection fraction, New York Heart functional class, β-blocker, Angiotensin-converting enzyme inhibitor, haemoglobin, leukocytes, estimated glomerular filtration rate, blood urea nitrogen, C-reactive protein (per mg/dL) and NT-proBNP.

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4. Resúmenes en castellano de los artículos

4.1 Artículo 1.

4.1.1 Título

Valor pronóstico complementario de los niveles plasmáticos de Cistatina C, fragmento N-terminal del propéptido natriurético tipo B y Troponina T en pacientes con insuficiencia cardiaca aguda descompensada.

4.1.2 Objetivo

El objetivo de este estudio fue comparar el valor pronóstico de la concentración plamática de cistatina C frente al de otros marcadores de función renal clásicos como la creatinina y la tasa de filtrado glomerular estimada mediante la ecuación de MDRD. Además, en el presente estudio también evaluamos si este nuevo biomarcador de función renal es capaz de proporcionar información pronóstica complementaria a la aportada por otros biomarcadores cardiacos en la estratificación de riesgo de una cohorte no seleccionada de pacientes hospitalizados por insuficiencia cardiaca aguda descompensada.

4.1.3 Material y Métodos

Entre el 1 de Septiembre de 2006 y el 28 de Febrero de 2008, se incluyeron prospectivamente 138 pacientes consecutivos admitidos en el departamento de Cardiología del Hospital Universitario Virgen de la Arrixaca con diagnóstico final de insuficiencia cardiaca aguda descompensada. El diagnóstico de insuficiencia cardiaca aguda descompensada se realizó en base a los criterios establecidos en las guías de práctica clínica vigentes en cada momento[1]. A la llegada a urgencias se obtuvieron muestras de plasma sanguíneo en todos los pacientes. Durante el ingreso hospitalario se realizó: a) una recogida exhaustiva de variables *clínicas* y *analíticas*. Los pacientes incluidos fueron tratados acorde a las guías de práctica clínica vigentes en cada momento y la toma de decisiones diagnóstico terapéuticas dependió de su cardiólogo

responsable que fue ciego para los niveles de cistatina C. Además se realizó un ecocardiograma previo al alta hospitalaria en el que se calculó la fracción de eyección ventricular izquierda mediante el método Simpson biplano.

Tras el ingreso hospitalario, todos los pacientes fueron seguidos al menos durante 6 meses (mediana 261 días [161-449]) registrándose la aparición de eventos clínicos adversos en todos. El seguimiento de los pacientes se llevó cabo mediante contacto telefónico con el propio paciente o sus familiares, contacto clínico y la revisión de su historia clínica. Los eventos clínicos de estudio fueron la re-hospitalización por descompensación cardiaca y la mortalidad por cualquier causa. La causa de la muerte fue averiguada a través de las historias clínicas y los certificados médicos de defunción. Todos los pacientes incluidos fueron ampliamente informados sobre el objeto del estudio, siendo necesario la firma del consentimiento de participación. Se mantuvo en todo momento la confidencialidad de los datos.

La determinación de los niveles plasmáticos de cistatina C se realizó con el analizador BN ProSpec (Dade Behring). El coeficiente intra-ensayo de variación fue 2.5% para 0.9 mg/L y 2.3% para 1.8 mg/L. El coeficiente inter-ensayo de variación fue 2.0% para 0.9 mg/L y 2.2% para 1.8 mg/L. La función renal también fue evaluada mediante los niveles séricos de creatinina y la ecuación simplificada de MDRD (mL/min/1.73 m^2, 186.3× [plasma creatinina]–1.154 × [edad]–0.203) (el factor de corrección para mujeres fue ×0.742)[11].

Mediante el test de Kolmogorov-Smirnov se comprobó la normalidad de la distribución en las variables cuantitativas; las variables con distribución no normal se expresaron como mediana (RIC) y el resto, como media ± desviación estándar (DE) para variables cuantitativas, y número (%) para cualitativas. Debido a que los rangos de normalidad de los niveles plasmáticos de cistatina C no están establecidos, la población de estudio fue clasificada en función de los terciles de cistatina C. A nivel basal las características clínicas, analíticas y ecocardiográficas de la población de estudio fueron comparadas mediante el test de ANOVA o de Kruskal-Wallis para

variables cuantitativas y el test de χ^2 para las variables categóricas. Se realizó un análisis de regresión de Cox para el estudio univariable de predictores de eventos clínicos adversos durante el seguimiento. Las variables con p <0.1 en el análisis univariable fueron introducidas en un modelo multivariable de Cox con el objetivo de identificar los predictores independientes de muerte y/o re-hospitalización por insuficiencia cardiaca aguda descompensada. Los niveles de cistatina C, creatinina y MDRD fueron introducidos en primer lugar de forma individual en el modelo multivariable. Posteriormente, los niveles de creatinina y MDRD fueron ajustados por cistatina C en un análisis por separado. Para comparar las diferentes medidas de función renal como predictores de eventos clínicos adversos, los niveles de cistatina C, creatinina y MDRD fueron divididos por terciles. Los terciles de creatinina se definieron en función del sexo para asegurar la adecuada representación de ambos sexos entre grupos. Se calcularon los riesgos para el 3º y 2º terciles respecto al 1º tercil para cada medida de función renal. Para testar si la valoración simultánea de cistatina C, troponina T y NT-proBNP mejora la estratificación de riesgo, los pacientes fueron categorizados en base al número de "biomarcadores elevados". Se definió como "biomarcador elevado" a la presencia de niveles de cistatina C, TnT o NT-proBNP por encima de la mediana (>1.21mg/L para cistatina C, >0.011ng/ml para troponina T y >3.345pg/ml para NT-proBNP). La incidencia acumulada de mortalidad y/o reingreso por insuficiencia cardiaca aguda descompensada fue estimada por el método de Kaplan–Meier y el estadístico de log-rank fue utilizado para las comparaciones. Se consideró significativo un valor de p <0.05. El análisis estadístico se realizó mediante el paquete de *software* estadístico para ciencias sociales (SPSS v. 15.0 para Windows, SPSS Inc., Chicago, Illinois, Estados Unidos).

4.1.4 Resultados

Un total de 138 pacientes fueron incluidos en el análisis de estudio. La mediana de la concentración de cistatina C fue 1.21 [0.97-1.67] mg/L, la mediana de creatinina

sérica fue 1.15 [0.87-1.45] mg/dl y la media de MDRD fue 63±25 ml/min por 1.73m^2. Las tablas 1 y 2 muestran la distribución de las características clínicas y de laboratorio en función de los terciles de cistatina C. Los pacientes con niveles más elevados de cistatina C presentaron una edad más avanzada, una mayor prevalencia de anemia y necesitaron de forma más frecuente soporte inotrópico durante el ingreso hospitalario. Los niveles séricos de creatinina, urea, ácido úrico y NT-proBNP fueron más elevados en los pacientes con niveles de cistatina C elevados; mientras que la albúmina sérica, la hemoglobina y el MDRD fueron inferiores en estos pacientes. Los niveles de cistatina C correlacionaron significativamente con la creatinina y el MDRD [Coeficiente de correlación de Spearman r=0.77 y -0.67 (p<0.001), respectivamente].

Durante el periodo de estudio, un total de 60 pacientes (43.5%) presentaron eventos clínicos adversos: 27 pacientes murieron y 40 pacientes fueron readmitidos debido a insuficiencia cardiaca aguda descompensada. En el análisis univariable de regression de Cox, todas las medidas de función renal se asociaron con un mayor riesgo de eventos clínicos adversos (Tabla 3). Sin embargo, tras el ajuste por otros predictores de eventos en el análisis multivariable de regresión de Cox, la concentración de cistatina C (mg/L, HR 1.46 95%CI 1.02-2.09; p=0.037) fue predictora independiente de eventos adversos, mientras que la creatinina sérica y el MDRD no alcanzaron la significación estadística. Cuando los niveles de cistatina C, creatinina y MDRD fueron introducidos de forma combinada en un modelo multivariable, la cistatina C fue también la única medida de función renal que permaneció como predictora independiente de eventos clínicos adversos (mg/dl, HR 1.46 95%CI 1.02-2.09; p=0.037). Otros predictores independientes de muerte y/o reingreso por insuficiencia cardiaca aguda descompensada fueron la clase funcional avanzada (NYHA III/IV), la presencia de diabetes mellitus y los niveles elevados de NT-proBNP y troponina T (Tabla 3). Tal y como muestra el análisis de supervivencia de Kaplan-Meier (Figure 1), los terciles de cistatina C se asociaron con un incremento de la tasa de eventos adversos. La mortalidad y/o re-hospitalización por descompensación

cardiaca al año de seguimiento fue 65% para el 3º tercil de cistatina C, 44.1% para el 2º tercil de cistatina C y 30.1% para el 1º tercil de cistatina C (log rank test p=0.001). En el análisis univariable de regresión de Cox y tras el ajuste multivariable, el tercil más elevado de cistatina C (>1.50 mg/L) se asoció de forma significativa con una mayor mortalidad y/o re-hospitalización por insuficiencia cardiaca aguda descompensada (HR 3.08 95%CI 1.54-6.14; p=0.004). Por el contrario, el tercil más elevado de creatinina y MDRD sólo alcanzó la significación en el análisis univariable (Tabla 4).

Al introducir en un mismo análisis de regresión multivariable de Cox los 3 biomarcadores como variables dicotómicas [cistatina C elevada (>1.21mg/L; HR 1.92, 95%IC 1.12-3.28; p=0.017), NT-proBNP elevado (>3.345pg/ml; HR 1.79, 95%IC 1.06-3.03; p=0.029) y troponina T elevada (>0.011ng/ml; HR 2.01, 95%IC 1.24-3.41; p=0.003)], encontramos que cada uno de ellos fue predictor independiente de eventos clínicos adversos. Tras estratificar a los pacientes en base al número de biomarcadores elevados encontramos que el 23% presentó los tres biomarcadores no elevados, el 25% un biomarcador elevado, el 28% dos biomarcadores elevados, y el 24% los tres biomarcadores elevados. Además existió un incremento gradual significativo del riesgo de muerte y/o reingreso por IC descompensada conforme aumentaba el número de biomarcadores elevados. De este modo, el 25.8%, 37.1%, 43.6% y 66.7% de pacientes con 0, 1, 2 y 3 biomarcadores elevados respectivamente presentaron eventos adversos; p para tendencia=0.015). La figura 2 muestra el riesgo relativo de muerte y/o reingreso por insuficiencia cardiaca aguda descompensada, de acuerdo al número de biomarcadores elevados.

4.1.5 Conclusiones

Los principales hallazgos de este estudio fueron: i) los niveles elevados de cistatina C se asociaron de forma independiente con la incidencia de mortalidad y / o reingreso por descompensación cardiaca en pacientes hospitalizados con insuficiencia

cardiaca aguda descompensada, ii) los niveles de cistatina C fueron superiores a los niveles séricos de creatinina y MDRD para la predicción de eventos clínicos adversos, iii) la evaluación simultánea de cistatina C, troponina T y NT-proBNP aportó información pronóstica complementaria y podría ayudar a la realización de una estratificación del riesgo más precisa en este tipo de pacientes.

4.1.6.1 Tabla 1. Características clínicas basales.

Variables	Terciles 1º (n=48)	2° (n=45)	3° (n=45)	*p*
Edad (años)	72 [56-78]	76 [68-78]	78 [71-84]	0.002
Varones	24 (50%)	27 (60%)	23 (51%)	0.576
IMC, Kg/m^2	28 [25-33]	29 [26-32]	28 [26-31]	0.522
PAS (mmHg)	149±40	151±32	153±38	0.937
PAD (mmHg)	86±21	86±20	82±19	0.828
Frecuencia cardiaca (latido/min)	112±30	100±35	101±23	0.371
FEVI	47 [37-60]	49 [30-63]	52 [33-65]	0.946
NYHA III-IV	11 (23%)	14 (31%)	19 (42%)	0.135
Insuficiencia cardiaca crónica	26 (54%)	29 (64%)	31 (69%)	0.321
Insuficiencia cardiaca isquémica	16 (33%)	14 (31%)	18 (40%)	0.534
Diabetes mellitus	23 (48%)	26 (58%)	21 (47%)	0.511
Hipertensión arterial	37 (79%)	39 (87%)	38 (84%)	0.574
Hiperlipidemia	16 (33%)	17 (38%)	21 (47%)	0.410
Tabaquismo activo	11 (23%)	7 (16%)	5 (11%)	0.303
Fibrilación/Flutter auricular	27 (59%)	33 (73%)	26 (58%)	0.228
Bloqueo de rama	13 (27%)	16 (36%)	13 (29%)	0.650
IAMEST previo	10 (21%)	8 (18%)	18 (40%)	0.033
Accidente cerebrovascular previo	7 (15%)	9 (20%)	6 (13%)	0.655
Anemia	15 (31%)	18 (40%)	31 (69%)	0.001
EPOC	3 (6%)	7 (16%)	8 (18%)	0.213
Uso de inotrópicos intrahospitalario	3 (6%)	4 (9%)	12 (27%)	0.009
Tratamiento al alta				
Beta-bloqueantes	31 (66%)	26 (59%)	17 (44%)	0.107
Inhibidores de la ECA /ARAII	40 (84%)	41 (91%)	35 (85%)	0.951
Estatinas	25 (53%)	24 (54%)	22 (56%)	0.956
Espironolactona/Eplerenona	18 (38%)	18 (41%)	11 (28%)	0.451
Diuréticos de ASA	43 (91%)	41 (93%)	38 (97%)	0.261

Datos expresados como media ± DE o mediana (cuartiles), y número (%). IMC = Índice de Masa Corporal, PAS = Presión arterial sistólica, PAD = Presión arterial diastólica, FEVI = Fracción de eyección ventricular izquierda, NYHA = New York Heart Association, IAMEST = infarto agudo de miocardio con elevación persistente del segmento ST, EPOC = Enfermedad pulmonar obstructiva crónica, IECA = Inhibidores de la enzima convertidora de angiotensina, ARA II = Antagonistas de los receptores de angiotensina II.

4.1.6.2 Tabla 2. Parámetros de laboratorio en función de los terciles de cistatina C.

Variables	Terciles 1° (n=48)	2° (n=45)	3° (n=45)	*p*
Hemoglobina (g/dL)	13.4±2.0	12.8±1.9	11.3±1.8	<0.001
Glucosa (mg/dL)	181±97	166±79	158±61	0.792
Cistatina C (mg/L)	0.86 [0.77-0.97]	1.21 [1.10-1.39]	1.85 [1.65-2.44]	<0.001
Creatinina (mg/dL)	0.82 [0.77-1.00]	1.14 [0.94-1.29]	1.61 [1.29-2.08]	<0.001
TFG estimada (ml/min/1.73^2)	83.6±19.9	63.9±19.2	41.1±16.8	<0.001
Urea (mg/dL)	38 [33-48]	51 [42-64]	79 [54-115]	<0.001
Albumina (g/dL)	4.2±0.4	4.1±0.5	3.9±0.4	0.006
Sodio(mEq/L)	139±4.5	139±6.8	138±5.4	0.754
Ácido úrico (mg/dL)	6.4±2.0	7.8±2.7	9.1±2.5	<0.001
Proteina C reactiva (mg/dL)	0.9 [0.4-2.1]	0.9 [0.5-5.0]	1.5 [0.7-4.3]	0.270
Troponina T (ng/mL)	0.01 [0.01-0.03]	0.01-[0.01-0.58]	0.02 [0.01-0.08]	0.094
NT-proBNP (pg/mL)	2358 [1359-3853]	3571 [1680-7597]	5255 [2968-14543]	<0.001

Datos expresados como media ± DE o mediana (cuartiles), y número (%). TFG = Tasa de filtrado glomerular.

4.1.6.3 Tabla 3. Análisis de regresión de Cox.

	Univariable		Multivariable*	
Variables	Hazard Ratio	*p*	Hazard Ratio	*p*
Edad (por año)	1.03 (1.01-1.05)	0.012	-	0.379
Hiperlipidemia	1.62 (0.97-2.69)	0.062	-	0.432
NYHA III-IV	3.17 (1.89-5.32)	<0.001	3.13 (1.85-5.29)	<0.001
Diabetes mellitus	1.78 (1.06-3.00)	0.030	1.81 (1.07-3.07)	0.028
IAMEST previo.	1.75 (1.01-3.05)	0.046	-	0.208
Anemia	1.69 (1.01-2.82)	0.046	-	0.757
Uso inotrópicos hospitalario	2.07 (1.10-3.90)	0.024	-	0.460
NT-proBNP (por 100 pg/dL)	1.003 (1.001-1.006)	0.013	1.004 (1.001-1.007)	0.032
Troponina T (por ng/mL)	2.48 (1.38-4.45)	0.002	2.46 (1.33-4.54)	0.004
Cistatina C (por mg/L)	1.64 (1.19-2.27)	0.003	1.46 (1.02-2.09)	0.037
Creatinina (por mg/dL)	1.55 (1.07-2.24)	0.019	-	0.114
TFG (por mL/min/1.73m^2)	0.96 (0.97-0.99)	0.011	-	0.104

Datos expresados como media±DE o mediana (cuartiles), y número (%).Abreviaturas como Tabla 1 y 2.

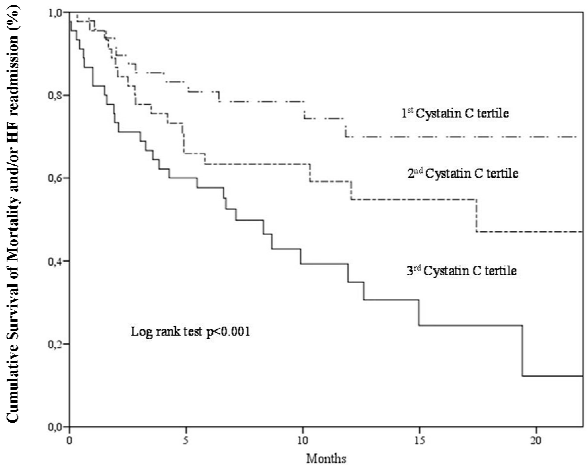
*Cistatina C (mg/L), creatinina (mg/dL) y TFG (mL/min/1.73m^2) fueron introducidos por separado en el análisis multivariable. Los "HR" y "p" para el resto de variables provienen del modelo que incluyó la cistatina C.

4.1.6.3 Tabla 4. Parámetros de función renal y riesgo de eventos adversos.

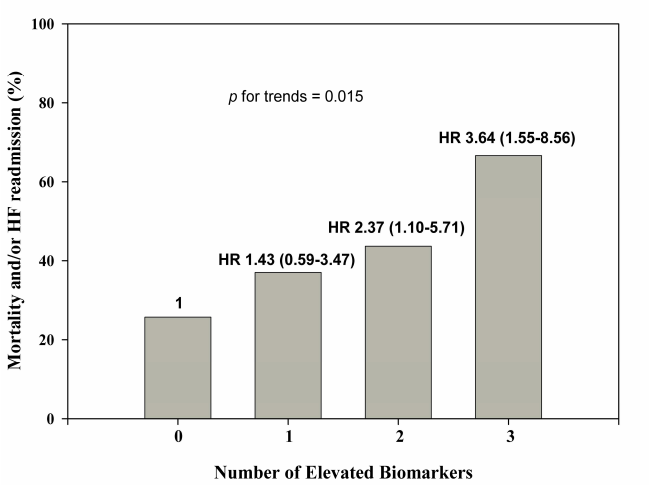
	Terciles			
Variables	1°	2°	3°	*p*
Cistatina C (por mg/L)				
Rango	0.63-1.04	1.04-1.50	1.50-3.97	
n	48	45	45	
Hazard Ratio no ajustado	1	1.89 (0.91-3.88)	3.43 (1.75-6.75)	0.001
Hazard Ratio ajustado*	1	1.61 (0.78-3.33)	3.08 (1.54-6.14)	0.004
Creatinina (por mg/dL)				
Rango en mujeres	0.52-0.82	0.82-1.20	1.20-2.48	
Rango en varones	0.60-1.13	1.13-1.41	1.41-3.69	
n	47	46	45	
Hazard Ratio no ajustado	1	1.38 (0.69-2.74)	2.28 (1.19-4.37)	0.034
Hazard Ratio ajustado*	1	1.62 (0.78-3.36)	1.76 (0.85-3.63)	0.280
TFG estimada (per mL/min/1.73^2)				
Rango	17-48	48-71	72-142	
n	46	46	46	
Hazard Ratio no ajustado	1	0.64 (0.36-1.14)	0.35 (0.18-0.69)	0.009
Hazard Ratio ajustado*	1	0.86 (0.47-1.58)	0.52 (0.25-1.08)	0.207

*Ajustado por edad, NYHA Functional Classification, diabetes mellitus, hiperlipidemia, IAMEST previo, anemia, uso de inotrópicos intrahospitalario, troponina T y NT-proBNP. Abreviaturas como en Tabla 1.

4.1.7.1 Figura 1. Análisis de supervivencia de Kaplan-Meier para la aparición de eventos clínicos adversos a lo largo del seguimiento en función de los terciles de cistatina C.



4.1.7.2 Figura 2. Eventos clínicos adversos en función del número de biomarcadores elevados *Ajustado por edad, NYHA, diabetes mellitus, hiperlipemia, historia previa de IAMEST, anemia y uso de inotrópicos hospitalario.



4.2 Artículo 2.

4.2.1 Título

Valor pronóstico a largo plazo de los niveles plasmáticos de β-traza proteína y cistatina C en pacientes con insuficiencia cardiaca aguda descompensada

4.2.2 Objetivo

La β-traza proteína (BTP) y la cistatina C son 2 nuevos biomarcadores de función renal cuyo papel pronóstico respecto al de otros marcadores estándar de función renal permanece desconocido. El objetivo de este estudio fue evaluar el valor pronóstico de estos 2 nuevos biomarcadores de función renal en pacientes con insuficiencia cardiaca aguda descompensada.

4.2.3 Material y Métodos

Desde Septiembre de 2006 hasta Marzo de 2009, se incluyeron prospectivamente 220 pacientes consecutivos admitidos en el departamento de Cardiología del Hospital Universitario Virgen de la Arrixaca con diagnóstico final de insuficiencia cardiaca aguda descompensada. El diagnóstico de insuficiencia cardiaca aguda descompensada se realizó en base a los criterios establecidos en las guías de práctica clínica vigentes en cada momento[1]. A la llegada a urgencias se obtuvieron muestras de plasma sanguíneo en todos los pacientes. Durante el ingreso hospitalario se realizó: a) una recogida exhaustiva de variables *clínicas* y *analíticas*. Los pacientes incluidos fueron tratados acorde a las guías de práctica clínica vigentes en cada momento y la toma de decisiones diagnóstico terapéuticas dependió de su cardiólogo responsable que fue ciego para los niveles de BTP y cistatina C. Además se realizó un ecocardiograma previo al alta hospitalaria en el que se calculó la fracción de eyección ventricular izquierda mediante el método Simpson biplano.

La determinación de los niveles plasmáticos de BTP y cistatina C se realizó con el analizador BN ProSpec (Dade Behring). Los coeficientes de variación intra-ensayo e inter-ensayo para BTP fueron 2.8% y 4.7% respectivamente; y para cistatina C 2.5% y 2%. La función renal también fue evaluada mediante los niveles séricos de creatinina y la ecuación simplificada de MDRD (mL/min/1.73 m^2, 186.3× [plasma creatinina]–1.154 × [edad]–0.203) (el factor de corrección para mujeres fue ×0.742)[18] y el nitrógeno ureico en sangre.

Tras el ingreso hospitalario, todos los pacientes fueron seguidos con el objetivo de registrar la aparición de eventos clínicos adversos. El seguimiento de los pacientes se llevó cabo mediante contacto telefónico con el propio paciente o sus familiares, contacto clínico y la revisión de su historia clínica. La mediana de seguimiento fue 500 días con un rango intercuartil de 231 a 796 días. Los eventos clínicos de estudio fueron la re-hospitalización por descompensación cardiaca y la mortalidad por cualquier causa. También se estudió la aparición de insuficiencia renal intrahospitalaria que fue definida como la presencia de un incremento de la creatinina sérica ≥0.3 mg/dL durante el periodo de hospitalización[19]. La causa de la muerte fue averiguada a través de las historias clínicas y los certificados médicos de defunción. Todos los pacientes incluidos fueron ampliamente informados sobre el objeto del estudio, siendo necesario la firma del consentimiento de participación. Se mantuvo en todo momento la confidencialidad de los datos.

Mediante el test de Kolmogorov-Smirnov se comprobó la normalidad de la distribución en las variables cuantitativas; las variables con distribución no normal se expresaron como mediana (RIC) y el resto, como media ± desviación estándar (DE) para variables cuantitativas, y número (%) para cualitativas. Las características clínicas, analíticas y ecocardiográficas de la población de estudio fueron comparadas mediante el test de la t de student o de la U de Mann-Whitney para variables cuantitativas y el test de χ^2 para las variables categóricas. La relación entre los niveles plasmáticos de BTP, cistatina C y otros parámetros cuantitativos clínicos y analíticos

fue valorada a través del coeficiente de correlación de Spearman. La relación entre las diferentes variables y la aparición de fallo renal intrahospitalario fue valorada usando modelos de regresión logística univariable y multivariable. Para comparar la precisión pronóstica de BTP y cistatina C se realizaron comparaciones entre las curvas ROC obtenidas para los diferentes parámetros de función renal evaluados. Para comparar los valores predictivos de cada uno de los parámetros de función renal se construyeron curvas ROC para sensibilidad, especificidad, valor predictivo positivo, y valor predictivo negativo. Además, se realizaron análisis de "Net reclassification improvement" (NRI) e "integrated discrimination improvement" (IDI) con los biomarcadores de función renal tomados como variables dicotómicas tal y como ha sido descrito por Pencina et al[20], donde las categorias de probabilidad para eventos fueron definidas en base al modelo de estratificación pronostico en insuficiencia cardiaca de Heart Failure Survival Score[21]. Por otro lado se realizó un análisis de regresión de Cox para el estudio univariable de predictores de eventos clínicos adversos durante el seguimiento. Las variables con $p < 0.1$ en el análisis univariable fueron introducidas en un modelo multivariable de Cox con el objetivo de identificar los predictores independientes de muerte y/o re-hospitalización por insuficiencia cardiaca aguda. Para evitar el efecto de la colinealidad, debido a su extramadamente alta correlación ($r > 0.80$), la creatinina sérica y el MDRD, así como la BTP y cistatina C, no fueron introducidos de manera conjunta en los modelos multivariables. La incidencia acumulada de mortalidad y/o reingreso por insuficiencia cardiaca aguda fue estimada por el método de Kaplan–Meier y el estadístico de log-rank fue utilizado para las comparaciones. Se consideró significativo un valor de $p < 0.05$. El análisis estadístico se realizó mediante el paquete de *software* estadístico para ciencias sociales (SPSS v. 15.0 para Windows, SPSS Inc., Chicago, Illinois, Estados Unidos).

4.2.4 Resultados

En la población de estudio, la concentración plasmática de BTP fue 0.97 [0.74-1.37] mg/L, la cistatina C plasmática fue 1.15 [0.90-1.59] mg/L, la creatinina sérica fue 1.14 [0.84-1.45] mg/dL, la tasa de filtrado glomerular estimada por MDRD fue 64 [45-78] mL/min per 1.73m^2, y el nitrógeno ureico en sangre fue 24 [18-34] mg/dL.

La concentración plasmática de BTP correlacionó positivamente con la concentración plasmática de cistatina C (r = 0.86; p <0.001). Además, las concentraciones plasmáticas de BTP y cistatina C correlacionaron positivamente con la creatinina sérica, el nitrógeno ureico en sangre, la edad, la clase funcional de la New York Heart Association, el NT-proBNP, el ácido úrico, la troponina T y la proteína C reactiva. Por el contrario, la tasa de filtrado glomerular estimada mediante MDRD, la albúmina sérica y la concentración de hemoglobina plasmática correlacionaron negativamente con los niveles de ambos marcadores de función renal. Las concentraciones de BTP y cistatina C no correlacionaron ni con el índice de masa corporal ni con la fracción de eyección ventricular izquierda (Tabla 1).

Durante el ingreso hospitalario 66 pacientes (30%) presentaron empeoramiento de la función renal. Los niveles basales de los marcadores de disfunción renal estudiados fueron similares entre los pacientes que presentaron empeoramiento de la función renal intrahospitalario y los que no: BTP: 1.03 [0.75-1.44] vs 0.96 mg/L [0.71-1.35]; p=0.35, cistatina: C 1.12 [0.87-1.71] vs 1.19 mg/L [0.90-1.54], p=0.73, creatinina sérica: 1.11 [0.84-1.49] vs 1.15 mg/L [0.85-1.44]; p=0.83, MDRD: 64 [44-77] vs 64 mL/min por 1.73m^2 [45-79]; p=0.77 y nitrógeno ureico sanguíneo: 24 [21-34] vs 24 mg/L [17-34]; p=0.32. Tras el ajuste multivariable en un modelo de regresión logística múltiple, sólo la historia previa de cirugía de revascularización coronaria (OR 3.87 95%CI 1.120-12.5; p=0.023) y el sexo femenino (OR 2.07 95%CI 1.13-3.78; p=0.019) se asociaron significativamente con un mayor riesgo de fallo renal intrahospitalario.

Durante el periodo de estudio (mediana 500 días, rango intercuartil 231 a 796), un total de 116 pacientes (53%) presentaron eventos clínicos adversos: 62 pacientes

murieron y 76 fueron readmitidos a un hospital por insuficiencia cardiaca aguda descompensada. Las tablas 2 y 3 muestran la distribución de las características clínicas y analíticas de la población de estudio en función de la presencia de eventos clínicos adversos durante el seguimiento. Los pacientes que sufrieron eventos presentaron una mayor edad, tuvieron más frecuentemente historia previa de insuficiencia cardiaca crónica, peor clase funcional, mayor necesidad de soporte inotrópico positivo y sufrieron más frecuentemente fallo renal intrahospitalario.

Los pacientes con eventos adversos tuvieron niveles plasmáticos más elevados de BTP (1.04 [0.80-1.49] vs 0.88 mg/L [0.68-1.17], p=0.003; Figura 1A) y de cistatina C (1.29 [1.00-1.71] vs 1.03 mg/L [0.86-1.43], p=0.001; Figura 1B) que los pacientes sin eventos. Los niveles de creatinina sérica, nitrógeno ureico en sangre, NT-proBNP, y troponina T fueron también más altos en los pacientes que presentaron eventos; mientras que la glucosa y la tasa de filtrado glomerular estimada mediante MDRD fueron menores en estos pacientes.

El análisis por terciles de las concentraciones de BTP y cistatina C en función de la tasa de eventos clínicos adversos reveló la existencia de un incremento gradual de la mortalidad y/o reingreso por insuficiencia cardiaca aguda descompensada conforme incrementaron las concentraciones de ambos biomarcadores (Figura 2).

Para definir la precisión pronóstica de los niveles de BTP y cistatina C para en la predicción de eventos, realizamos análisis de curvas ROC. Además, para realizar la comparación con el resto de medidas de función renal realizamos el mismo análisis de curvas ROC con creatinina, MDRD y nitrógeno ureico en sangre. Tal y como aparece detallado en la Tabla 4, las concentraciones plasmáticas de BTP y cistatina C presentaron áreas bajo la curva comparables, con características de rendimiento general similares, si no ligeramente superiores a las medidas convencionales de función renal. El NRI tras la adicción de cistatina C a la tasa de filtrado glomerular estimada por MDRD fue 0.28 (95% IC 0.13 a 0.43, p =0.002), mientras que el IDI fue 0.05 (95% IC 0.02 a 0.08, p =0.001). La probabilidad de predecir correctamente

muerte y/o reingreso por insuficiencia cardiaca aguda al añadir la cistatina C sobre la tasa de filtrado glomerular estimada por MDRD se vio reflejada particularmente en el porcentaje de no eventos correctamente reclasificados (44%), mientras que el % de eventos reclasificados fue -16%. Comparado con los niveles de cistatina C, la BTP presentó un NRI de 0% tanto para eventos como para no eventos, con un IDI de 0.00008; lo cuál indica que la BTP ofreció una información similar a la cistatina C.

En el análisis univariado de regresión de Cox, todas las medidas de función renal se asociaron con un mayor riesgo de eventos clínicos adversos (Tabla 5). Sin embargo, tras ajustar por factores de confusión, las concentraciones plasmáticas de BTP y cistatina C permanecieron como factores predictores significativos de eventos adversos, mientras que la creatinina sérica, el MDRD y el nitrógeno ureico en sangre no fueron predictores (Tabla 5).Esto siguió siendo el caso cuando las medidas de función renal fueron evaluadas como variables dicotómicas en el análisis de regresión multiple de Cox: los niveles de BTP y cistatina C fueron predictores significativos de eventos adversos (HR 1.54, 95%IC 1.10-2.38; p = 0.02 para BTP >0.96 mg/L y HR 1.73 95%IC 1.15-2.62; p = 0.009 para cistatina C >1.05 mg/L), pero la creatinina, la tasa de filtrado glomerular estimada y el nitrógeno ureico no lo fueron (p >0.2). Además, cuando los niveles de BTP y cistatina C fueron ajustados por otras medidas de función renal clásicas, ambos permanecieron como predictores independientes de eventos adversos (Log_{10} BTP, HR 3.01, 95% IC 1.08-7.87, p = 0.033 y Log_{10} cistatina C, HR 3.56, 95% IC 1.22-9.35, p = 0.018).

Tal y como muestran los análisis de supervivencia de Kaplan-Meier, los niveles elevados en plasma de BTP (>0.96 mg/L) y cistatina C (>1.05 mg/L) se asociaron con un incremento del riesgo de eventos clínicos adversos (Figura 3; log rank test p <0.05). Además, tras realizar un análisis estratificado en los pacientes pacientes con MDRD >60 mL/min/1.73m^2 (n = 146), los niveles elevados de ambos marcadores de función renal fueron también predictores de un mayor riesgo de muerte y/o re-hospitalización por insuficiencia cardiaca aguda descompensada (Figura 4; log rank test p <0.05).

4.2.5 Conclusiones

Los principales hallazgos de este estudio fueron: i) En pacientes con insuficiencia cardiaca aguda descompensada los niveles plasmáticos de BTP y cistatina C medidos en urgencias añaden valor pronóstico complementario al aportado por otras variables clínicas ii) Los niveles plasmáticos de BTP y cistatina C fueron prácticamente equiparables en cuanto a su capacidad de predicción de eventos adversos; y superiores a los marcadores de función renal estándar como la creatinina, el MDRD y el nitrógeno ureico en sangre, todas ellos marcadores pronósticos bien establecidos en este contexto clínico iii) Además, los niveles plasmáticos elevados de BTP y cistatina C se asociaron con un riesgo elevado de eventos adversos en aquellos pacientes con tasas de filtrado glomerular >60 mL/min iv) Los niveles plasmáticos en urgencias de BTP y cistatina C, así como los del resto de marcadores de función renal examinados, no fueron predictores de fallo renal intrahospitalario. Esto sugiere que la función renal basal puede ser menos importante para la predicción de fallo renal intrahospitalario que otros factores clínicos, hemodinámicas y terapéuticos presentes en los pacientes con insuficiencia cardiaca aguda descompensada.

4.2.6.1 Tabla 1. Análisis de correlación.

	β-traza proteína		Cistatina C	
Variables	r	*p*	r	*p*
Edad (año)	0.32	<0.001	0.29	<0.001
Albumina (g/dL)	-0.13	0.05	- 0.19	0.004
Índice de Masa Corporal (Kg/m^2)	- 0.06	0.42	0.09	0.23
Proteína C reactiva (mg/dL)	0.16	0.026	0.21	0.003
Creatinina (mg/dL)	0.70	<0.001	0.73	<0.001
Cistatina C (mg/L)	0.86	<0.001	-	-
TFG estimada ($mL/min/1.73^2$)	- 0.71	<0.001	- 0.76	<0.001
Hemoglobina (g/dL)	- 0.38	<0.001	- 0.33	<0.001
FEVI	0.07	0.28	0.04	0.56
NYHA (I-IV)	0.22	0.001	0.25	<0.001
NT-proBNP (pg/mL)	0.29	<0.001	0.37	<0.001
Troponina T (ng/dL)	0.13	0.06	0.16	0.02
Nitrógeno ureico sangre (mg/dL)	0.67	<0.001	0.72	<0.001
Ácido úrico (mg/dL)	0.40	<0.001	0.45	<0.001

TFG = Tasa de filtrado glomerular, FEVI = Fracción de eyección ventricular izquierda y NYHA = New York Heart Association.

4.2.6.2 Tabla 2. Características clínicas basales.

Variables	Eventos (n=116)	No eventos (n=104)	*p*
Edad (años)	75±10	69±13	0.002
Hombres	67 (57.3)	49 (47.2)	0.15
Índice de Masa Corporal (Kg/m^2)	29±5	29±4	0.65
PAS (mmHg)	150±37	153±34	0.69
Frecuencia cardiaca (latido/min)	100±28	100±34	0.96
FEVI	45±17	48±16	0.26
NYHA III-IV	53 (45.3)	12 (11.7)	<0.001
Insuficiencia cardiaca crónica	85 (72.6)	50 (48.5)	<0.001
Bypass coronario previo	6 (5.1)	7 (6.8)	0.60
ICP previo	32 (27.4)	20 (19.4)	0.17
Insuficiencia cardiaca isquémica	40 (34.2)	32 (31.1)	0.62
Diabetes mellitus	66 (56.4)	48 (46.6)	0.15
Hipertensión arterial	95 (81.2)	85 (82.5)	0.80
Hiperlipiemia	50 (42.7)	43 (41.7)	0.88
Tabaquismo activo	14 (12)	17 (16.5)	0.33
Enfermedad arterial periférica	11 (9.4)	6 (5.8)	0.32
Fibrilación o fluter auricular	72 (61.5)	54 (52.4)	0.17
Bloqueo de rama	39 (34.2)	30 (29.1)	0.42
IAMEST previo	36 (30.8)	21 (20.4)	0.08
ACV previo	15 (12.8)	13 (12.6)	0.96
Anemia	60 (51.3)	43 (41.7)	0.16
EPOC	31 (26.5)	22 (21.4)	0.37
Uso inotrópicos intrahospitalario	20 (17.1)	3 (2.9)	0.001
Fallo renal intrahospitalario	43 (37)	23 (22)	0.016
Tratamiento al alta			
Betabloqueantes	58 (55.2)	74 (72.5)	0.01
IECA/ARA II	88 (83.8)	87 (85.3)	0.77
Estatinas	58 (55.2)	55 (53.9)	0.85
Antagonistas de la aldosterona	34 (32.4)	39 (38.2)	0.38
Diuréticos de ASA	100 (95.2)	91 (89.2)	0.11

Datos expresados como media ± DE o mediana (cuartiles), y número (%). IMC = Índice de Masa Corporal, PAS = Presión arterial sistólica, ICP = Intervencionismo coronario percutáneo, IAMEST = infarto agudo de miocardio con elevación persistente del segmento ST, EPOC = Enfermedad pulmonar obstructiva crónica, IECA = Inhibidores de la enzima convertidora de angiotensina, ARA II = Antagonistas de los receptores de angiotensina II. Otras abreviaturas como en tabla 1.

4.2.6.3 Tabla 3. Parámetros de laboratorio.

Variable	Eventos (n=116)	No eventos (n=104)	*p*
Hemoglobina (g/dL)	12.1 [10.9-13.6]	12.3 [11.4-14.6]	0.09
Leucocitos	8350 [6735-11125]	8050 [6370-9590]	0.08
Glucosa (mg/dL)	163 [114-214]	131 [109-188]	0.03
Creatinina (mg/dL)	1.18 [0.92-1.59]	1.02 [0.82-1.37]	0.02
TFG estimada (mL/min/1.73^2)	62 [43-74]	66 [48-80]	0.03
Nitrógeno ureico sangre (mg/dL)	27 [20-37]	22 [16-29]	0.001
Albumina (g/dL)	3.9±0.4	4.0±0.4	0.57
Sodio (mEq/L)	137±5	138±4	0.08
Ácido úrico (mg/dL)	7.7±2.9	7.4±2.2	0.29
Proteína C reactiva (mg/dL)	1.30 [0.60-4.02]	0.90 [0.40-2.20]	0.11
Troponina T (ng/mL)	0.018 [0.010-0.057]	0.010 [0.010-0.030]	0.008
NT-proBNP (pg/mL)	4010 [2021-7768]	2502 [1291-5357]	0.006

Datos expresados como media **±** DE o mediana (cuartiles), y número (%). Abreviaturas como en Tabla 1.

4.2.6.4 Tabla 4: Rendimiento de las medidas de función renal y el NT-proBNP para predecir eventos a 1 año.

Variables	ABC	95% CI	P. corte	S	E	VPP	VPN	p
BTP (mg/dL)	0.62	0.55-0.68	0.96	0.61	0.60	0.66	0.55	
Cistatina C (mg/dL)	0.63	0.56-0.69	1.05	0.72	0.55	0.64	0.63	0.73
Creatinina (mg/dL)	0.58	0.52-0.65	1.07	0.64	0.54	0.61	0.57	0.34
TFG (mL/min/1.73^2)	0.57	0.50-0.64	72	0.65	0.44	0.57	0.53	0.26
BUN (mg/dL)	0.60	0.53-0.66	25	0.53	0.65	0.63	0.55	0.71
NT-proBNP (pg/mL)	0.60	0.53-0.67	3041	0.62	0.57	0.62	0.57	0.70

ABC = área bajo la curva, S = sensibilidad, E = especificidad, VPP = valor predictivo positivo y VPN = valor predictivo positivo. Los valores de p mostrados corresponden a la comparación de BTP con el resto de medidas de función renal. Abreviaturas como en Tabla 1.

4.2.6.5 Tabla 5. Análisis de regresión de Cox para la predicción de eventos adversos.

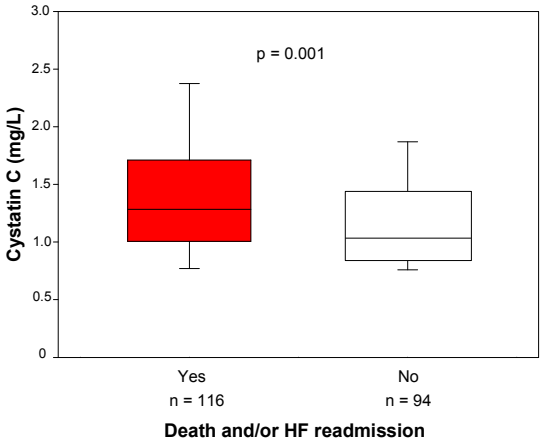
	Univariable		Multivariable	
Variables	Hazard Ratio	p	Hazard Ratio	p
Edad (por año)	1.03 (1.01-1.05)	0.001	1.04 (1.01-1.06)	0.001
FEVI	0.99 (0.98-1.01)	0.06	0.98 (0.97-0.99)	0.023
NYHA III-IV	2.91 (2.01-4.22)	<0.001	2.11 (1.48-3.01)	<0.001
Diabetes mellitus	1.39 (0.96-2.00)	0.08	-	0.51
Insuficiencia cardiaca crónica	1.94 (1.29-2.91)	0.001	-	0.14
IAMEST previo	1.56 (1.05-2.32)	0.03	-	0.19
Uso inotrópicos hospitalario	2.43 (1.50-4.01)	<0.001	2.16 (1.26-3.72)	0.005
Log_{10} glucosa	2.71 (1.05-7.02)	0.04	-	0.33
Sodio (por mEq/L)	0.96 (0.93-1.01)	0.09	-	0.11
Log_{10} leucocitos	5.26 (1.40-19.8)	0.014	8.58 (2.21-33.3)	0.002
Log_{10} NT-proBNP	1.75 (1.18-2.59)	0.006	-	0.47
Log_{10} troponina T	1.51 (1.09-2.11)	0.014	2.66 (1.51-4.71)	0.001
Fallo renal hospitalario	1.52 (1.04-2.21)	0.031	-	0.14
Log_{10} β-traza proteina	3.30 (1.39-7.84)	0.007	3.19 (1.15-8.92)	0.026
Log_{10} cistatina C	4.57 (1.66-12.6)	0.003	4.20 (1.31-13.3)	0.015
Log_{10} creatinina	4.21 (1.34-11.8)	0.006	-	0.14
Log_{10} TFG	0.47 (0.34-0.85)	0.007	-	0.30
Log_{10} BUN	2.58 (1.05-8.8)	0.009	-	0.26

Abreviaturas como en tabla 1.

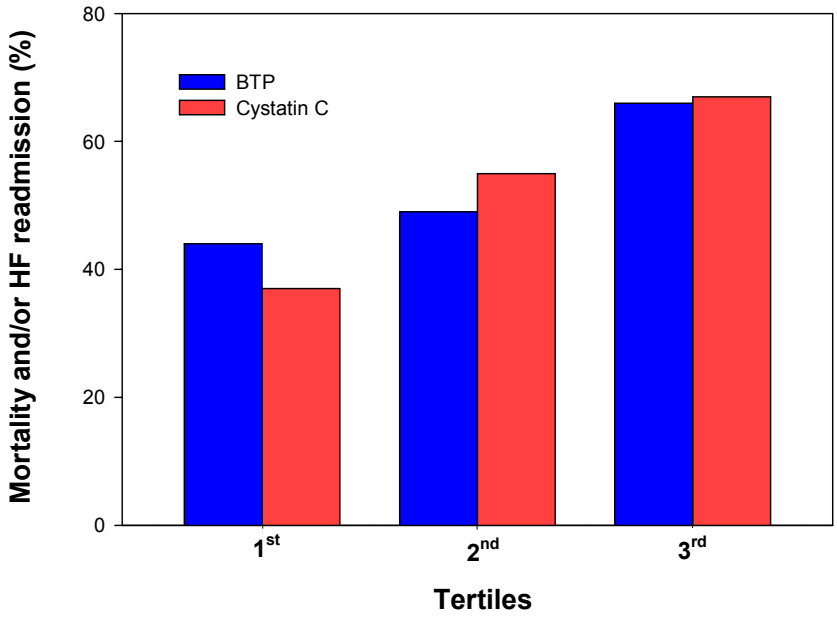
4.2.7.1 Figura 1. Diagrama de cajas que muestra las concentraciones de A) β-traza proteína y B) cistatina C en pacientes con y sin eventos clínicos adversos durante el seguimiento.

A)

B)

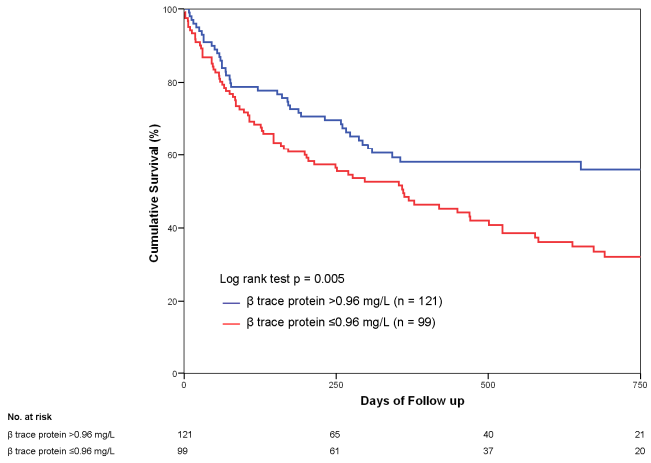


4.2.7.2 Figura 2. Eventos clínicos adveros en función de los terclies de β-traza proteína y cistatina C.



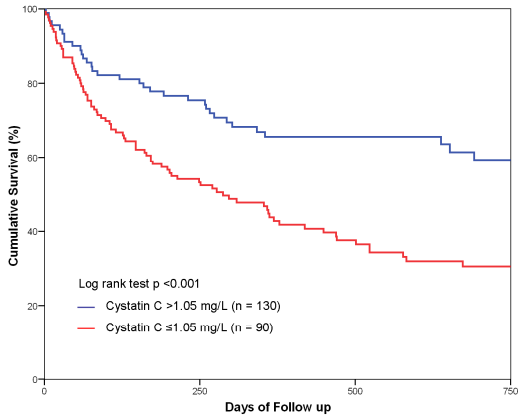
4.2.7.3 Figura 3. Análisis de supervivencia de Kaplan–Meier para eventos clínicos adversos en función de los niveles plasmáticos de A) β-traza proteína y B) cistatina C.

A)



No. at risk				
β trace protein >0.96 mg/L	121	65	40	21
β trace protein ≤0.96 mg/L	99	61	37	20

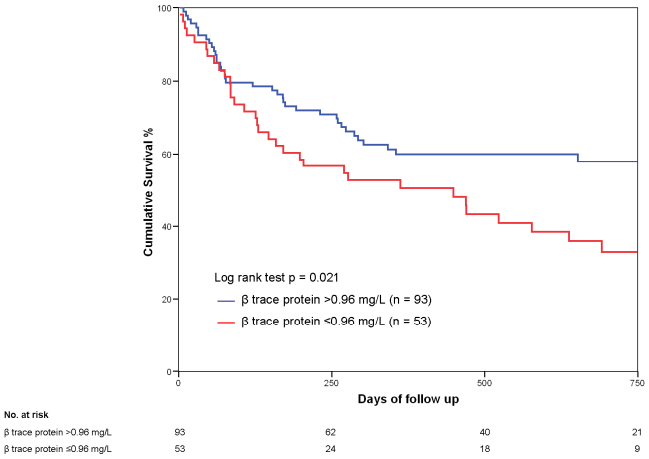
B)



No. at risk				
Cystatin C >1.05 mg/L	130	64	42	22
Cystatin C ≤1.05 mg/L	90	62	35	19

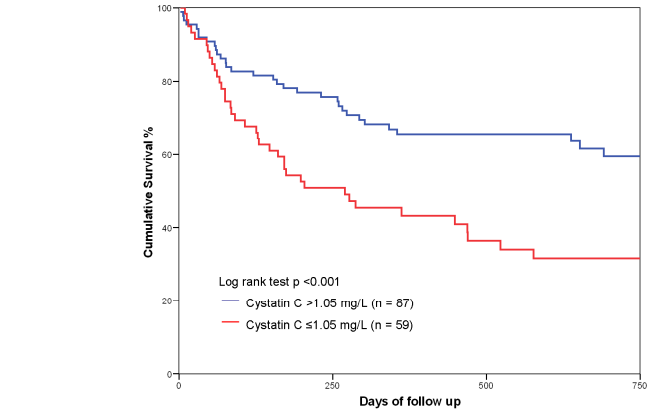
4.2.7.4 Figura 4. Análisis de supervivencia de Kaplan–Meier para eventos clínicos adversos en función de los niveles plasmáticos de A) β-traza proteína y B) cistatina C.en una tasa de filtrado glomerular estimada >60mL/min/1.73m^2.

A)



No. at risk				
β trace protein >0.96 mg/L	93	62	40	21
β trace protein ≤0.96 mg/L	53	24	18	9

B)



No. at risk				
Cystatin C >1.05 mg/L	87	62	42	22
Cystatin C ≤1.05 mg/L	59	29	16	8

4.3 Resumen en castellano del artículo 3.

4.3.1 Título: Utilidad pronóstica de la concentración plasmática de ST2 en pacientes con insuficiencia cardiaca aguda descompensada y fracción de eyección ventricular izquierda reducida vs preservada.

4.3.2 Objetivo

El objetivo de este estudio fue determinar si el riesgo de mortalidad asociado a la presencia de concentraciones plasmáticas elevadas de ST2 difiere en los pacientes ingresados por insuficiencia cardiaca aguda con fracción de eyección ventricular izquierda (FEVI) preservada frente a los que poseen FEVI reducida.

4.3.3 Material y Métodos

La población de estudio la constituyeron 447 pacientes hospitalizados con insuficiencia cardiaca aguda descompensada pertenecientes a 3 estudios prospectivos previamente publicados[9-11]. Estos estudios emplearon criterios de inclusión y exclusión compatibles y recogieron similares características clínicas y analíticas, incluidos ST2, troponina T, NT-proBNP y proteína C reactiva. Se definió insuficiencia cardiaca con FEVI preservada como la presencia de síntomas y signos de insuficiencia cardiaca aguda descompensada y una FEVI ≥50% en ecocardiograma transtorácico (método biplano de Simpson)[12, 13]. Los pacientes incluidos fueron tratados acorde a las guías de práctica clínica vigentes en cada momento y la toma de decisiones diagnóstico terapéuticas dependió de su cardiólogo responsable que fue ciego para los niveles de ST2. Las concentraciones de ST2 fueron medidas mediante inmunoensayo ligado a enzima (Medical & Biological Laboratories, Woburn, Massachusetts), en muestras

sanguíneas congeladas a -80°C. Tras el ingreso los pacientes fueron seguidos durante 1 año y el estado vital fue registrado en todos.

Las variables cuantitativas con distribución normal fueron presentadas como media ± desviación estándar y las no normales como mediana y rangos intercuartílicos. Las diferencias en las características basales fueron comparadas usando el test de la t de student para las variables continuas y el test de χ^2 para las variables categóricas. El test de la U de Mann-Whitney fue utilizado para comparar las variables continuas no normales. El test de Kruskal-Wallis fue utilizado para comparar las concentraciones de ST2 en los diferentes grados funcionales de la clasificación de la New York Heart Association (NYHA). La correlación entre las concentraciones de ST2 y el resto de variables clínicas, analíticas y ecocardiográficas fue evaluada mediante el coeficiente de correlación de Spearman. Para evaluar el valor pronóstico del ST2 en pacientes con y sin disfunción sistólica ventricular izquierda se emplearon diversos métodos. Los pacientes fueron divididos en terciles, evaluándose la frecuencia de aparición de eventos fatales en función de las concentraciones de ST2 y del estado de la FEVI. Se realizaron análisis de curvas ROC para la mortalidad a 1 año, y se calcularon las consiguientes Áreas Bajo la Curva (ABC) para la concentración de ST2 en la población total y tras estratificar por FEVI. La aportación adicional de la concentración de ST2 sobre los niveles de NT-proBNP para predecir muerte a 1 año fue evaluada usando el estadístico C y los análisis de NRI e IDI[14] , donde las categorías de probabilidad para los eventos fueron definidas basándose en el esquema pronóstico de Heart Failure Survival Score[15]. Para identificar los predictores independientes de mortalidad al año realizamos un análisis de regresión múltiple de Cox. Las variables incluidas en el análisis multivariable fueron aquellas que alcanzaron la significación estadística en el análisis univariable ($p < 0.05$). La incidencia acumulativa de muerte fue estimada mediante el análisis de supervivencia de Kaplan–Meier y el estadístico de log-rank fue empleado para las comparaciones. Los valores de $p < 0.05$ fueron aceptados como estadísticamente significativos. Los análisis

estadísticos se llevaron a cabo utilizando la versión 15.0 de SPSS 15.0 para Windows (SPSS, Inc., Chicago, IL, USA). Las curvas ROC se realizaron usando la versión 14.0 de MedCalc statistical software para Windows (MedCalc Software, Broekstraat 52, Mariakerke, Belgium).

4.3.4 Resultados

Un total de 447 sujetos fueron incluidos en el análisis: 197 (44%) pacientes presentaron FEVI preservada y 250 (56%) reducida. La tabla 1 muestra la distribución de las características clínicas y analíticas en función de la FEVI.

Los pacientes con FEVI preservada presentaron una edad más avanzada, fueron más frecuentemente mujeres y presentaron historia previa de hipertensión arterial de forma más frecuente. Por el contrario, los pacientes con FEVI reducida tuvieron más frecuentemente historia previa de insuficiencia cardiaca crónica y enfermedad coronaria conocida. De acuerdo con esto, un porcentaje más alto de pacientes con FEVI reducida estaba bajo tratamiento con inhibidores de la enzima convertidora de angiotensina, diuréticos de asa y digoxina. Sin embargo, no se observaron diferencias en cuanto al uso de beta-bloqueantes en ambos grupos. Los síntomas fueron similares, con una clase funcional de la NYHA comparable en ambos grupos. Los pacientes con FEVI preservada presentaron niveles más elevados de proteína C reactiva y menores niveles de hemoglobina y tasa de filtrado glomerular estimada. Las concentraciones de NT-proBNP y troponina T también fueron menores en los pacientes con FEVI preservada.

Tal y como muestra la Tabla 2, la concentración de ST2 correlacionó significativamente con diversas características clínicas y analíticas. Así, encontramos una correlación positiva moderada entre la concentración plasmática de ST2 y los niveles de proteína C reactiva, troponina T y NT-proBNP. Además, las concentraciones de ST2 también correlacionaron de forma negativa con la FEVI ($r = -0.12$; $p = 0.01$); por lo que los pacientes con insuficiencia cardiaca sistólica

presentaron concentraciones de ST2 más elevadas (0.55 [0.30-1.03] ng/mL vs. 0.38 [0.26-0.79] ng/mL, p <0.001). Cuando los pacientes fueron clasificados en función de la clase funcional de la NYHA, las concentraciones medias de ST2 fueron más elevadas en aquellos pacientes con síntomas más avanzados independientemente del estado de la FEVI (≥50% (n = 197): NYHA II 0.28 [0.17-0.36] ng/mL, NYHA III 0.43 [0.30-0.84] ng/mL, NYHA IV 0.49 [0.29-0.96] ng/mL; p = 0.001 and <50% (n = 250): NYHA II 0.33 [0.19-0.72] ng/mL, NYHA III 0.59 [0.31-1.01] ng/mL, NYHA IV 0.63 [0.35-1.38] ng/mL; p <0.001).

Durante 1 año de seguimiento, un total de 117 pacientes (26%) fallecieron. La mediana de concentración de ST2 fue significativamente más elevada entre los fallecidos (0.80 [0.42 to 1.83] ng/mL vs. 0.38 [0.24 to 0.72] ng/mL; p <0.001), permaneciendo invariable este patrón de niveles más elevados entre los fallecidos en los pacientes con FEVI preservada (0.57 [0.26-1.28] ng/mL vs. 0.35 [0.22-0.66] ng/mL; p <0.001) y en aquellos con FEVI reducida (0.98 [0.57-2.48] ng/mL vs. 0.42 [0.26-0.78] ng/mL; p <0.001) (Fig. 1).

Tal y como aparece detallado en la Tabla 3, la concentración de ST2 presentó un área bajo la curva comparable en los pacientes con FEVI reducida y preservada. En el análisis de regresión múltiple de Cox encontramos que los niveles elevados de ST2, tomados como variable cuantitativa, se asociaron con un riesgo mayor de mortalidad al año tanto en pacientes con FEVI preservada (por ng/mL, HR 1.41 95%CI 1.14-1.76, p = 0.002) como en pacientes con FEVI reducida (por ng/mL, HR 1.20 95%CI 1.10-1.32, p <0.001) (Tabla 4). Además, el análisis por terciles de la concentración de ST2 también reveló un incremento gradual de la tasa anual de muerte conforme aumentaban las concentraciones de ST2 independientemente del estado de la función ventricular izquierda (Fig. 2). El análisis de supervivencia de Kaplan-Meier a 1 año mostró una separación precoz de las curvas de mortalidad en los pacientes con niveles de ST2 por encima y por debajo de los puntos de corte óptimos en la población total (sST2 ≥0.53 ng/mL (n = 201): 35% vs. <0.53 ng/mL (n =

246): 12%; log rank test p <0.001), así como tras la estratificación por FEVI (FEVI preservada, sST2 ≥0.35 ng/mL (n = 114): 31% vs. sST2 <0.35 ng/mL (n = 83): 9.6%; log rank test p <0.001; y FEVI reducida, sST2 ≥.0.56 ng/mL (n = 121): 37% vs. sST2<0.56 ng/mL (n = 129): 14%; log rank test p <0.001).

La aportación adicional de la concentración de ST2 sobre los niveles de NT-proBNP para predecir muerte a 1 año fue evaluada usando el estadístico C y los análisis de NRI e IDI. Tras añadir el valor de la concentración plasmática de ST2 sobre los niveles de NT-proBNP la capacidad para predecir mortalidad a 1 año mejoró significativamente independientemente del estado de la FEVI y del método de evaluación empleado (Tabla 5).

4.3.5 Discusión

En el presente estudio, mostramos datos novedosos respecto a la relación existente entre los niveles plasmáticos de ST2 soluble y las características clínicas y analíticas de pacientes hospitalizados por insuficiencia cardiaca aguda descompensada, en función de la presencia o no de disfunción sistólica ventricular izquierda. Los principales hallazgos de este estudio fueron: i) las concentraciones de ST2 correlacionaron significativamente con diversos biomarcadores séricos relacionados con el pronóstico y proceso deletéreo de remodelado cardiaco, así como con la severidad de los síntomas de insuficiencia cardiaca independientemente del estado de función ventricular izquierda ii) el análisis de curvas ROC mostró diferentes puntos de corte de ST2 para la predicción de mortalidad a 1 año en pacientes con FEVI preservada (>0.35 ng/mL) y reducida (>0.56 mg//mL), los cuales tuvieron una precisión pronóstica similar (Área Bajo la Curva 0.69 vs. 0.73; p >0.05) iii) los niveles de ST2 representaron un potente factor predictor de mortalidad al año en pacientes ingresados con insuficiencia cardiaca tanto con FEVI preservada como reducida, y todo esto independientemente de la presencia de las concentraciones de los péptidos natriuréticos en el análisis multivariado. De hecho, es de resaltar que en presencia de

ST2 en el modelo, que las concentraciones de NT-proBNP no fueron predictoras de muerte en el grupo de pacientes con FEVI preservada iv) la concentración de ST2 mejoró la predicción de riesgo de muerte al año sobre el NT-proBNP, valorada mediante el estadístico C así como a través de NRI e IDI

4.3.6.1 Tabla 1: Características basales de los pacientes en función de la FEVI

Variables	Población Total (n = 447)	FEVI ≥50% (n = 197)	FEVI <50% (n = 250)	*p*
Edad (año)	73±13	74±12	72±13	0.035
Varón	290 (65%)	83 (42%)	207 (83%)	<0.001
ÍMC (Kg/m^2)	27 [24-31]	28 [25-32]	26 [23-30]	<0.001
PAS (mmHg)	142±32	149±32	136±31	<0.001
PAD (mmHg)	80±18	79±17	80±19	0.46
Frecuencia cardiaca (lpm)	92±27	88±27	96±26	0.002
Hipertensión	306 (69%)	148 (75%)	158 (63%)	0.007
Diabetes mellitus	183 (41%)	79 (40%)	104 (42%)	0.75
Enfermedad coronaria	202 (45%)	64 (33%)	138 (55%)	<0.001
Insuficiencia cardiaca previa	239 (54%)	81 (41%)	158 (63%)	<0.001
Enfermedad pulmonar obstructiva crónica	103 (23%)	49 (25%)	54 (22%)	0.42
Tabaquismo activo	63 (14%)	22 (11%)	41 (16%)	0.11
FEVI %	46 [32-60]	60 [55-65]	34 [25-42]	<0.001
NYHA functional class				
II	102 (23%)	45 (23%)	57 (23%)	0.46
II	156 (35%)	63 (32%)	93 (37%)	
IV	189 (42%)	89 (45%)	100 (40%)	
Fibrilación/flutter auricular	189 (42%)	82 (42%)	107 (43%)	0.80
β-bloqueantes	233 (52%)	105 (53%)	128 (51%)	0.66
IECAS	208 (47%)	71 (36%)	137 (55%)	<0.001
ARA II	57 (13%)	34 (17%)	23 (9%)	0.011
Digoxina	104 (23%)	31 (16%)	73 (29%)	0.01
Diureticos de asa	309 (69%)	124 (63%)	185 (74%)	0.012
Hemoglobina (g/dL)	12.7±2.2	12.1±2.3	13.1±2.1	<0.001
Leucocitos (per 10^3)	8.7 [7.0-10.9]	8.6 [7.1-10.6]	8.7 [6.7-11.1]	0.79
Creatinina (mg//dL)	1.10 [0.83-1.50]	1.10 [0.82-1.49]	1.14 [0.88-1.56]	0.53
TFG (mL/min/1.73m^2)	63 [43-86]	61 [40-83]	65 [45-90]	0.029
Nitrógeno ureico sangre (mg/dL)	25 [18-34]	24 [18-33]	25 [18-35]	0.36
Proteína C reactiva (mg/dL)	3.5 [0.9-16.3]	5.2 [1-22]	2.65 [0.80-9.95]	0.013
Troponina T (ng/mL)	0.01 [0.01-0.04]	0.01 [0.01-0.037]	0.016 [0.01-0.062]	0.004
NT-proBNP plasmático (pg/mL)	3558 [1646-9250]	2749 [1344-6634]	4709 [2099-11159]	<0.001
ST2 (ng/mL)	0.47 [0.28-0.94]	0.38 [0.26-0.79]	0.55 [0.30-1.03]	<0.001

Los datos están expresados como media+DE o mediana (cuartiles), y número (%). IMC = Índice de Masa Corporal, PAS = Presión arterial sistólica, PAD = Presión arterial diastólica, FEVI = Fracción de eyección ventricular izquierda, NYHA = New York Heart Association, IECA = Inhibidores de la enzima convertidora de angiotensina, ARA II = Antagonistas de los receptores de angiotensina II, TFG = Tasa de filtrado glomerular

4.3.6.2 Tabla 2. Correlaciones entre ST2 y variables cuantitativas.

	Población Total (n = 447)		FEVI ≥50% (n = 197)		FEVI <50% (n = 250)	
Variable	r	*p*	r	*p*	r	*p*
Edad (año)	-	0.69	-	0.65	0.10	0.12
ÍMC (Kg/m^2)	-	0.23	-	0.94	-	0.42
PAS (mmHg)	-0.10	0.040	-	0.47	-0.10	0.11
PAD (mmHg)	-	0.68	-0.11	0.13	-	0.59
Frecuencia cardiaca (latido/min)	0.20	<0.001	-	0.19	0.25	<0.001
Hemoglobina (g/dL)	-0.11	0.018	-0.12	0.09	-0.26	0.006
Leucocitos	0.23	<0.001	0.19	0.01	0.26	<0.001
Creatinina (mg/dL)	0.25	<0.001	0.18	0.015	0.30	<0.001
TFG (mL/min/1.73m^2)	-0.23	<0.001	-0.18	0.01	-0.30	<0.001
Nitrógeno ureico en sangre (mg/dL)	0.26	<0.001	0.13	0.08	0.36	<0.001
Proteína C reactiva (mg/dL)	0.40	<0.001	0.36	<0.001	0.47	<0.001
NT-proBNP plasmático (pg/mL)	0.41	<0.001	0.35	<0.001	0.43	<0.001
Troponina T (ng/mL)	0.31	<0.001	0.25	0.001	0.34	<0.001
DVITs(mm)	0.15	0.033	-	0.l6	0.23	0.034
DVITd (mm)	-	0.382	-	0.49	-	0.39
PSVD (mmHg)	0.22	<0.001	0.16	0.08	0.27	<0.001

DVITs = diámetro ventricular izquierdo telesistólico, DVITd = Diámetro ventricular izquierdo telediastólico, PSVD = presión sistólica ventricular derecha. Otras abreviaturas como en Tabla 1.

4.3.6.3 Tabla 3: Rendimiento del ST2 (ng/mL) para predecir mortalidad a 1 año.

Variables	ABC	95% CI	Puntos de corte	S	E	VPP	VPN
Población Total (n = 447)	0.71	0.67-0.76	0.53	0.69	0.64	0.41	0.85
FEVI ≥50% (n = 197)	0.69	0.62-0.75	0.35	0.82	0.49	0.37	0.88
FEVI <50% (n = 250)	0.73	0.67-0.79	0.56	0.76	0.62	0.42	0.88

ABC = área bajo la curva, S = sensibilidad, E = especificidad, VPP = valor predictivo positivo y VPN = valor predictivo negativo.

4.3.6.4 Tabla 4: Análisis de regresión de Cox univariable y multivariable para la predicción de mortalidad a 1 año.

	Población Total (n=447)				FEVI ≥50% (n=197)		FEVI <50% (n=250)	
	Univariable		Multivariable		Multivariable		Multivariable	
Variables	Hazard Ratio	*p*	Hazard Ratio	*p*	Hazard Ratio	*p*	Hazard Ratio	*p*
Edad (por año)	1.006 (1.004-1.008)	<0.001	1.05 (1.03-1.07)	<0.001	1.07 (1.03-1.11)	<0.001	1.03 (1.008-1.06)	0.008
ÍMC (por Kg/m2)	0.94 (0.90-0.97)	0.001						
PAS(por mmHg)	0.985 (0.979-0.992)	<0.001	0.988 (0.982-0.994)	<0.001			0.986 (0.977-0.995)	0.002
PAD (por mmHg)	0.981 (0.971-0.992)	0.001						
Insuficiencia cardiaca previa	1.72 (1.18-2.50)	0.005						
NYHA (por clase funcional)	1.43 (1.12-1.82)	0.004						
Beta-bloqueantes	0.68 (0.48-0.98)	0.038	0.66 (0.45-0.97)	0.035			0.56 (0.34-0.91)	0.019
IECA	0.64 (0.44-0.93)	0.018						
Hemoglobina (por g/dL)	0.87 (0.80-0.94)	<0.001						
Leucocitos (por U)	1.07 (1.02-1.03)	0.003			1.13 (1.03-1.24)	0.008		
Creatinina (por mg//dL)	1.74 (1.43-2.12)	<0.001						
TFG (por mL/min/1.73^2)	0.985 (0.979-0.992)	<0.001			0.98 (0.97-0.99)	0.012		
Nitrógeno urico sangre (por mg/dL)	1.02 (1.01-1.03)	<0.001	1.02 (1.01-1.03)	<0.001			1.03 (1.01-1.04)	<0.001
Proteína C reactiva (por mg/dL)	1.006 (1.004-1.008)	<0.001						
NT-proBNP (por 100pg/mL)	1.003 (1.002-1.004)	<0.001	1.002 (1.001-1.003)	<0.001			1.002 (1.001-1.003)	<0.001
ST2 (por ng/mL)	1.74 (1.43-2.12)	<0.001	1.23 (1.13-1.34)	<0.001	1.37 (1.11-1.68)	0.003	1.20 (1.10-1.32)	<0.001
ST2 > puntos de corte								
Población total *(>0.53 mg/mL)*	3.31 (2.23-4.89)	<0.001	2.43 (1.60-3.69)	<0.001				
FEVI ≥50% (>0.35 mg/mL)					3.26 (1.50-7.05)	0.003		
FEVI <50% (>0.56 mg/mL)							2.94 (1.66-5.19)	<0.001

Abreviaturas como en Tabla 1.

4.3.6.5.1 Tabla 5A. Valor incremental de los biomarcadores para detectar muerte a 1 año

	Estadístico C (modelo 1)	Estadístico C (modelo 2)	∆Estadístico C (95% IC)	*p*
Población total (n=447)	0.64	0.71	0.07 (0.02, 0.11)	0.002
FEVI <50% (n = 250)	0.65	0.74	0.08 (0.03, 0.14)	0.08
FEVI ≥50% (n =197)	0.60	0.68	0.08 (0.02, 0.15)	0.01

4.3.6.5.2 Tabla 5B. Evaluación de la capacidad predictiva añadida del Modelo 2 sobre el Modelo 1 para predecir muerte a 1 año usando NRI

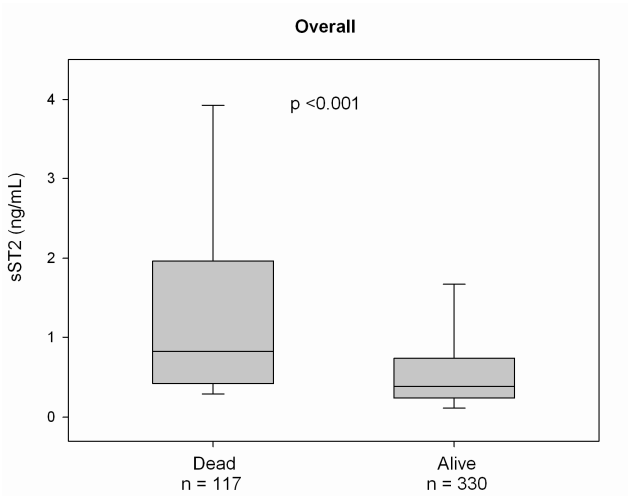
	NRI (95% IC)	% eventos correctamente reclasificados	% no eventos correctamente reclasificados	*p*
Población total (n=447)	0.56 (0.39, 0.73)	9%	48%	<0.0001
FEVI <50% (n = 250)	0.71 (0.49, 0.92)	26%	45%	<0.0001
FEVI ≥50% (n =197)	0.47 (0.22, 0.71)	16%	31%	0.0002

4.3.6.5.3 Tabla 5C. Evaluación de la capacidad predictiva añadida del Modelo 2 sobre el Modelo 1 para predecir muerte a 1 año usando IDI

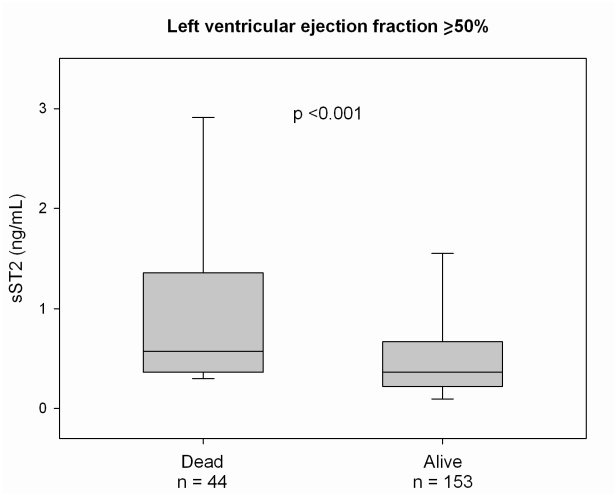
	IDI (95% IC)	Probabilidad de cambio eventos	Probabilidad de cambio no eventos	P
Población total (n=447)	0.04 (0.02, 0.06)	3%	-1.1%	<0.0001
FEVI <50% (n = 250)	0.07 (0.04, 0.10)	5%	-2.1%	<0.0001
FEVI≥50% (n =197)	0.04 (0.01, 0.07)	3%	-0.9%	0.004

4.3.7.1 Figura 1: Niveles de ST2 soluble y mortalidad a 1 año en la A) población total, y en pacientes con FEVI B) preservada y C) reducida.

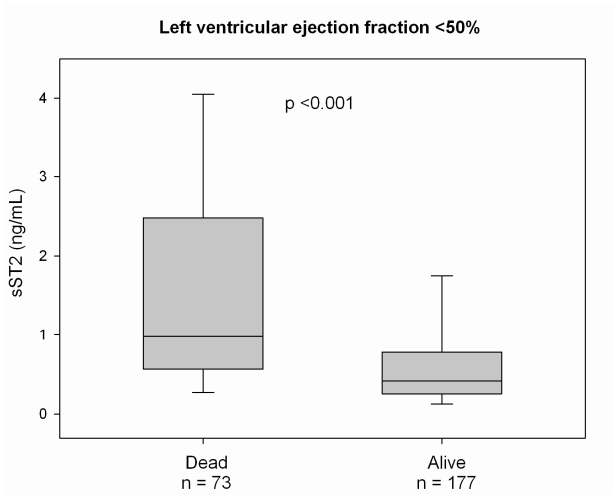
A



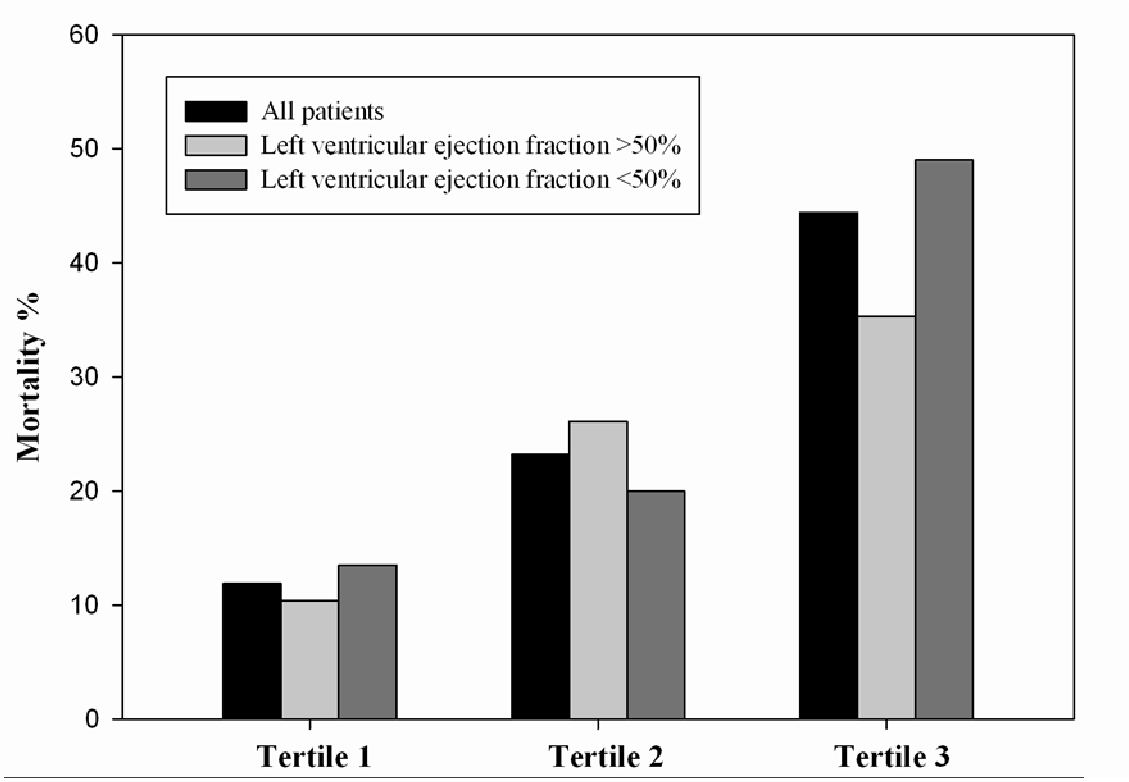
B



C



4.3.7.2 Figura 2: Análisis por terciles de ST2, comparando las concentraciones de ST2 soluble en relación a la mortalidad a 1 año en la población total, así como en los pacientes con FEVI preservada y reducida. El análisis univariable y multivariable de regresión de Cox para los terciles de ST2 como predictores de mortalidad a 1 año están representados en la figura.



	Tertile			P value
	1st ≤0.32 ng/mL	2nd 0.33-0.71 ng/mL	3rd ≥0.72 ng/mL	
All patients (n = 447)				
Mortality %	12	23	44	
*Unadjusted Hazard Ratio**	1	2.04 (1.15-3.60)	4.77 (2.83-8.03)	<0.001
Adjusted Hazard Ratio	1	1.68 (0.94-3.00)	3.08 (1.78-5.34)	<0.001
Left ventricular ejection fraction ≥50% (n = 197)				
Mortality %	10	26	35	
*Unadjusted Hazard Ratio**	1	2.67 (1.16-6.15)	4.07 (1.77-9.35)	0.004
Adjusted Hazard Ratio	1	2.63 (1.13-6.12)	4.18 (1.79-9.76)	0.004
Left ventricular ejection fraction <50% (n = 250)				
Mortality %	13	20	49	
*Unadjusted Hazard Ratio**	1	1.38 (0.69-2.74)	2.28 (1.19-4.37)	<0.001
Adjusted Hazard Ratio	1	0.90 (0.40-2.04)	2.64 (1.29-5.41)	<0.001

Adjusted for age, body mass index, systolic blood pressure, diastolic blood pressure, prior heart failure, left ventricular ejection fraction, New York Heart functional class, β-blocker, Angiotensin-converting enzyme inhibitor, haemoglobin, leukocytes, estimated glomerular filtration rate, blood urea nitrogen, C-reactive protein (per mg/dL) and NT-proBNP.

5. Apendice

5.1 Copias de las cartas de aceptación de las publicaciones.

5.1.1 Carta de aceptación del artículo 1.

Date: Feb 15, 2009
To: "Sergio Manzano-Fernández" sergiosmf13@hotmail.com
From: "AJC Editorial Office" ajc@baylorhealth.edu
Subject: Your Submission
Ms. Ref. No.: AJC-D-08-02671R1
Title: Complementary Prognostic Value of Cystatin C, N-terminal pro-B-type Natriuretic Peptide and Cardiac Troponin T in Patients with Acute Heart Failure
American Journal of Cardiology

Dear Dr Sergio Manzano-Fernández,

Your manuscript is accepted and scheduled for publication in June 2009. Thanks for the changes.

Sincerely,

William C. Roberts, MD
Editor-in-Chief

5.1.2 Carta de aceptación del artículo 2.

Manuscript #	JACC050510-1967DR
Current Revision #	1
Other Version	JACC050510-1967D
Submission Date	2010-07-13
Current Stage	Manuscript Ready for Publication
Title	β-trace Protein and Cystatin C as Predictors of Long Term Outcomes in Patients with Acute Heart Failure
Running Title	β-trace Protein,Cystatin C and Acute Heart Failure
Manuscript Type	New Research Papers
Special Issues	N/A
Corresponding Author	James Louis Januzzi (Massachusetts General Hospital)
Contributing Authors	Sergio Manzano-Fernandez , James Januzzi , Miguel Boronat-García , Juan Carlos Bonaque-González , Quynh Truong , Francisco José Pastor-Pérez , Carmen Muñoz-Esparza , Patricia Pastor , María Dolores Albaladejo-Otón , Teresa Casas , Mariano Valdes , Domingo A. Pascual-Figal
Abstract	Objective: To evaluate the prognostic importance of novel markers of renal dysfunction among patients with acutely destabilized heart failure (ADHF). Background: β-trace protein (BTP) and cystatin C are newer biomarkers for renal dysfunction; the prognostic importance of these tests, particularly BTP, relative to standard measures of renal function remains unclear. Methods: Two hundred and twenty consecutive hospitalized patients with ADHF were prospectively studied. Blood samples were collected on presentation. In-hospital worsening renal function, as well as mortality and/or HF hospitalization over a median follow-up period of 500 days was examined as a function of BTP or cystatin C concentrations; results were compared to creatinine, estimated glomerular filtration rate (eGFR) and blood urea nitrogen (BUN). Results: Neither BTP nor cystatin C were associated with worsening renal function during index hospitalization. 116 subjects (53%) suffered either death/HF hospitalization during follow up. Those with adverse outcomes had higher BTP (1.04 [0.80-1.49] vs 0.88 mg/L [0.68-1.17], p=0.003) and cystatin C (1.29 [1.00-1.71] vs 1.03 mg/L [0.86-1.43], p=0.001). After multivariable adjustment, both BTP (HR 1.41 95%CI 1.06-1.88; p=0.018) and cystatin C (HR 1.50 95%CI 1.13-2.01; p=0.006) were significant predictors of death/HF hospitalization, whereas serum creatinine, eGFR, and BUN were no longer significant. In patients with eGFR>60mL/min, elevated concentrations of BTP and cystatin C were still associated with significantly higher risk of adverse clinical events (p <0.05). Net reclassification index analysis suggested cystatin C and BTP to deliver comparable information regarding prognosis. Conclusion: Among patients hospitalized with ADHF, BTP and cystatin C predict risk for death and/or HF hospitalization, and are superior to standard measures of renal function for this indication.
Key Words	β-trace Protein, cystatin C, prognosis, acute heart failure
Categories	Cardiac Function and Heart Failure/Cardiac Transplantation/Assist Devices--Basic and Clinical, Cardiac Function and Heart Failure/Myocardial Function/Heart Failure--Clinical Pharmacological Treatment, Cardiac Function and Heart Failure/Myocardial Function/Heart Failure--Basic/Molecular
Relationship with Industry	**Yes,** having read the above statement, there is at least one relationship with industry to disclose. This has been fully detailed in my cover letter.
Clinical Trial	No
Decision	Accept, no revision / 2010-08-10
Copyright Release Date	2010-08-30

5.1.3 Carta de aceptación del artículo 3.

Date: Sep 06, 2010
To: "Sergio Manzano-Fernández" sergiosmf13@hotmail.com
From: "AJC Editorial Office" ajc@baylorhealth.edu
Subject: Your Submission
Ms. Ref. No.: AJC-D-10-01442R1
Title: Usefulness of Soluble Concentrations of the Interleukin Family Member ST2 as Predictor of Mortality in Patients with Acutely Decompensated Heart Failure Relative to Left Ventricular Ejection Fraction
American Journal of Cardiology

Dear Dr Sergio Manzano-Fernández,

Your manuscript is accepted and scheduled for publication in January 2011. Thanks for the changes.

Sincerely,

William C. Roberts, MD
Editor-in-Chief

For further assistance, please visit our customer support site at http://epsupport.elsevier.com. Here you can search for solutions on a range of topics, find answers to frequently asked questions and learn more about EES via interactive tutorials. You will also find our 24/7 support contact details should you need any further assistance from one of our customer support representatives.

5.2 Índice de impacto de las publicaciones.

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