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Associació de diferents biomarcadors en pacients amb insuficiència cardíaca controlats a una Unitat d'Insuficiència Cardíaca Multidisciplinar.

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El projecte de treball d'investigació presentat al 2011 tenia per objectiu general analitzar en pacients ambulatoris amb insuficiència cardíaca de diverses etiologies i en variada situació funcional, controlats a una Unitat d'Insuficiència Cardíaca multidisciplinar, la utilitat de combinar diferents biomarcadors de diferents aspectes fisiopatològics de la IC, per tal de valorar si la seva combinació millorava el rendiment i precisió en detectar els pacients amb més risc de mortalitat i identificar les millors combinacions.

Al llarg de l'any 2011-2012 s'han assolit els següents objectius del projecte de d'investigació:

En primer lloc s'ha actualitzat la base de dades de la cohort de pacients seguits a la Unitat d'Insuficiència Cardíaca de l'Hospital Germans Trias i Pujol.

Seguidament, amb la col·laboració dels Serveis de Bioquímica i Hematologia del nostre centre s'han determinat els valors dels biomarcadors NT-pro BNP, ST2, troponina T i I d'alta sensibilitat, cistatina C, ferritina i índex de saturació de transferrina.

En tercer lloc i treballant conjuntament amb el Servei d'Estadística del programa de Processos Inflamatoris i Cardiovasculars de l'Institut de Recerca IMIM-Hospital del Mar s'ha analitzat el valor pronòstic de tots els biomarcadors de forma aïllada i la combinació d'alguns d'ells. Fruit de totes aquests estudis s'han publicat 2 articles originals (s'adjunten al final del document):

- Bayes-Genis A, de Antonio M, Galán A, Sanz H, Urrutia A, Cabanes R, Cano L, González B, Díez C, Pascual T, Elosúa R, Lupón J. Combined use of high-sensitivity ST2 and NTproBNP to improve the prediction of death in heart failure. Eur J Heart Fail. 2012 Jan;14(1):32-8. L'addició de ST2 i NT-proBNP a un model de 11 factors de risc clàssics (edat, sexe, fracció d'ejeccció del ventricle esquerre, classe funcional NYHA, diabetis, filtrat glomerular, etiologia isquèmica, sodi, hemoglobina, tractament amb beta-blocadors i tractament amb inhibidors de l'enzim convertidor d'angiontensina) va millorar la predicció de mortalitat global en la cohort de pacients.

- de Antonio M, Lupon J, Galan A, Vila J, Urrutia A, Bayes-Genis A. Combined use of high-sensitivity cardiac troponin T and N-terminal pro-B type natriuretic peptide improves measurements of performance over established mortality risk factors in chronic heart failure. Am Heart J. 2012 May;163(5):821-8. La incorporació del NT-proBNP i la troponina T d'alta sensibilitat (hs-cTnT) al mateix model de factors de risc clàssics va augmentar la capacitat del model en l'estratificació del risc de mortalitat global.

Hi ha 3 articles que estan en procés de revisió en diferents revistes:

- Una primera aproximació a l'estratègia multimarcador en què hem valorat la combinació de l'ST2, troponina T d'alta sensibilitat i NT-proBNP. En aquesta anàlisi ha estat superior el valor pronòstic de l'ST 2 i hs-cTnT que el del NT-proBNP.

- El segon treball en revisió correspon a la comparació directa entre les dures troponines. Les dues troponines són predictores de mortalitat però la hs-cTnT s'ha mostrat superior.

- Pel que fa a la comparació de cistatina C (com a marcador de funció renal) amb el filtrat glomerular calculat per la fórmula Cockroft-Gault no es van observer diferències significatives en la capacitat pronòstica. La combinació dels dos va millorar l'estratificació pronòstica en graus moderats d'insuficiència renal.

En una primera anàlisi el deficit de ferro també ha demostrat ser predictor de mortalitat en la nostra cohort de pacients.

Resta pendent analitzar de forma més exhaustiva el valor pronòstic de la ferritina i índex de saturació de transferrina i la combinació de tots els biomarcadors junt amb les variables clíniques i ecocardiogràfiques per tal d'establir un model pronòstic en pacients amb insuficiència cardíaca.

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Combined use of high-sensitivity ST2 and NTproBNP to improve the prediction of death in heart failure

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Aims To address the incremental usefulness of biomarkers from different disease pathways for predicting risk of death in heart failure (HF).

Methods and results We used data from consecutive patients treated at a structured multidisciplinary HF unit to investigate whether a combination of biomarkers reflecting ventricular fibrosis, remodelling, and stretch [ST2 and N-terminal pro brain natriuretic peptide (NTproBNP)] improved the risk stratification of a HF patient beyond an assessment based on established mortality risk factors (age, sex, ischaemic aetiology, left ventricular ejection fraction, New York Heart Association functional class, diabetes, glomerular filtration rate, sodium, haemoglobin, and beta-blocker and angiotensin-converting enzyme inhibitor/angiotensin II receptor blocker treatments). ST2 was measured with a novel high-sensitivity immunoassay. During a median follow-up time of 33.4 months, 244 of the 891 participants in the study (mean age 70.2 years at baseline) died. In the multivariable Cox proportional hazards model, both ST2 and NTproBNP significantly predicted the risk of death. The individual inclusion of ST2 and NTproBNP in the model with established mortality risk factors significantly improved the C statistic for predicting death [0.79 (0.76–0.81); $P < 0.001$]. The net improvement in reclassification after the separate addition of ST2 to the model with established risk factors and NTproBNP was estimated at 9.90% [95% confidence interval (CI) 4.34–15.46; $P < 0.001$] and the integrated discrimination improvement at 1.54 (95% CI 0.29–2.78); $P = 0.015$).

Conclusions Our data suggest that in a real-life cohort of HF patients, the addition of ST2 and NTproBNP substantially improves the risk stratification for death beyond that of a model that is based only on established mortality risk factors.

Keywords Heart failure • ST2 • Prognosis

Introduction

Chronic heart failure (HF) is a major public health problem, with an increasing incidence and prevalence of the disease.[1] Despite successful treatment achievements in recent decades, the mortality of patients with HF continues to be high. The use of established mortality risk factors including physician-assessed New York Heart Association (NYHA) functional class, specific medication use, laboratory values, and left ventricular ejection fraction (LVEF) does not fully explain the risk of death in HF patients.[2–4] A more refined approach to risk assessment might include the use of biological markers of pathophysiological processes not directly reflected by these established mortality risk factors, such as myocardial fibrosis and stretch, conditions that are associated with an increased risk of death in patients with HF.[5,6] An enhanced risk assessment would be of great clinical value if it could more

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accurately identify HF patients at increased risk of death and who could then be targeted for more intensive treatment and monitoring.[7] We hypothesized that the addition of two biomarkers from these pathophysiological pathways could add substantial prognostic information with respect to the risk of death. ST2, a biomarker reflective of myocardial fibrosis and remodelling, was able to predict mortality in acutely decompensated HF patients, and may identify HF patients at higher risk of sudden cardiac death.[8–10] N-terminal pro brain natriuretic peptide (NTproBNP), which indicates myocardial stretch, is currently recognized as a robust prognostic marker at all stages of HF, and for all related clinical outcomes.[11,12] Accordingly, we investigated whether the incorporation of ST2 (using a novel high-sensitivity assay) in a model with established mortality risk factors and NTproBNP improved the prediction of death in a real-life cohort of ambulatory patients with HF.

Methods

Study population

From May 2006 to July 2010, 891 ambulatory patients treated at a multidisciplinary HF unit were consecutively included in the study. Most patients were referred to the unit by cardiology or internal medicine departments and, to a lesser extent, from the emergency or other hospital departments.

Blood samples were obtained by venipuncture between 09:00 and 12:00 h during conventional ambulatory visits, and after adequate centrifugation the serum samples were stored at −80°C. NTproBNP and ST2 were analysed from the same blood sample.

All participants provided written informed consent, and the study was approved by the local ethics committee. All study procedures were in accordance with the ethical standards outlined in the Declaration of Helsinki of 1975, as revised in 1983.

Follow-up and outcomes

All patients were followed-up at regular pre-defined intervals, with additional visits as required in the case of decompensation. The regular schedule of visits included a minimum of quarterly visits with nurses, biannual visits with physicians, and elective visits with geriatricians, psychiatrists, and rehabilitation physicians. Those who did not attend the regular visit were contacted by telephone.

Death from all causes was the main outcome. Fatal events were identified from clinical records or by reviewing the electronic clinical history of the Catalan Institute of Health. The median follow-up time was 33.4 months (range 15.8–50.2 months).

ST2 assay

ST2 was measured from banked plasma samples using a high-sensitivity sandwich monoclonal immunoassay (Presage® ST2 assay, Critical Diagnostics, San Diego, CA, USA).

This platform offers improved accuracy in quantifying ST2 levels, particularly at lower concentrations. The antibodies used in the Presage assay were generated from recombinant protein based on the human cDNA clone for the complete soluble ST2 sequence.[13] The ST2 assay had a within-run coefficient of <2.5% and a total coefficient of variation of 4%.

N-terminal pro brain natriuretic peptide assay

NT-proBNP levels were determined using an immuno-electrochemiluminisence method (Elecsys®, Roche Diagnostics, Switzerland). This assay has <0.001% cross-reactivity with bioactive BNP, and in the constituent studies in this report the assay had inter-run coefficients of variation ranging from 0.9% to 5.5%.[14]

Statistical analysis

Categorical variables were expressed as percentages. Continuous variables were expressed as the mean (standard deviation) or median (interquartile range) according to normal or non-normal distribution. Statistical differences between groups were compared using the χ^2 test for categorical variables, and the Student *t*-test or Mann–Whitney test for continuous variables (given the deviation from the assumptions of normality of the underlying distribution).

Survival analyses were performed using Cox regression models. In order to fulfil the assumption of linearity of the co-variables ST2 and NT-proBNP, a quadratic term of ST2 and the logarithmic function of NT-proBNP were used in the Cox models. The following variables were incorporated in the model: age, gender, LVEF (in %), estimated glomerular filtration rate (eGFR; mL/min/1.73 m^2), NYHA functional class, presence of diabetes mellitus, ischaemic aetiology, plasma haemoglobin (g/dL), serum sodium (mmol/L), beta-blocker treatment, angiotensin-converting enzyme inhibitor or angiotensin II receptor blocker treatment, ST2 (ng/mL) level, and NTproBNP level.

The best cut-off points for ST2 and NTproBNP were found by bootstrapping the value that maximized the log-likelihood of the non-adjusted Cox models. Density distributions of these values from the bootstrapping were also plotted. Log-rank tests for Kaplan–Meier survival curves were performed for testing differences between the best ST2 and NTproBNP cut-off point groups.

We used three different statistics to assess the potential value of including these biomarkers in mortality risk prediction: (i) the goodness-of-fit of the models using the Hosmer–Lemeshow test; (ii) the improvement in the discrimination capacity of the model that included the biomarkers with respect to a model without them computing the concordance index (C statistic); and (iii) the reclassification with the method described by Pencina and D'Agostino.[15]

There are two main statistics to assess reclassification; the first one [net reclassification improvement (NRI)] requires the *a priori* definition of meaningful risk categories (we have used tertiles for the risk of death). The NRI considers changes in the estimated mortality prediction probabilities that imply a change from one category to another. The second version [integrated discrimination improvement (IDI)] considers the changes in the estimated mortality prediction probabilities as a continuous variable.

P-values of < 0.05 from two-sided tests were considered to indicate statistical significance. The analyses were performed using the software R (version 2.11.1) statistical package (Foundation for Statistical Computing, Vienna, Austria).

Results

A total of 891 consecutive patients with a median age of 70.2 years (range 60.5–77.2 years) were included. Baseline characteristics of the entire sample are shown *Table 1*. In summary, over two-thirds were men in NYHA class II (75.5%), with HF of mainly ischaemic aetiology (52.5%), a median LVEF of 34%, and accepted treatments

for HF were used extensively. During the follow-up period (median, 33.4 months; range, 15.8–50.2 months), 244 patients died. Among cardiovascular causes of death, refractory HF was responsible in 76 (31.3%) patients, sudden death in 25 (10.3%) patients, and acute myocardial infarction in 12 (4.9%) patients. No patients were lost to follow-up.

Table 1 Demographic and clinical baseline characteristics and treatments during follow-up

	n = 891
Age, median (IQR), years	70.2 (60.5–77.2)
Males, *n* (%)	638 (71.6)
White, *n* (%)	886 (99.4)
Aetiology	
Ischaemic heart disease, *n* (%)	468 (52.5)
Dilated cardiomyopathy, *n* (%)	87 (9.8)
Hypertensive, *n* (%)	83 (9.3)
Alcohol, *n* (%)	50 (5.6)
Toxic, *n* (%)	23 (2.6)
Valvular, *n* (%)	103 (11.6)
Other, *n* (%)	77 (8.6)
Heart failure duration, median (IQR), months	27 (4–72.4)
LVEF, median (IQR), %	34 (26–43)
eGFR, median (IQR), mL/min/1.73 m^2	41.5 (28.5–57.9)
BMI, median (IQR), kg/m^2	26.9 (24.2–30.5)
NYHA functional class II/III, *n* (%)	584 (65.5)/232 (26.0)
Hypertension, *n* (%)	544 (61.1)
Diabetes mellitus, *n* (%)	321 (36.0)
Chronic pulmonary lung disease, *n* (%)	149 (16.7)
Smoker, *n* (%)	
Current	130 (14.6)
Past	370 (41.5)
Treatments, *n* (%)	
ACEI or ARB	801 (89.9)
Beta-blocker	782 (87.8)
Spironolactone/eplerenone	349 (39.2)
Loop diuretic	754 (84.6)
Digoxin	272 (30.5)
Statin	607 (68.1)
Oral anticoagulant	382 (42.9)
Antiplatelet	449 (50.4)
ICD	94 (10.5)
CRT	48 (5.4)
Sodium, median (IQR), mmol/L	139 (137–142)
Haemoglobin, mean ± SD, g/dL	12.9 ± 1.8
NTproBNP, median (IQR), ng/mL	1376 (527.1–3024)
ST2, median (IQR), ng/mL	38.1 (30.8–50.9)

ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; BMI, body mass index; CRT, cardiac resynchronization therapy; eGFR, estimated glomerular filtration rate; ICD, implantable cardiac defibrillator; IQR, interquartile range; LVEF, left ventricular ejection fraction; NTproBNP, N-terminal pro brain natriuretic peptide; NYHA, New York Heart Association; SD, standard deviation.

Cox regression and modelling

Both NT-proBNP [hazard ratio (HR) 1.632, 95% confidence interval (CI) 1.484–1.795, $P < 0.001$] and ST2 (HR 1.040, 95% CI 1.029–1.051, $P < 0.001$) predicted death from all causes in the bivariable analysis as continuous variables. In multivariable analysis, both biomarkers remained significant independent predictors of mortality together with age, sex, NYHA functional class, beta-blocker treatment, sodium, and haemoglobin (*Table 2*).

Density plots of the best cut-off point in non-adjusted Cox models were calculated using bootstrap methodology to identify optimal prognostic cut-off points for ST2 [50 ng/mL (95% CI 37–75); *Figure 1A*] and NTproBNP [1829 ng/mL (95% CI 449–3127), *Figure 1B*]. To determine the potential utility of simultaneous ST2 and NTproBNP assessment, we divided the sample into four groups based upon ST2 and NTproBNP cut-off points. As shown in *Figure 2*, patients with either an elevated ST2 or NTproBNP level had an increased risk compared with the reference group that had low levels of both markers (HR 3.48, 95% CI 2.30–5.25, $P < 0.001$; and HR 3.35, 95% CI 2.39–4.70, $P < 0.001$, respectively). Patients with elevated levels of both ST2 and NTproBNP had a markedly increased risk (HR 6.38 95% CI 4.67–9.25, $P < 0.001$), indicating that assessment of both ST2 and NTproBNP is more effective at identifying a high-risk subgroup than individual assessments of either biomarker.

Table 2 Multivariable Cox regression analysis

	HR	95% CI	*P*-value
Age	1.041	1.024–1.059	<0.001
Female gender	0.676	0.490–0.934	0.018
Ischaemic aetiology of HF	0.980	0.741–1.297	0.889
LVEF	0.996	0.984–1.007	0.432
NYHA functional class	1.704	1.284–2.262	<0.001
eGFR, mL/min/1.73 m^2	0.994	0.984–1.004	0.252
BMI, kg/m^2	1.004	0.975–1.034	0.787
HF hospitalizations previous year	0.757	0.557–1.027	0.074
Diabetes mellitus	1.231	0.940–1.612	0.132
COLD	1.189	0.864–1.636	0.287
ACEI or ARB treatment	0.835	0.559–1.247	0.378
Beta-blocker treatment	0.588	0.410–0.842	0.004
NTproBNP, ng/mL	1.241	1.089–1.413	0.001
ST2, ng/mL[a]	1.026	1.014–1.039	<0.001
Na, mmol/L	0.943	0.908–0.980	0.003
Hb, g/dL	0.915	0.845–0.991	0.028

ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; BMI, body mass index; COLD, chronic obstructive lung disease; eGFR, estimated glomerular filtration rate; Hb, plasma haemoglobin; HF, heart failure; ICD, implantable cardiac defibrillator; IQR, interquartile range; LVEF, left ventricular ejection fraction; Na, serum sodium; NTproBNP, N-terminal pro brain natriuretic peptide; NYHA, New York Heart Association (I–II vs. III–IV); SD, standard deviation.
[a]The quadratic term of ST2 has a *P*-value of 0.003.

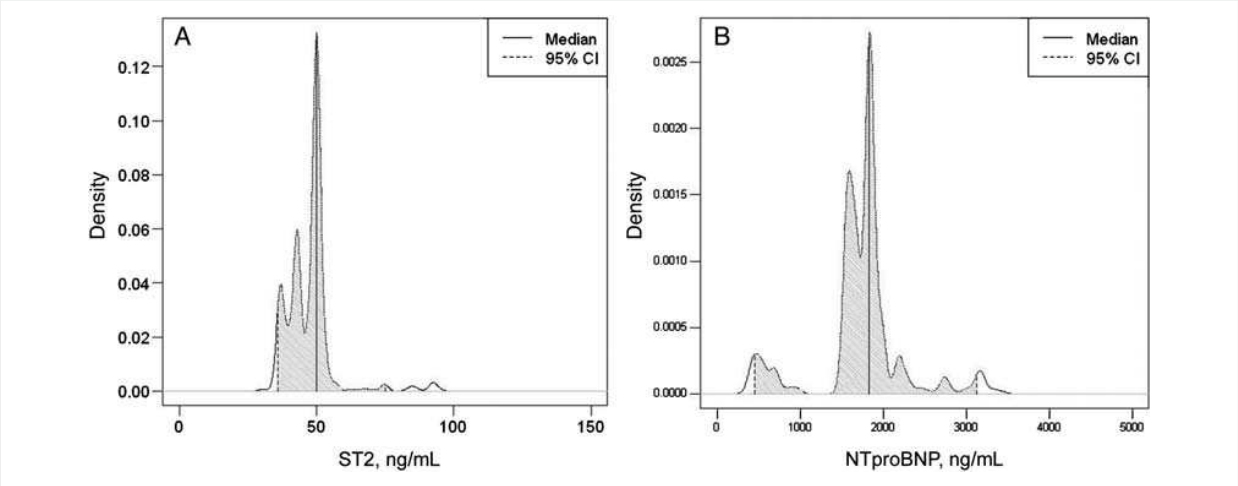

Figure 1 Bootstrap density plot of best cut-off points for ST2 (*A*) and N-terminal pro brain natriuretic peptide (NTproBNP) (*B*); values are expressed in ng/mL.

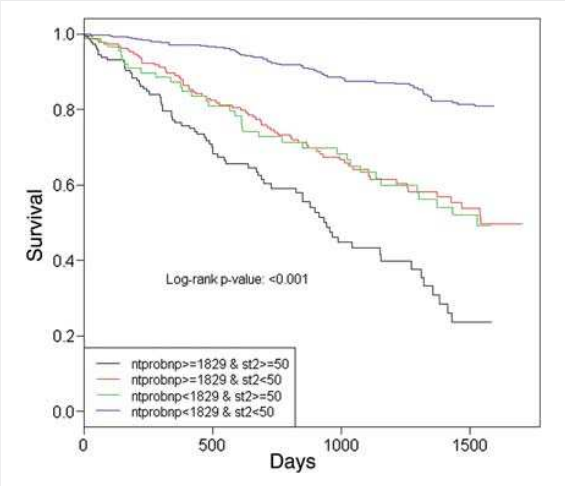

Figure 2 Kaplan–Meier survival curves according to ST2 and N-terminal pro brain natriuretic peptide (NT-proBNP) levels.

Discrimination

The C statistic for the prediction of death increased significantly when the two measured biomarkers were incorporated into a model with established mortality risk factors (age, sex, LVEF, NYHA functional class, diabetes, eGFR, ischaemic aetiology, sodium, haemoglobin, beta-blocker treatment, and angiotensin-converting enzyme inhibitor or angiotensin II receptor blocker treatment) (*Table 3*). The individual inclusion of NTproBNP or ST2 in the model also significantly improved the C statistic for predicting death from all causes.

Table 3 C statistic for Cox regression models predicting death in ambulatory patients with HF

HF mortality risk factors and biomarkers	C statistic for death	*P*-value[a]
Mortality risk factors[b]	0.76 (0.73–0.79)	Referent
Mortality risk factors plus NTproBNP	0.77 (0.74–0.80)	0.040
Mortality risk factors plus ST2	0.78 (0.75–0.81)	0.001
Mortality risk factors plus NproBNP and ST2	0.79 (0.76–0.81)	<0.001

HF, heart failure; LVEF, left ventricular ejection fraction; NTproBNP, N-terminal pro brain natriuretic peptide; NYHA, New York Heart Association.
[a]*P*-values are for the comparison with the model with mortality risk factors.
[b]Heart failure mortality risk factors include: age, sex, LVEF, NYHA functional class, diabetes, eGFR, ischaemic aetiology, plasma haemoglobin, serum sodium, and beta-blocker and angiotensin-converting enzyme inhibitor or angiotensin II receptor blocker treatments.

Reclassification

Reclassification of HF patients into risk categories according to the occurrence of death during follow-up is summarized in *Table 4*. The NRI after the individual inclusion of ST2 in the model with established mortality risk factors and NTproBNP was 9.90% (95% CI 4.34–15.46; $P < 0.001$), and the IDI was 1.54 (95% CI 0.29–2.78); $P = 0.015$). The NRI for those who died was 5.44% (95% CI 1.01–9.87; $P = 0.014$) and the NRI for survivors was 4.46% (95% CI 1.66–7.26; $P = 0.004$) (*Table 4*).

Calibration

The *P*-values for the Hosmer–Lemeshow statistics indicated good calibration for the model with and without the two biomarkers ($P > 0.18$ for all comparisons).

Table 4 Reclassification of patients with heart failure who died or who did not die[a]

Model with mortality risk factors + NTproBNP	Model with mortality risk factors + NTproBNP + ST2[b]			
	Low tertile (<13%)	Medium tertile (13–32%)	High tertile (>32%)	Total no.
Patients who died				
Low tertile (<13%)	11	5	0	16
Medium tertile (13–32%)	1	53	14	68
High tertile (>32%)	0	5	151	156
Total no.	12	63	165	239
Patients who did not die				
Low tertile (<13%)	256	14	1	271
Medium tertile (13–32%)	33	171	14	218
High tertile (>32%)	0	24	115	139
Total no.	289	209	130	629

Dark-shaded boxes show patients in whom reclassification was more accurate when the model with NTproBNP + ST2 was used; light-shaded boxes show patients in whom reclassification became less accurate.

[a]Calculated at 3 years. Model with mortality risk factors included age at baseline, sex, left ventricular ejection fraction, New York Heart Association functional class, presence or absence of diabetes, estimated glomerular filtration rate, ischaemic aetiology, plasma haemoglobin, serum sodium and use or non-use of beta-blocker and angiotensin-converting enzyme inhibitor or angiotensin II receptor blocker treatments.

[b]The interaction between ST2 and N-terminal pro brain natriuretic peptide (NTproBNP) is also incorporated into the model

Global model fit

The model that included the two biomarkers showed better global fit than models with only the established mortality risk factors and NTproBNP, as evaluated by likelihood ratio tests ($P < 0.001$)

Discussion

In this ambulatory, real-life cohort of HF patients, the incorporation of ST2 (reflective of myocardial fibrosis and remodelling) and NTproBNP (indicative of myocardial stretch) into a model with established mortality risk factors improved the risk stratification for death. The improvement in risk assessment remained strong when it was estimated by means of statistical measures that evaluate model discrimination and reclassification, model calibration, and global model fit.

NTproBNP is well recognized as an important prognostic biomarker in HF but, beyond natriuretic peptides, the use of biomarkers for risk assessment is still being debated. ST2 is emerging as a novel biomarker for patient stratification in different clinical settings. Under the induction of separate promoters, the ST2 gene expresses two unique proteins: soluble ST2, the circulating form of ST2 (as assessed in this study); and ST2L, which is the transmembrane form of the protein that signals through a complex involving interleukin-33.[11,17] The role of ST2 in the heart remains to be entirely elucidated; however, experimental disruption of the ST2 gene in a murine model resulted in severe cardiac hypertrophy, fibrosis, dilatation of the ventricular chamber, and reduced contractility.[16]

ST2 is a powerful and reliable prognostic biomarker in patients admitted with acute cardiac decompensation.[18] In both acute HF patients and in acute myocardial infarction patients, ST2 proved to be an independent and complementary biomarker of risk together with NTproBNP.[8,19] In a nested case–control study in chronic HF patients, Pascual-Figal *et al.* found that ST2 was useful for identifying patients at risk of sudden cardiac death.[10] In our population of consecutive patients treated at a multidisciplinary HF unit, high-sensitivity ST2 added independent prognostic information to predict death from all causes over the other variables studied, including NTproBNP. Therefore, this study provides new data about the prognostic value of ST2 in the ambulatory setting of chronic HF, and the complementary roles of ST2 and NTproBNP. Furthermore, this is a reasonably sized cohort of elderly HF patients with a high mortality rate, and in this way offers incremental information.

Such a multimarker predictive approach was also evaluated by Ky *et al.* in a younger, healthier HF cohort.[20] These authors found that the combination of ST2 and NTproBNP offered moderate improvement in risk stratification, but, in contrast to our findings, they did not find a substantial improvement in risk stratification after the addition of ST2 to a clinical model with NTproBNP (as assessed by C statistics and NRI). Risk estimates may differ in patients with different demographics, such as the elderly (56.3 years vs.70.2 years in our cohort) or in populations with less severe disease (median values for ST2 and NTproBNP: 27.5 ng/mL and 566 ng/mL vs. 38.1 ng/mL and 1376 ng/mL in our cohort, respectively). Moreover, besides differences in cohort characteristics there was also a difference in length of mortality follow-up (1 vs. 3 years in NRI analysis), which could have also explained in part the differences observed in both studies. Nevertheless, in our study, after addition of the quadratic form of ST2 to the model, the predictive value of ST2 remained proportional during follow-up. Finally, the clinical model in the study by Ky *et al.*[20] had an area under the curve (AUC) of 0.81, and in our

study the AUC for the clinical model was 0.76. Improvement in risk prediction should indeed be more difficult to achieve with a higher baseline AUC.

The two studied biomarkers were analysed in frozen samples. Consequently, there is a risk that the absolute levels of biomarkers could have been affected by having been measured from frozen rather than fresh samples. However, there is evidence that freeze–thaw cycles do not significantly modify NTproBNP[21] or ST2 (manufacturer's disclosure).

Although it has been shown that modification of some mortality risk factors may decrease the risk of HF hospitalizations and death,[22–24] there is currently less evidence that reducing the levels of ST2 and NTproBNP will reduce the risk. Data for NTproBNP from pilot studies and randomized clinical trials suggest that targeting therapy to lower NTproBNP levels may facilitate more optimal use of proven HF therapies and may reduce adverse clinical outcomes.[25–27] No evidence in this regard is yet available for ST2. Thus, our data should not be construed as implying that there is a direct benefit from reducing biomarker levels.

Conclusion

Our data suggest that the simultaneous addition of ST2 and NTproBNP to a model that includes established mortality risk factors substantially improves the risk stratification for death in HF patients. If these results are validated, the incorporation of these biomarkers into clinical practice for the prediction of death could be accomplished quickly.

Acknowledgements

ST2 assays were performed by Critical Diagnostics, which had no role in the design of the study, or the collection, management, analysis, or interpretation of the data.

Conflict of interest: A.B.-G. reports having received lecture honoraria from Roche Diagnostics. All other authors declare no conflict of interest.

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Combined use of high-sensitivity cardiac troponin T and N-terminal pro-B type natriuretic peptide improves measurements of performance over established mortality risk factors in chronic heart failure

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Background Heart failure still maintains a high mortality. Biomarkers reflecting different pathophysiological pathways are under evaluation to better stratify the mortality risk. The objective was to assess high-sensitivity cardiac troponin T (hs-cTnT) in combination with N-terminal pro-B type natriuretic peptide (NT-proBNP) for risk stratification in a real-life cohort of ambulatory heart failure patients.

Methods We analyzed 876 consecutive patients (median age 70.3 years, median left ventricular ejection fraction 34%) treated at a heart failure unit. A combination of biomarkers reflecting myocyte injury (hs-cTnT) and myocardial stretch (NT-proBNP) was used in addition to an assessment based on established mortality risk factors (age, sex, left ventricular ejection fraction, New York Heart Association functional class, diabetes, estimated glomerular filtration rate, ischemic etiology, sodium, hemoglobin, β-blocker treatment, and angiotensin converting enzyme inhibitor or angiotensin II receptor blocker treatment).

Results During a median follow-up of 41.4 months, 311 patients died. In the multivariable Cox proportional hazards model, hs-cTnT and NT-proBNP were independent prognosticators (P = .003 each). The combined elevation of both biomarkers above cut-off values significantly increased the risk of death (HR 7.42 [95% CI, 5.23-10.54], P < .001). When hs-cTnT and NT-proBNP were individually included in a model with established mortality risk factors, measurements of performance significantly improved. Results obtained for hs-cTnT compared with NT-proBNP were superior according to comprehensive discrimination, calibration, and reclassification analysis (net reclassification indices of 7.7% and 1.5%, respectively).

Conclusions hs-cTnT provides significant prognostic information in a real-life cohort of patients with chronic heart failure. Simultaneous addition of hs-cTnT and NT-proBNP into a model that includes established risk factors improves mortality risk stratification. (Am Heart J 2012;163:821-8.)

Chronic heart failure (HF) is a major and growing public health problem, with increasing incidence and prevalence.[1] Although significant advances have been made in the treatment of HF in recent decades, mortality remains high.[2] Outcomes in HF are highly variable and established risk markers such as New York Heart Association (NYHA) functional class, treatment, laboratory variables, and left ventricular ejection fraction (LVEF) do not fully explain the mortality risk of HF patients and fail to estimate an individual's prognosis.[3-5] Biomarkers of different pathophysiological processes of HF, such as myocardial stretch and injury, both associated with worse prognosis,[6-8] may help in mortality prediction. Accurate identification of high-risk patients is a prerequisite to indicate intensive monitoring or aggressive treatment.

Cardiac troponin, a marker of myocyte injury, predicts adverse clinical outcomes in acute[9-11] and chronic HF.[12] A high-sensitivity assay for cardiac troponin T (hs-cTnT) has recently become available; this assay detects low troponin concentrations and improves precision at the

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lower limit of detection.[13] Some reports suggest that hs-cTnT also provides relevant prognostic information in HF, yet these are small studies with short follow-up[14,15] or derive from randomized clinical trials.[16] N-terminal pro-B type natriuretic peptide (NT-proBNP), which indicates myocardial stretch, is currently recognized as a robust prognostic marker at all stages of HF, and for all related clinical outcomes.[17]

In the present study we evaluated the value of hs-cTnT and NT-proBNP levels in a large real-life cohort of ambulatory patients with HF and whether the incorporation of hs-cTnT on top of established mortality risk factors and NT-proBNP improved long-term mortality prediction.

Methods

Study population

From May 2006 to July 2010, ambulatory patients treated at a multidisciplinary HF unit were consecutively included in the study. Patients were referred to the unit by cardiology or internal medicine departments and, to a lesser extent, from the emergency or other hospital departments. The principal referral criterion was HF according to the European Society of Cardiology guidelines irrespective of etiology, at least one HF hospitalization and/or reduced LVEF.[18]

Blood samples were obtained by venipuncture between 09:00 am and 12:00 PM during conventional ambulatory visits, and after adequate centrifugation serum samples were stored at −80°C. NT-proBNP and hs-cTnT were analyzed from the same blood sample.

All participants provided written informed consent, and the study was approved by the local ethics committee. All study procedures were in accord with the ethical standards outlined in the Helsinki Declaration of 1975, as revised in 1983.

Follow-up and outcomes

All patients were followed at regular pre-defined intervals, with additional visits as required in case of decompensation. The regular visitation schedule included a minimum of quarterly visits with nurses, biannual visits with physicians, and elective visits with geriatricians, psychiatrists, and rehabilitation physicians.[18] Patients who did not attend the regular visits were contacted by telephone.

Death from all causes was the main outcome. Fatal events were identified from clinical records or by reviewing the electronic clinical history of the Catalan Institute of Health.

hs-cTnT assay

Troponin levels were measured by an electrochemiluminescence immunoassay using an hs-cTnT assay on the Modular Analytics E 170 (Roche Diagnostics). This assay uses two monoclonal antibodies that recognize epitopes located in the central region of the cTnT protein. The assay's sensitivity is improved by increasing the sample volume, heavier ruthenylation of the detection antibody, and lowering the background signal by buffer optimization.[13] The hs-cTnT assay had an analytic range from 3 to 10,000 ng/L. At the 99th percentile value of 13 ng/L, the coefficient of variation was 9%. The analytic performance of this assay has been validated and complies with the recommendations of the Global Task Force for use in the diagnosis of myocardial necrosis.[13]

NT-proBNP assay

NT-proBNP levels were determined using an inmmuno-electrochemiluminescence assay on the Modular Analytics E 170 (Roche Diagnostics). This assay has <0.001% cross-reactivity with bioactive BNP, and in the constituent studies in this report, the assay had inter-run coefficients of variation ranging from 0.9% to 5.5%.[19]

Statistical analysis

Categorical variables were expressed as percentages. Continuous variables were expressed as the mean (standard deviation) or median (perecentiles 25th and 75th [P_{25}-P_{75}]) according to normal or non-normal distribution. Statistical differences between groups were compared using the Chi-square test for categorical variables, and Student *t* test or Mann-Whitney and Kruskal Wallis tests for continuous variables (given the deviation from the assumptions of normality of the underlying distribution). Correlations between hs-cTnT and continuous variables were evaluated using the Spearman ρ coefficient. Multivariable logistic regression analysis was performed to ascertain which variables were independently associated with hs-cTnT levels.

The best cut-off points for hs-cTnT and NT-proBNP were found by bootstrapping the value that maximized the log-likelihood of the non-adjusted Cox models. The density distributions of these values from the bootstrapping were also plotted. Log-rank tests for Kaplan-Meier survival curves were performed for testing differences between the best hs-cTnT and NT-proBNP cut-off point groups.

Survival analyses were performed using Cox regression models. To fulfill the assumption of linearity of the co-variables hs-cTnT and NT-proBNP, the logarithmic functions of both NT-proBNP and hs-cTnT and the quadratic terms of the logarithmic functions of hs-cTnT were used in the Cox models. The following variables were incorporated into the model: age, sex, LVEF (in %), estimated glomerular filtration rate (eGFR; in mL/min per 1.73 m^2), NYHA functional class, presence of diabetes mellitus, ischemic etiology, plasma hemoglobin (g/dL), serum sodium (mmol/L), β-blocker treatment, angiotensin converting enzyme inhibitor (ACEI) or angiotensin II receptor blocker (ARB) treatment, hs-cTnT (ng/L) level, and NT-proBNP (ng/L) level.

We used different measurements of performance to test the potential incremental prognostic value of these biomarkers:

Discrimination. The improvement in the discrimination capacity of a model that included biomarkers compared with a model that did not was obtained by computing the concordance index (*C* statistic). The area under the receiver operating characteristic curve (AUC) summarized the diagnostic discrimination. Discrimination refers to a model's ability to correctly distinguish the two classes of outcomes. We used the index of rank correlation, Somers' D, which equals $2\times(c-1/2)$, where *c* is the concordance (discrimination) probability. This test already incorporates information of censored data. AUCs between models were compared using the *U*-statistic test for equality concordance.

Table I. Demographic and clinical baseline characteristics and treatments during follow-up

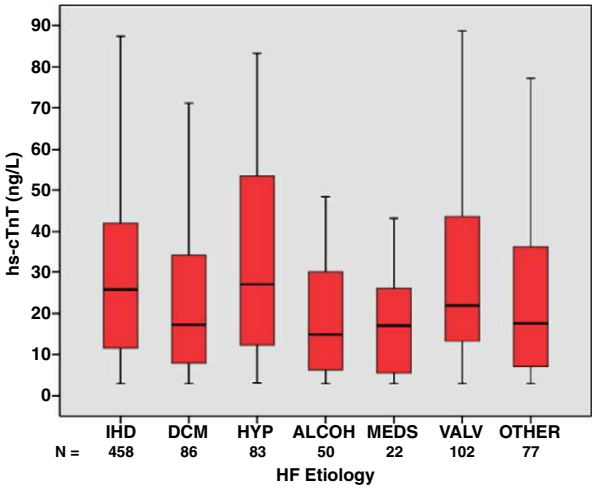
	n = 876
Age, years*	70.3 (60.5-77.2), 68.0 ± 12.3
Males, n (%)	630 (71.9)
White, n (%)	871 (99.4)
Etiology	
Ischemic heart disease, n (%)	458 (52.1)
Dilated cardiomyopathy, n (%)	86 (9.8)
Hypertensive, n (%)	83 (9.5)
Etoh, n (%)	50 (5.7)
Toxic, n (%)	22 (2.5)
Valvular, n (%)	102 (11.6)
Other, n (%)	77 (8.8)
Heart failure duration, months*	27.3 (4.9-73.8), 50.2 ± 61.3
LVEF, %*	34 (26-43), 35.9 ± 13.7
eGFR, mL/min per 1.73m^{2}*	44.3 (31.4-62.3), 48.4 ± 24.1
BMI, kg/m^{2}*	26.9 (24.2-30.5), 1.82 ± 0.22
NYHA functional class, n (%)	
I	63 (7.2)
II	574 (65.5)
III	230 (26.3)
IV	9 (1.0)
Hypertension, n (%)	536 (61.2)
Diabetes mellitus, n (%)	314 (35.8)
COLD, n (%)	148 (16.9)
Treatments, n (%)	
ACEI or ARB	785 (89.6)
β-Blocker	767 (87.6)
Spironolactone/eplerenone	344 (39.3)
Loop diuretic	743 (84.8)
Digoxin	269 (30.7)
Statin	595 (67.9)
Oral anticoagulant	378 (43.2)
Antiplatelet	440 (50.2)
Sodium, mmol/L*	139 (137–142), 139.2 ± 3.5
Hemoglobin, g/dL*	13 (11.7-14.3), 12.9 ± 1.8
NT-proBNP, ng/L*	1361 (510.4-3012.5), 3212 ± 6779
hs-cTnT, ng/L*	22.6 (10.6-40.6), 34.9 ± 51.1

BMI, Body mass index; *COLD*, chronic obstructive lung disease.
*Data in median (P_{25}-P_{75}) and mean ± SD.

Calibration

1) The D'Agostino-Nam version of the Hosmer and Lemeshow calibration test was used to calculate a χ^2 value. Calibration describes how closely the predicted probabilities agree numerically with the actual outcomes. A model is well calibrated when predicted and observed values agree for any reasonable grouping of the observation (no statistically significant differences in the Hosmer-Lemeshow test).
2) The Bayesian information criterion (BIC), the Akaike information criterion (AIC), and the Brier score were calculated for each model. Given any two estimated models, the model with the lower BIC, AIC, and Brier scores was preferred, because a lower score represents higher accuracy. No statistical tests compare different BIC, AIC, or Brier estimations; lower values indicate a better model.
3) The global goodness-of-fit of the models was evaluated by likelihood ratio tests. A significant *P* value in this test means that adding a new variable to the model significantly improves the accuracy of the model.

Figure 1



hs-cTnT levels according to heart failure etiology. **IHD**, ischemic heart disease; **DCM**, dilated cardiomyopathy; **HYP**, hypertensive cardiomyopathy; **ALCOH**, alcoholic cardiomyopathy; **MEDS**, drug-related cardiomyopathy; **VALV**, valvular disease.

Reclassification. We used the method described by Pencina et al.[20] There are two main statistics to assess reclassification. The integrated discrimination improvement (IDI) considers the changes in the estimated mortality prediction probabilities as a continuous variable. *P* values of less than .05 from 2-sided tests were considered to indicate statistical significance. The net reclassification improvement (NRI) requires a previous definition of meaningful risk categories (we used tertiles for the risk of death: <18.5%, 18.5−41%, and >41%). The NRI considers changes in the estimated mortality prediction probabilities that imply a change from one category to another.

All analyses were performed using the software R (version 2.11.1) statistical package (Foundation for Statistical Computing, Vienna, Austria).

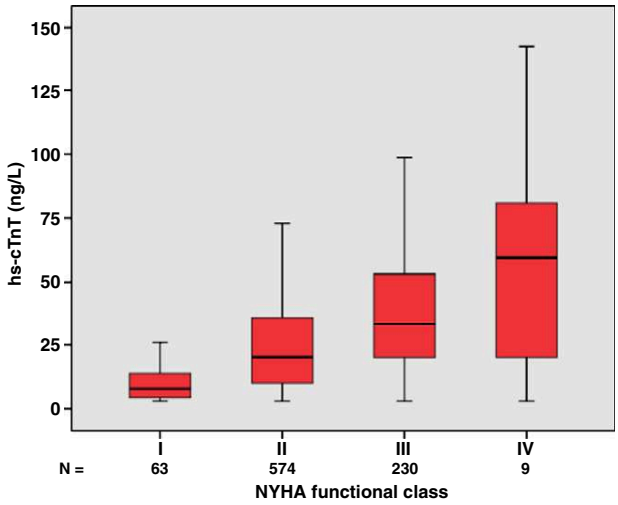
Results

Eight hundred seventy-six consecutive patients with a median age of 70.3 years (P_{25}-P_{75} 60.5−77.2 years) were included. Baseline characteristics of the entire sample are shown in Table I. During a median follow-up period of 41.4 months (P_{25}-P_{75} 22.1−60.5), 311 patients died. Among cardiovascular causes of death, refractory HF was responsible in 91 (45.7%) patients, sudden death in 30 (15.1%) patients, and acute myocardial infarction in 15 (7.5%) patients. Two patients were lost during follow-up and adequately censored.

hs-cTnT and clinical parameters

In this HF cohort, all patients had detectable levels of hs-cTnT (median 22.6 ng/L, [P_{25}-P_{75} 10.6−40.6]). Levels

Figure 2



hs-cTnT serum levels according to New York Heart Association functional class.

of hs-cTnT inversely and weakly correlated with LVEF ($\rho = -0.13$, $P < .001$) and HF duration ($\rho = -0.12$, $P = .001$), and were positively associated with age ($\rho = 0.35$, $P < .001$), eGFR ($\rho = 0.52$, $P < .001$), and NT-proBNP ($\rho = 0.62$, $P < .001$). Men tended to have higher values than women (24 [P_{25}-P_{75} 11–40.9] vs. 20.4 [P_{25}-P_{75} 9.7–38.1]; $P = .086$). Patients with HF of ischemic etiology had higher hs-cTnT levels than the non-ischemic subgroup (25.9 [P_{25}-P_{75} 11.5–42.1] vs. 20.1 [P_{25}-P_{75} 9.3–36.5]; $P = .004$). Among non-ischemic patients, the highest hs-cTnT levels were observed in those with hypertensive cardiomyopathy (Figure 1). Diabetic patients had significantly higher levels of hs-cTnT (28.4 [P_{25}-P_{75} 15.2–44.9] vs. 19 [P_{25}-P_{75} 8.9–36.9]; $P < .001$). Finally, levels of hs-cTnT were correlated significantly with NYHA functional class ($P < .001$, Figure 2).

In a multivariable logistic regression analysis the variables that remained independently associated with a hs-cTnT level ≥16 ng/L (the best cut-off point) were male sex, NYHA functional class, diabetes, eGFR and NT-proBNP.

Table II. Multivariable Cox regression analysis

	HR	95% CI	P
Age	1.04	1.02–1.05	<.001
Female sex	0.73	0.55–0.98	.033
Ischemic etiology of HF	1.04	0.81–1.33	.768
LVEF	1.00	0.99–1.01	.791
NYHA functional class	1.75	1.37–2.24	<.001
eGFR, ml/min/1.73m^2	1.00	0.99–1.01	.741
BMI, kg/m^2	1.00	0.98–1.03	.915
HF hospitalizations previous year	0.80	0.62–1.04	.092
Diabetes mellitus	1.19	0.94–1.51	.148
COLD	1.16	0.88–1.54	.296
ACEI or ARB treatment	0.72	0.51–1.01	.058
β-Blocker treatment	0.54	0.39–0.73	<.001
Na, mmol/L	0.96	0.92–0.99	.009
Hb, g/dL	0.92	0.86–0.99	.028
ln(NT-proBNP)	1.21	1.07–1.37	.003
ln(hs-cTnT)	3.55	1.53–8.23	.003

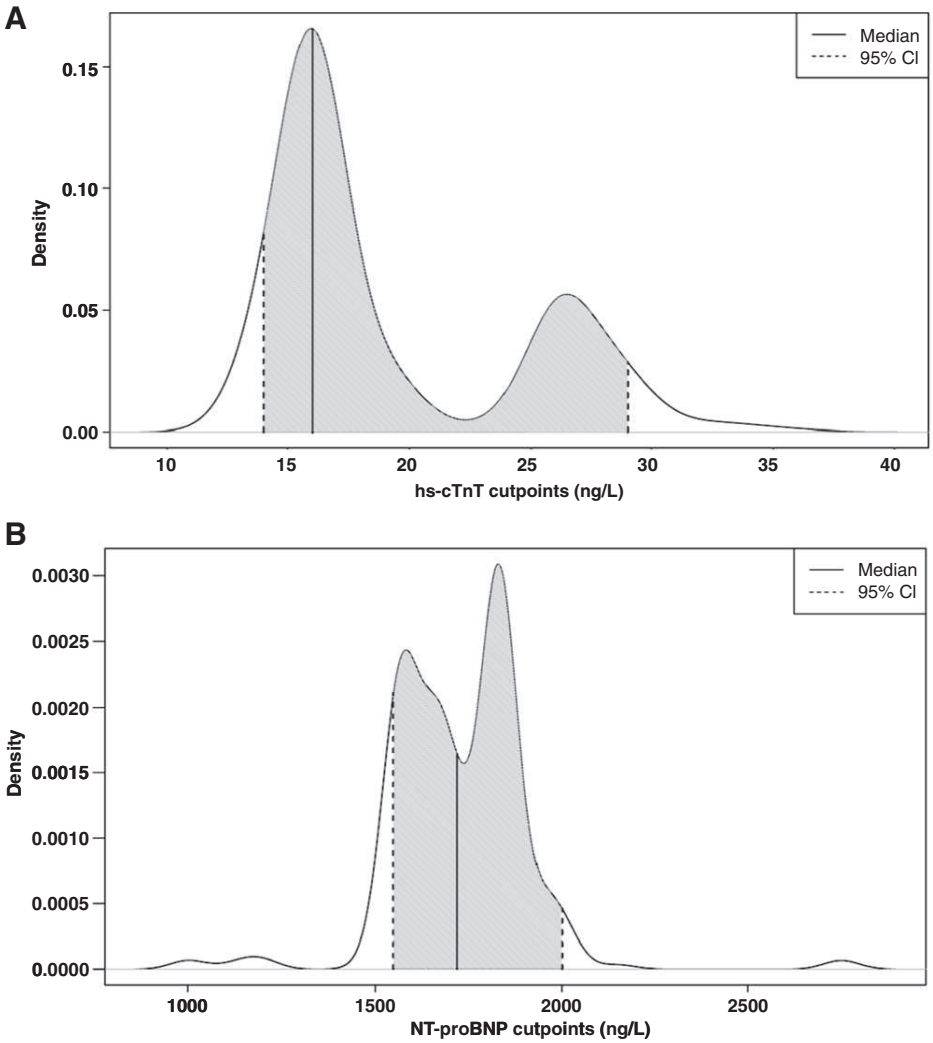
Hb, Plasma hemoglobin.

Cox regression and modeling

In the bivariable analysis, both hs-cTnT (HR 10.68 [95% CI, 4.70-24.26], $P < .001$) and NT-proBNP (HR 1.63 [95% CI, 1.50-1.78], $P < .001$) predicted death from all causes as continuous variables. In multivariable analysis, the two biomarkers remained independent predictors of mortality together with age, sex, NYHA functional class, β-blocker treatment, sodium, and hemoglobin (Table II).

Density plots of the best cut-off point in non-adjusted Cox models were calculated using bootstrap methodology to identify optimal prognostic cut-off points for hs-cTnT (16 ng/L [95% CI, 14-29]; Figure 3A) and NT-proBNP (1720 ng/L [95% CI, 1550-2000]; Figure 3B). To determine the potential utility of simultaneous hs-cTnT and NT-proBNP assessment, we divided the sample into four groups based on hs-cTnT and NT-proBNP cut-off points. As shown in Figure 4, patients with elevated hs-cTnT levels had higher risk than patients with elevated NT-proBNP levels when compared with the reference group, which had low levels of both markers (HR 3.68 [95% CI, 2.51–3.59], $P < .001$ and HR 1.73

Figure 3



Bootstrap density plots of best cut-off points for hs-cTnT (panel A) and NT-proBNP (panel B); values are expressed in ng/L.

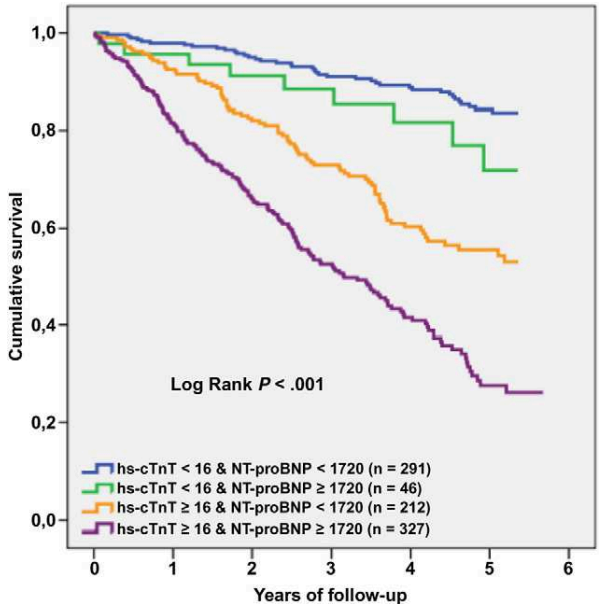
[95% CI, 0.84–3.58], $P = .136$], respectively). Patients with elevated levels of both hs-cTnT and NT-proBNP had a markedly increased risk (HR 7.42 [95% CI, 5.23-10.54], $P < .001$), indicating that assessment of both hs-cTnT and NT-proBNP is more effective at identifying a high-risk subgroup than individual assessments of either biomarker.

Measurements of performance

Discrimination. The *C* statistic for the prediction of death increased significantly when either of the two biomarkers were incorporated into a model with established mortality risk factors (age, sex, LVEF, NYHA functional class, diabetes, eGFR, ischemic etiology, sodium, hemoglobin, β-blocker treatment, and ACEI or ARB treatment) (Table III). Moreover, the addition of both biomarkers significantly improved the C statistic for predicting death from all causes.

Calibration. The *P* values for the Hosmer-Lemeshow statistics indicated good calibration for the model with and without one or the two biomarkers ($P > .14$ for all comparisons) (Table III). BIC, AIC, and Brier scores were lower in the model that included hs-cTnT than in the model that included NT-proBNP. However, lower BIC, AIC, and Brier scores were obtained in the model that included both biomarkers (Table III). Models including

Figure 4



Kaplan-Meier survival curves according to hs-cTnT and NT-proBNP levels.

Table III. Performance of the models at 4 years

	Model 1†	Model 2‡	Model 3§	Model 4‖
Discrimination				
AUC*	0.76 [0.74–0.79]	0.77 [0.75–0.79] $P = .017$	0.78 [0.75–0.80] $P = .002$	0.78 [0.76–0.81] $P = .004$
Calibration				
H-L	$X^2 = 8.6$ $P = .38$	$X^2 = 9.8$ $P = .28$	$X^2 = 2.2$ $P = .98$	$X^2 = 12.1$ $P = .14$
Brier Score	0.161	0.155	0.152	0.150
AIC	3591	3570	3559	3553
BIC	3643	3627	3620	3619
Reclassification				
IDI*	Reference	1.4 [0.3–2.4] $P = .011$	2.8 [1.6–4.0] $P < .001$	3.1 [1.7–4.5] $P < .001$
NRI*	Reference	1.5 [−5.2 to 8.2] $P = .67$	7.7 [0.7–14.7] $P = .03$	4.2 [−3.0 to 11.3] $P = .25$

H-L, Hosmer and Lemeshow test.
* *P* values vs. Model 1.
† Model 1: Age, sex, ischemic etiology, LVEF, NYHA functional class, eGFR, diabetes mellitus, ACEI/ARB treatment, β-blocker treatment, sodium, hemoglobin.
‡ Model 2: Model 1 + NT-proBNP.
§ Model 3: Model 1 + hs-cTnT.
‖ Model 4: Model 1 + NT-proBNP + hs-cTnT.

biomarkers (either one or both) showed better global goodness-of-fit than the model with only established mortality risk factors as evaluated by likelihood ratio tests ($P < .001$).

Table IV. Direct comparison of performance of models containing biomarkers

	Model 2 vs model 4		Model 3 vs model 4	
Discrimination				
AUC	0.77 [0.75–0.79]	0.78 [0.76–0.81] $P = .037$	0.78 [0.75–0.80]	0.78 [0.76–0.81] $P = .28$
Calibration				
Brier Score	0.155	0.150	0.152	0.150
AIC	3570	3553	3559	3553
BIC	3627	3619	3620	3619
Likelihood ratio		$P < .001$		$P = .005$
Reclassification				
IDI	Reference	1.7 (0.9-2.6) $P < .001$	Reference	0.3 (−0.3 to 0.9) $P = .28$
NRI	Reference	4.2 (−1.9 to 10.3) $P = .174$	Reference	−2.4 (−6.7 to 1.8) $P = .26$

Footnotes as in Table III.

Reclassification. IDI (risk as a continuous variable) increased significantly with the incorporation of each biomarker compared with the model with established mortality risk factors, yet the net increase was higher with the addition of hs-cTnT compared with NT-proBNP (2.8 and 1.4, respectively; Table III). The highest IDI was obtained with the combination of the two biomarkers (Table III). NRI (reclassification according to predefined risk categories) was significant after the individual inclusion of hs-cTnT, while NT-proBNP reclassified a negligible number of patients to the model with established mortality risk factors (7.7% and 1.5%, respectively; Table III).

The separate addition of hs-cTnT into the model that already combined established mortality risk factors + NT-proBNP (Model 4 vs. Model 2) also significantly improved the studied measurements of performance (AUC, likelihood ratio and IDI) (Table IV). The combination of the two biomarkers also showed better calibration results than hs-cTnT alone (Table IV).

Discussion

This study provides a comprehensive analysis of the prognostic value of hs-cTnT (a marker of myocardial damage), alone or in combination with NT-proBNP (a marker of myocardial stretch), in a real-life cohort of chronic HF patients. Both biomarkers improved risk stratification for death above and beyond a model with eleven well established risk factors.

Our study findings are in agreement with previous reports that assessed the relationship between hs-cTnT and clinical variables.[14–16] There were remarkable findings in this cohort. First, hs-cTnT levels increased

very significantly with the severity of HF (NYHA class), suggesting ongoing myocardial damage and progression of HF in sicker patients. Second, although subgroup analysis should be interpreted with caution, the high hs-cTnT levels observed in hypertensive cardiomyopathy came as a surprise. However, Setsuta et al[21] previously reported that elevated cTnT in hypertensive patients was an important predictor of future cardiovascular events. One possible hypothesis is that subendocardial ischemia caused by hypertensive left ventricular hypertrophy drives myocyte injury, resulting in higher levels of hs-cTnT and ultimately patchy fibrosis. In the general population, even in asymptomatic individuals, high hs-cTnT levels were predictive of future cardiovascular events and correlated with structural heart disease.[22–24] Finally, the association between cTnT and chronic kidney disease is a consistent finding. Detectable cTnT levels by means of conventional assays in patients with end stage chronic kidney disease are associated with a poor prognosis, even in the absence of coronary heart disease. The clearance and degradation of cTnT remains undefined.[25] However, in a small study, Tsutamoto et al[26] demonstrated a significant correlation between eGFR and serum cTnT levels in HF patients, suggesting that decreased cTnT clearance could contribute to elevated troponin levels in these patients.

The mechanisms of troponin release in HF are not well established, and several processes are likely involved. Although higher troponin levels were observed in patients with HF of ischemic etiology, it has been consistently reported that patients with non-ischemic HF also have elevated troponin levels. Multiple mechanisms may be involved,[27] such as subendocardial ischemia due to increased transmural wall stress and stiffening of the myocardium, myocyte necrosis (induced by ischemia, inflammation, and oxidative stress), myocyte apoptosis, cellular release of proteolytic troponin degradation products, and increased cellular wall permeability because of reversible injury.

Several studies have demonstrated a consistent association between cTnT elevation and prognosis in acute[9–11] and chronic HF[12] using conventional assays. Latini et al[16] first evaluated the prognostic value of very low cTnT levels using a precommercial version of the hs-cTnT assay in patients enrolled in the Valsartan Heart Failure Trial. Ninety-two percent had detectable hs-cTnT levels, and the risk of death and HF hospitalization increased seven- to eight-fold across increasing deciles of hs-cTnT, and remained strongly associated with these outcomes after adjustment for standard risk predictors and BNP levels. These authors[16] used 12 ng/L (the median value in their population) as the cut-off. Two additional studies[14,15] that evaluated hs-cTnT in chronic HF, both small studies with limited follow-up, used the upper reference limit of the assay (between 10 and 15 ng/L) to define elevated hs-cTnT levels. In this cohort (a large, prospective, real-life, ambulatory HF population followed for 41 months), the median value of hs-cTnT was 22.6 ng/L. Nevertheless, the optimal cut-off (set at 16 ng/L), was obtained using state-of-the-art statistics by bootstrapping the value that maximized the log-likelihood of the non-adjusted Cox models. This novel approach provided a more precise cut-off for prognostic purposes. To the best of our knowledge, this is the first study in HF that uses this method to select more accurate biomarker cut-off points.

NT-proBNP is well recognized as an important prognostic biomarker in HF. However, beyond natriuretic peptides, the use of other biomarkers for risk assessment is being debated. In this study, the predictive accuracy of hs-cTnT was even higher than that of NT-proBNP according to comprehensive discrimination, calibration, and reclassification analyses. However, the combination of both biomarkers was associated with a substantially higher risk compared with either biomarker alone, reaching a very significant HR of 7.42. Above their respective cut-off points, both biomarkers allowed us to identify a very high-risk subgroup of HF patients with a 5-year predicted survival of 28% (compared with 86% survival for both biomarkers below their respective cut-off points) as assessed by Kaplan-Meier.

Limitations

There is a risk that the absolute levels of hs-cTnT could have been affected by having been measured from frozen rather than fresh samples. There is little information about long-term stability of frozen hs-cTnT. We have analyzed only one blood sample per patient and cannot comment on the prognostic value of serial determinations. The use of bootstrap method to determine the cut-off points for NT-proBNP and hs-cTnT allows to optimize the prognostic prediction but limits its comparison with other analyses.

Our population was a general HF population treated at a specific and multidisciplinary HF unit in a tertiary hospital, and most patients were referred from the cardiology department, resulting in relatively young men with HF of ischemic etiology and reduced LVEF. As such, the obtained results cannot necessarily be extrapolated to a global HF population.

Conclusions

Hs-cTnT provides significant prognostic information in a real-life cohort of patients with chronic HF. The simultaneous addition of hs-cTnT and NT-proBNP into a model that includes established risk factors improves mortality risk stratification.

Disclosures

Dr. M. de Antonio received a competitive research grant from the Catalan Society of Cardiology. hs-cTnT and NT-proBNP assays were kindly provided by Roche

Diagnostics, which had no role in the design of the study, or the collection, management, analysis, or interpretation of the data.

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