Universitat Autònoma de Barcelona
Departament de Medicina

TESI DOCTORAL

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

Doctoranda

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El Dr. Josep Lupón i Rosés, Cap de Secció del Servei de Cardiologia, Coordinador de la Unitat d'Insuficiència Cardíaca de l'Hospital Universitari Germans Trias i Pujol de Badalona, Professor Associat del Departament de Medicina de la Universitat Autònoma de Barcelona; i el Dr. Antoni Bayés i Genís, Cap del Servei de Cardiologia de l'Hospital Universitari Germans Trias i Pujol de Badalona, President de la Societat Catalana de Cardiologia, Professor Titular del Departament de Medicina de la Universitat Autònoma de Barcelona,

CERTIFIQUEN: Que Marta de Antonio i Ferrer, Llicenciada en Medicina, ha realitzat sota la seva direcció la Tesi titulada "ASSOCIACIÓ DE DIFERENTS BIOMARCADORS EN PACIENTS AMB INSUFICIÈNCIA CARDÍACA CONTROLATS A UNA UNITAT D'INSUFICIÈNCIA CARDÍACA MULTIDISCIPLINÀRIA", per optar al Grau de Doctora i que dita tesi compleix tots els criteris necessaris per ser defensada al Tribunal corresponent.

Badalona, 22 de Setembre del 2014

Dr. Josep Lupón Rosés

Dr. Antoni Bayés Genís

Als meus pares

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- Lupón J, de Antonio M, Vila J, Peñafiel J, Galán A, Zamora E, Urrutia A, Bayes-Genis. Development of a novel heart failure risk tool: the barcelona bio-heart failure risk calculator (BCN bio-HF calculator). PLoS One. 2014;9(1):e85466.

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1. AGRAÏMENTS

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2. ACRÒNIMS

ACCF/AHA	*American College of Cardiology Foundation/American Heart Association*
ANP	pèptid natriurètic auricular
ARA-II	Antagonistes del Receptors de l'Angiotensina-II
AUC	Àrea sota la corba
b-blocadors	Beta-blocadors
BNP	Pèptid natriurètic cerebral
cTn	Troponina cardíaca mesurada amb assaig convencional
DAI	Desfibril·lador Automàtic Implantable
ESC	European Society of Cardiology
FE	Fracció d'ejecció del ventricle esquerre
Hs-cTn	Troponina cardíaca mesurada amb assaig d'alta sensibilitat
IC	Insuficiència Cardíaca
IDI	*Integrated Discrimination Improvement o millora de la discriminació integrada*
IECAS	Inhibidors de l'Enzim Convertidor de l'Angiotensina-II
MMP	Metal·loproteïnasses de la matriu extracel·lular
NRI	*Net Reclassification Index o millora de la discriminació integrada*
NT-proBNP	Fragment amino terminal del pro pèptid natriurètic cerebral
NYHA	*New York Heart Association*
sc-cTn	Troponina cardíaca mesurada amb assaig de sensibilitat contemporània
ST2	*Soluble toll-like receptor-2*
TIMPs	Inhibidors Tissulars de les metal·loproteïnases de la matriu extracel·lular
TRC	Teràpia de Resincronització Cardíaca
VO_2	Consum d'Oxigen

3. INTRODUCCIÓ

LA INSUFICIÈNCIA CARDÍACA

3.1. Concepte i diagnòstic

Les darreres guies clíniques d'insuficiència cardíaca de la *European Society of Cardiology* (1) (ESC) i de *l'American College of Cardiology Foundation/American Heart Association* (2) (ACCF/AHA) defineixen la insuficiència cardíaca (IC) com una síndrome clínica resultant d'una alteració estructural o funcional en l'ompliment ventricular o en l'ejecció de sang. És el destí final de moltes cardiopaties. El diagnòstic pot ser complex i es basa en una història clínica detallada i exploració física que evidenciïn la presència de símptomes (dispnea, fatiga, intolerància a l'exercici, edemes) i signes clínics típics (com crepitants pulmonars i ingurgitació jugular). Els criteris diagnòstics clínics més utilitzats clàssicament han estat els de Fragmingham, requerint 2 criteris majors o 1 major i 2 menors (taula 1). Alguns dels símptomes clínics són inespecífics (3) i els signes congestius derivats de la retenció hidrosalina es poden modificar amb el tractament diürètic, per tot això és important objectivar la disfunció cardíaca. Dita disfunció acostuma a ser miocardíaca (disfunció ventricle esquerre sistòlica i/o diastòlica) però altres alteracions cardíaques com les valvulopaties, malalties del pericardi i alteracions del ritme cardíac entre d'altres poden causar IC.

Taula 1. Criteris de Framingham

CRITERIS MAJORS	CRITERIS MENORS
- Dispnea paroxística nocturna/ortopnea - Ingurgitació jugular (o augment de pressió venosa) - Reflux hepato-jugular - Estertors - Edema agut de pulmó - Cardiomegàlia - Galop i tercer soroll - Resposta al tractament diürètic (pèrdua de pes de més de 4,5 Kg)	- Edemes maleolars - Tos nocturna - Dispnea d'esforç - Hepatomegàlia - Vessament pleural - Taquicàrdia (>120x')

A tot pacient amb el diagnòstic o sospita d'IC se li hauria de practicar un electrocardiograma de 12 derivacions, una analítica completa amb hemograma, ionograma (incloent calci i magnesi), paràmetres de funció renal i hepàtica, lípids, estudi

del ferro i funció tiroïdal i una ecocardiografia-Doppler color. Aquesta darrera prova aporta informació sobre la mida de les cavitats cardíaques, el gruix miocardíac, el funcionament valvular i les funcions sistòlica i diastòlica. La radiografia de tòrax pren valor fonamentalment en el context d'IC aguda per identificar congestió venocapil·lar i/o edema pulmonar i per descartar altres possibles causes de dispnea. Cal destacar que l'absència de cardiomegàlia a la radiografia de tòrax no descarta en cap cas disfunció ventricular.

Diversos estudis han demostrat la utilitat dels pèptids natriurètics en el diagnòstic da la IC, sobretot en la situació de dispnea aguda (4,5), però també en el pacient ambulatori (6,7), malgrat que la sensibilitat i l'especificitat és menor en el context crònic . No obstant, no hi ha un acord unànime en el punt de tall que permeti descartar o confirmar el diagnòstic d'IC, ja que els seus valors circulants varien en funció de diverses condicions com l'edat, funció renal, obesitat, presència de fibril·lació auricular, tractament específic de la IC i altres patologies no cardiològiques. A les guies de l'ESC 2012 els pèptids natriurètics prenen especial protagonisme en el diagnòstic inicial, sobretot quan la disponibilitat de l'ecocardiografia és limitada, i es proposen uns nivells punts de tall pel diagnòstic d'IC aguda i crònica.

Les causes d'IC són nombroses, sent la cardiopatia isquèmica l'etiologia més freqüent als països occidentals. La hipertensió arterial contribueix a l'aparició d'IC en molts pacients, la majoria amb malaltia coronària, i és la principal causa d'IC amb FE preservada. Fins un 20-30% de casos es desconeix l'etiologia però en alguns d'aquests poden estar associades a alteracions genètiques. És important identificar la causa, sobretot aquelles etiologies que són corregibles. Cal tenir en compte que moltes de les possibles causes se solapen. A la Taula 2 s'enumeren algunes d'aquestes causes, sense ser una llista exhaustiva.

Taula 2 Causes freqüents d'IC

ETIOLOGIES

- Cardiopatia isquèmica
- Hipertensió arterial
- Miocardiopaties:
  o Genètiques: Dilatada (idiopàtica), hipertròfica, no compactada, displàsia aritmogènica.
  o Adquirides: miocarditis, tòxiques (alcohol, fàrmacs cardiotòxics), nutricionals, infiltratives, peri-part, autoimmunes.
- Valvulopaties
- Malalties del pericardi: Pericarditis constrictiva.
- Cardiopaties congènites

Per tal d'establir el diagnòstic etiològic i segons la sospita clínica es poden realitzar altres proves complementàries com tests de detecció d'isquèmia (tomogammagrafia per emissió de fotó únic (SPECT), ecocardiografia d'esforç o dobutamina), caracterització de l'anatomia coronària (coronariografia, tomografia computada), ressonància magnètica cardíaca, estudi de pressions endocavitàries i puntualment biòpsia endomiocàrdica.

Existeixen diferents tipus classificacions per a la IC. La distinció entre IC amb fracció d'ejecció del ventricle esquerre (FE) preservada o reduïda és la principal. La FE és un paràmetre que resulta de dividir el volum ejectiu (diferència entre el volum telediastòlic i el telesistòlic) pel volum telediastòlic i es determina per tècniques d'imatge cardíaca (ecocardiografia, G-SPECT o ressonància magnètica). En molts pacients coexisteixen anormalitats de la funció sistòlica i diastòlica. La classificació de la IC per la FE és important per diferents motius: defineix dues poblacions de pacients amb característiques diferents, té valor pronòstic i perquè la majoria d'assaigs clínics seleccionaren els pacients segons aquest paràmetre. Tanmateix, el punt de tall per diferenciar entre FE preservada i reduïda ha estat dispar (del 40 al 55%), arbitrari i s'ha modificat al llarg del temps, tant als assaigs clínics com a les guies de pràctica clínica. Cal destacar que la majoria d'estudis aleatoritzats sobre els tractaments en IC que han demostrat millorar el pronòstic van seleccionar pacients amb FE ≤ al 35%. Els pacients amb FE entre el 35 i el 50% es troben en una zona gris. A les guies de l'ACC/AHA 2013 (2) estableixen com a punt de tall per FE reduïda ≤ al 40 i a les guies europees no queda ben definit el punt de tall però consideren com "normal" una FE >50%. El diagnòstic del pacients amb IC i FE preservada és més complex perquè són poblacions d'edat avançada amb múltiples

comorbiditats , que també poden causar símptomes similars als de la IC (8). A més a més, cal demostrar disfunció diastòlica per ecocardiografia o cateterisme cardíac i/o alguna alteració estructural cardíaca com hipertròfia ventricular o dilatació auricular (9).

La classificació funcional de la New York Heart Association (NYHA) es divideix en 4 estadis segons la gravetat dels símptomes (Taula 1). S'utilitza per guiar el tractament i també és un criteri àmpliament utilitzat per seleccionar als pacients pels assaigs clínics. Tot i ser un paràmetre subjectiu, difícil de reproduir i no correlacionat amb el grau de disfunció ventricular, ha demostrat una clara associació amb el pronòstic (10).

L'ACC/AHA (2) proposa un sistema de 4 estadis clínics progressius, on al primer estadi es troben aquells individus asimptomàtics i sense cardiopatia estructural però amb factors de risc per presentar IC i en el darrer estadi aquells amb IC refractària (Taula 3). A estudis comunitaris s'ha demostrat que la progressió d'un estadi a l'altre es correlaciona amb un augment de 5 vegades la mortalitat a 5 anys i amb un augment de la concentració de pèptids natriurètics (11). Tanmateix, és controvertit el diagnòstic d'IC en pacients asimptomàtics i sense cardiopatia.

Taula 3. Estadis IC ACCF/AHA i classificació funcional NYHA

<table>
<tr><th colspan="2">ESTADIS ICC ACCF/AHA</th><th colspan="2">CLASSE FUNCIONAL</th></tr>
<tr><td>A</td><td>Alt risc de patir IC sense cardiopatia estructural ni símptomes</td><td colspan="2">-</td></tr>
<tr><td>B</td><td>Cardiopatia estructural sense signes ni símptomes d'IC</td><td rowspan="2">I</td><td rowspan="2">No limitació de l'activitat física. L'activitat física ordinària no causa símptomes IC.</td></tr>
<tr><td rowspan="2">C</td><td rowspan="2">Cardiopatia estructural amb símptomes previs o actuals d'IC</td></tr>
<tr><td>II</td><td>Limitació lleugera de l'activitat física. Confortable en repòs però l'activitat física ordinària provoca símptomes IC.</td></tr>
<tr><td rowspan="2">D</td><td rowspan="2">IC refractària que precisa maneig especialitzat</td><td rowspan="2">III</td><td rowspan="2">Limitació marcada de l'activitat física. Confortable en repòs però activitat físiques menys que ordinàries provoquen símptomes.</td></tr>
<tr><td>IV</td><td>Incapaç de fer qualsevol activitat física sense símptomes d'IC o bé símptomes en repòs.</td></tr>
</table>

Pel que fa a la cronologia de la IC, es denomina IC de nova aparició quan és el primer episodi, que es pot presentar de forma aguda o bé amb símptomes més larvats. La IC persistent (no hi ha un punt de tall clar, però generalment es demanen almenys 3 mesos d'evolució) s'anomena crònica. Un pacient amb simptomatologia estacionària durant un mes es descriu com estable. Si un pacient amb IC es deteriora es descriu com a IC aguda o descompensada.

3.2. Epidemiologia

La IC constitueix actualment un dels problema de Salut Pública de primer ordre als països desenvolupats on la prevalença se situa entre l'1 i el 3%, augmentant fins el 10% en majors de 70 anys (12). Es calcula que hi ha 23 milions de persones al món amb insuficiència cardíaca (13). Els estudis a Espanya (14,15) estimen xifres més altes de prevalença entre el 5 i el 6.8% en la població general i del 16% en majors de 75 anys. Aquesta disparitat amb països del nostre entorn podria ser real o bé deguda a les limitacions metodològiques dels estudis practicats al nostre medi. Tot i que la incidència de la IC sembla estar estabilitzada la prevalença de la IC creix any rere any per diversos motius: l'envelliment de la població, l'augment de supervivència en pacients amb hipertensió arterial i post infart de miocardi i dels pacients amb IC.

L'agudització de la IC és la primera causa d'hospitalització en majors de 65 anys, fet que condiciona en gran mesura l'elevat impacte econòmic de la mateixa, al voltant del 2% de la despesa sanitària en països desenvolupats (16). L'estància hospitalària mitja és de 9±5 dies (17) i la taxa de reingressos és molt elevada, pot arribar al 16-50% després d'una primera admissió per IC (18). El nombre d'hospitalitzacions per IC ha augmentat progressivament, sobretot en més grans de 65 anys.

Malgrat les millores significatives en el tractament de la IC i la disminució de la taxa de mortalitat ajustada per edat per IC (19), la IC continua presentant una elevada mortalitat, que es xifra al voltant del 30-45% el primer any després d'un primer ingrés i del 50% als 5 anys (20), pitjor que la de diversos tipus de càncer (21). A l'Estat Espanyol, segons les dades registre del Instituto Nacional de Estadística del 2010 (22), la IC va constituir el 3% del total de defuncions en homes i el 10% en les dones. La mortalitat hospitalària arreu és també elevada i a un estudi al nostre país és va estimar en el 10% (23,24)

Al voltant del 50% dels pacients presenten IC amb FE preservada i tenen un perfil clínic característic: predomini del sexe femení, edat avançada, major prevalença d'hipertensió arterial, fibril·lació auricular, obesitat i anèmia entre d'altres comorbiditats (25,26). El pronòstic és força semblant a la IC amb FE reduïda però un metanàlisi recent ha observat un mortalitat menor (27).

És important destacar que la IC afecta de forma important a la qualitat de vida (28).

3.3. Fisiopatologia

Diferents processos poden ocasionar un dany miocardíac que comporta una disfunció ventricular que desencadena tot un seguit de mecanismes de compensació per conservar la funció cardíaca. El manteniment perllongat de dits mecanismes és deleteri i perpetua un cercle viciós que deteriora progressivament la funció ventricular (Figura 1). Al llarg dels anys s'han proposat diferents models en funció dels diversos mecanismes implicats en la gènesi de la IC. A dia d'avui sabem que no hi ha un model únic sinó que la fisiopatologia de lC és un complex fenomen on participen múltiples "sistemes" que interactuen entre ells (29). Els avenços en el coneixement de la fisiopatologia han permès el desenvolupament i aplicació de tractaments específics que han millorat significativament el pronòstic de la malaltia.

El **model cardiorenal** va ser el primer descrit i fixava l'èmfasi en la interrelació del cor i el ronyó. Així, la disfunció sistòlica ventricular desencadenava alteracions del flux renal que resultaven en la retenció hidrosalina.

El **model hemodinàmic** va derivar de l'observació de la disminució de la contractilitat del múscul cardíac i en la resposta vasoconstrictora perifèrica excessiva. En la IC amb FE reduïda, com a resposta a la disminució de la contractilitat, el ventricle esquerre es dilata per mantenir el cabal cardíac segons el mecanisme de Frank-Starling. La dilatació progressiva però provoca finalment una disminució de la contractilitat. En la IC amb FE preservada acostuma la principal causa és una hipertròfia ventricular que comporta un augment de rigidesa, alteració de la relaxació del ventricle esquerre i disminució de la *compliance*.

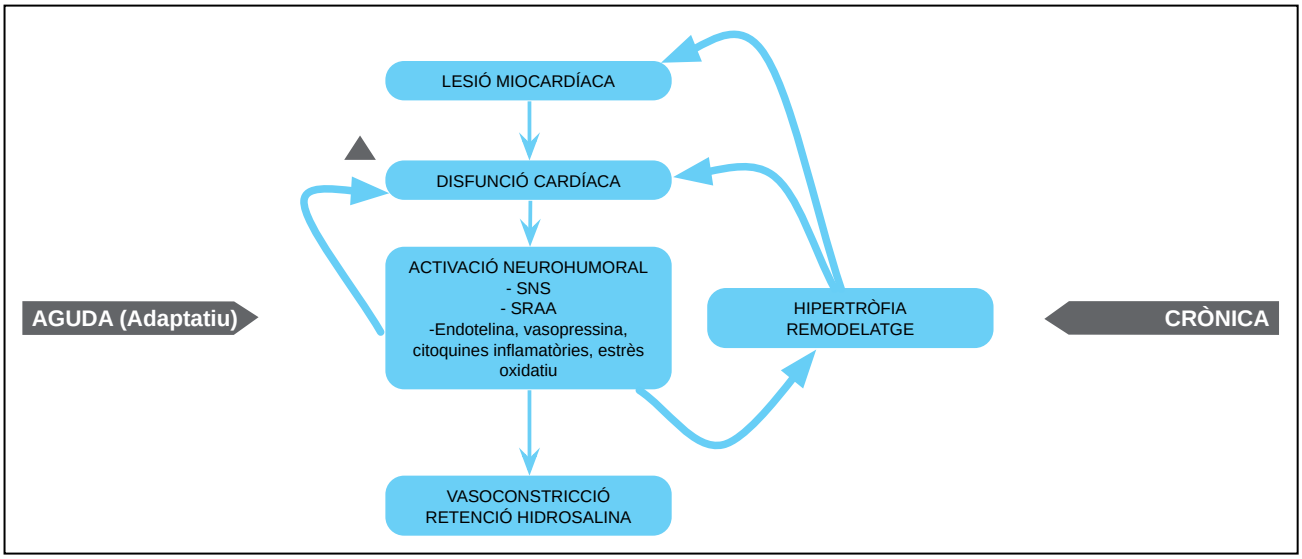
Cap les models anteriors explicava per si sol de forma eficient la progressió del a malaltia. El **model neurohumoral** descriu com la disfunció ventricular posa en marxa l'activació del sistema adrenèrgic (SNS) i el sistema renina-angiotensina-aldosterona (SRAA) entre d'altres per millorar la perfusió vital mitjançant la vasoconstricció sistèmica, estimulació de la contractilitat i freqüència cardíaca i expansió del volum extracel·lular. Aquesta resposta és beneficiosa a curt termini però l'activació neurohumoral perllongada té conseqüències deletèries i sobrepassa la resposta vasodilatadora i diürètica d'altres substàncies alliberades com els pèptids natriurètics, òxid nítric, prostaglandines i citoquines. Les conseqüències maladaptatives de l'activació neurohumoral no són únicament modificacions funcionals (augment de la contractilitat, postcàrrega) sinó també estructurals (hipertròfia, apoptosi, fibrosi, remodelat) que condueixen a l'empitjorament de la disfunció ventricular.

L'alteració en la funció i estructura cardíaca (forma, massa i volum) com a resposta a una lesió miocardíaca o a una sobrecàrrega de pressió es coneixen com a **remodelatge cardíac**. El procés de remodelatge implica canvis cel·lulars, moleculars i d'expressió gènica a nivell dels miòcits i de la matriu extracel·lular. La matriu extracel·lular proporciona un esquelet estructural i funcional als ventricles i conté nombroses molècules que intervenen en el funcionament cardíac com les citoquines i els factors de creixement. Es compon fonamentalment de fibres de col·lagen i altres proteïnes estructurals produïdes pels miofibroblasts. Un desequilibri del metabolisme del col·lagen de la matriu extracel·lular promou canvis en l'arquitectura i funció cardíaca. Així, un excés de proteïnes estructurals o fibrosi provoca un augment de la rigidesa miocardíaca i disfunció diastòlica, com en la IC amb FE preservada, mentre que una destrucció de la mateixa pot promoure dilatació com en la miocardiopatia dilatada. Diversos sistemes neurohumorals actuen a nivell de la matriu extracel·lular

Altres mecanismes fisiopatològics implicats en la IC són la inflamació, alteracions en la regulació del cicle del calci intracel·lular dels miòcits, l'apoptosi o mort cel·lular programada desencadenada per diferents estímuls, els processos inflamatoris i el mecanismes genètics. El camp de la genètica engloba des de l'aproximació clàssica de detecció de gens puntuals responsables de la síntesi de proteïnes anòmales (miocardiopaties familiars) fins a la genòmica, on l'exploració completa del genoma permet identificar múltiples variants genètiques en la IC i obre vies d'investigació. En aquest sentit cal destacar la investigació sobre els microRNAs, molècules de nucleòtids curtes que estan implicades en la regulació gènica postranscripcional .

S'han identificat múltiples mRNAs implicats en processos fisiopatològics de la IC com l'acoblament excitació-contracció, hipertròfia, dilatació ventricular, apoptosi i fibrosi. Les possibles aplicacions clíniques dels mRNA són àmplies, podrien ser utilitzats com a biomarcadors (alguns es poden detectar en sang perifèrica) i com a dianes terapèutiques.

Figura 1. Interacció entre la funció cardíaca i sistema neurohumoral (Adaptat de Braunwald [29]).



3.4. Biomarcadors

Els avenços en el coneixement de la IC han permès identificar múltiples substàncies biològiques circulants i mesurables en sang perifèrica que reflecteixen diferents aspectes de la fisiopatologia de la IC i que genèricament es coneixen com biomarcadors. Estrictament un biomarcador és qualsevol característica que es pugui mesurar de forma objectiva i permeti avaluar un procés biològic normal, un procés patogènic o una resposta farmacològica a intervencions terapèutiques (30) i per tant inclou variables genètiques, proves d'imatge, proves fisiològiques i característiques anatomopatològiques entre d'altres. No obstant això, en general quan hom parla de biomarcadors, la majoria de la literatura fa referència a substàncies mesurables en líquids corporals. Ens referirem exclusivament a substàncies biològiques circulants fonamentalment plasmàtiques. L'interès creixent pels biomarcadors es veu exemplificat per l'augment del nombre de publicacions en la matèria, que ha crescut de forma exponencial a la darrera dècada (31).

Morrow i de Lemos (32) van introduir tres criteris per avaluar la utilitat d'un biomarcador en les malalties cardiovasculars: 1) la seva mesura ha de ser de ràpida obtenció, precisa i reproduïble a un cost raonable; 2) ha d'aportar nova informació no disponible per altres mètodes; i 3) ha d'ajudar en el maneig clínic ja sigui en el diagnòstic, pronòstic o el tractament. Revisions posteriors (31) han suggerit altres característiques que ha de complir un nou biomarcador: 1) el biomarcador s'ha d'avaluar en ventall ampli de pacients i l'anàlisi estadística ha de ser contemporània, estandarditzada i rigorosa; 2) Els mètodes de determinació analítics han de tenir un variabilitat biològica definida i un nivell d'imprecisió analític baix; 3) Ha de reflectir processos fisiopatològics de la IC i de la progressió de la mateixa. Fins a l'actualitat, els únics biomarcadors que s'han mostrat aplicables en la pràctica clínica tant per al diagnòstic, pronòstic, com per a guiar el tractament de la IC són els pèptids natriurètics.

La classificació més comú dels biomarcadors es fa d'acord amb el procés fisiopatològic en el qual intervenen, però molts d'ells es poden trobar en més d'una categoria ja que actuen a més d'un nivell. A la taula 4 s'exemplifica una classificació.

A continuació s'exposen alguns dels biomarcadors més comuns.

3.4.1 Marcadors d'estrès o estirament miocardíac

Pèptids natriurètics. Són els biomarcadors més ben estudiats i els únics establerts a la pràctica clínica diària. Són prohormones o fragments de la degradació dels precursors que s'alliberen en situacions d'estrès hemodinàmic per sobrecàrrega de pressió, dilatació i hipertròfia ventricular (33), situacions que es donen en la IC i altres afectacions cardíaques com les cardiopaties estructurals, valvulars i arítmies entre d'altres. Tenen un efecte vasodilatador, diürètic, natriurètic i contraposen l'activitat del sistema renina-angiotensina-aldosterona i el sistema nerviós simpàtic. Per les seves accions es consideren també neurohormones. El pèptid natriurètic cerebral (BNP) s'allibera als ventricles i prové de l'escissió seqüencial d'un precursor hormonal, alliberant-se també el fragment amino terminal del proBNP (NT-proBNP) que no té cap funció biològica coneguda. El pèptid natriurètic auricular (ANP) es produeix a les aurícules també en situacions de distensió auricular però és menys estable a plasma i difícil de mesurar. Els nivells dels pèptids natriurètics augmenten en relació amb l'edat (34), insuficiència renal (35), hipertensió pulmonar (36) i tenen una relació inversa amb l'índex de massa corporal (34). Les seves concentracions disminueixen en relació al tractament amb beta-blocadors (b-blocadors) i els inhibidors de l'enzim convertidor de l'Angiotensina-II (IECAs) (37). Cal tenir present que el pèptids natriurètics es poden trobar elevats en altres situacions clíniques com la malaltia pulmonar obstructiva crònica, el tromboembolisme pulmonar i la patologia tiroïdal entre d'altres.

Tant el BNP com el NT-proBNP han demostrat al seva utilitat en múltiples situacions: en l'*screening* o detecció de pacients amb risc de patir IC (33), en el diagnòstic d'IC en situació de dispnea aguda a Urgències (4,5) o bé en pacients amb dispnea crònica (6,7) i com a marcadors pronòstics en IC aguda i crònica (38-40). La seva aplicació com a guia de tractament de la IC és encara controvertida. Generalment els nivells de BNP i NT-proBNP estan ben correlacionats i qualsevol dels dos és adequat, sempre i quan no s'intercanviïn els valors absoluts i punts de tall respectius. No obstant això, l'aparició de tractaments amb inhibidors de la neprilisina, substància que degrada el BNP (41), pot fer que això canviï, ja que els valors de BNP es veuran alterats per aquest tipus de tractament.

En l'avaluació dels pacients amb dispnea destaca l'estudi *Breathing not properly Multinational Study* (4) on van demostrar que els nivells de BNP incrementaven la precisió diagnòstica de IC en pacients que acudien a Urgències per dispnea, de forma

que nivells per sota de 100 pg/ml feien poc probable el diagnòstic d'IC mentre que valors per sobre de 400 pg/ml suggerien el diagnòstic amb alta probabilitat. A l'estudi PRIDE (5) es van obtenir resultats semblants però amb NT-proBNP, però es van formular punts de tall diferents segons l'edat. Es punts de tall pel diagnòstic en IC crònica difereixen dels de la situació aguda però les dades disponibles són més dispars. Així, a les darreres guies de la Societat Europea de Cardiologia (1) proposen un punt de tall de 35 pg/ml pel BNP i 125 pg/ml per l'NT-proBNP. Altres estudis proposen punts de tall diferents segons l'edat (42). Els nivells de pèptids natriurètics en el moment de l'hospitalització i abans de l'alta així com el grau de reducció durant l'ingrés son predictius de mortalitat intrahospitalària i risc de re-ingrés (43-45).

En el context del pacient ambulatori amb diferents graus de severitat també estratifiquen el pronòstic. A l'estudi Val-HeFT (*Valsartan Heart Failure Trial*) (39) un augment de 500 pg/ml per sobre dels nivells basals de NT-proBNP va comportar un augment del 3.8% de la mortalitat i 3% de l'hospitalització. A l'estudi COPERNICUS (*Carvedilol Prospective Randomized Cumulative Survival*) (40) els nivells de NT-proBNP foren predictors potents de mortalitat a un any i de l'objectiu combinat de mortalitat global i hospitalització.

El resultats dels assaigs clínics dirigits a analitzar la utilitat dels pèptids natriurètics per guiar el tractament de la IC han estat inconsistents (46-49). Els estudis són heterogenis en quant a la població d'estudi i la majoria de les mostres són petites. Els treballs que van resultar positius coincidien en que la cohort d'estudi era jove i amb IC amb FE deprimida. Dues metanàlisis posteriors han conclòs que el tractament guiat pels pèptids natriurètics disminueix la mortalitat per qualsevol causa en pacients menor de 75 anys (50,51) però no es va produir una disminució de les hospitalitzacions. Part del benefici reportat en els estudis positius podria estar en relació amb un tractament més optimitzat.

La inestabilitat analítica de l'ANP ha fet que es desestimés el seu ús a la pràctica clínica. La mesura la fracció regional mitja del precursor de l'ANP (MR-proANP) ha demostrat ser útil en el diagnòstic de la IC (52).

Adrenomodulina. És una neurohormona vasoactiva que es sintetitza al cor i a altres teixits, i s'allibera en resposta la sobrecàrrega de volum i pressió. A través de l'estimulació de producció d'òxid nítric produeix vasodilatació. Se li atribueix també un efecte natriurètic i inotròpic positiu. Els seus nivells augmenten en la IC i es relacionen amb el pronòstic però té una vida mitja molt curta i és difícil de mesurar. El fragment mig

de la proadrenomedulina (MR-proADM) és més estable i fàcil de mesurar i també ha mostrat tenir significació pronòstica en la IC aguda (52,53). El seus nivells augmenten amb l'edat, classe funcional i filtrat glomerular.

sST2 (*soluble toll-like receptor-2/growth STimulation expressed gene 2*). L'ST 2 és un membre de la família dels receptors de la interleuquina 1, amb un component unit a la membrana (ST2L) i una forma soluble (sST2) mesurable en plasma i s'expressa als miòcits, cèl·lules endotelials, fibroblasts i cèl·lules del sistema immunitari. Participa en múltiples processos biològics. A nivell miocardíac no s'ha aclarit íntegrament la seva funció. S'expressa en resposta a diferents estímuls entre els quals es troba l'estirament miocardíac i la isquèmia (54-56). El lligand de la ST2L és la interleuquina 33 (IL-33) i a través d'una complexa cascada de senyals es postula que té una acció cardioprotectora pel seu efecte antihipertròfic, antifibròtic i antiapoptòtic a nivell miocardíac (55). A nivell experimental, en un model murí una deleció del gen ST2 resultà en hipertròfia, fibrosi, dilatació i disfunció ventricular (57). El sST2 sembla actuar com un receptor esquer neutralitzant els efectes de la IL-33. S'inclou en diverses categories dins la classificació dels biomarcadors: estirament, fibrosi i inflamació A la resta del treball ens referirem a la forma soluble del ST2 com ST2.

El ST2 ha demostrat la seva capacitat pronòstica en un ampli ventall de situacions. Els seus nivells es correlacionen amb el grau de severitat de la IC i és un biomarcador pronòstic de mortalitat tant en IC aguda (58, 59) com crònica (60-62), de forma independent i com a valor additiu als pèptids natriurètics. En pacients ambulatoris amb IC descompensada, el grau de reducció dels nivells de ST2 amb el tractament es va relacionar amb el pronòstic, pel que podria ser un marcador potencial de guia del tractament (61). Un altre treball que apunta a la potencial utilització de l'ST2 com a guia del tractament va valorar l'efecte de l'eplerenona sobre el remodelat ventricular. Aquest fàrmac va atenuar de forma més marcada el remodelat en els pacients amb nivells més alts de ST2 (63). No aporten valor complementari als pèptids natriurètics en quant al diagnòstic d'IC.

Els seus nivells es poden trobar augmentats en altres situacions con l'infart de miocardi, la malaltia pulmonar obstructiva crònica, la pneumònia i la sepsis (61).

El desenvolupament d'una tècnica d'alta sensibilitat podria estendre les aplicacions de l'ST2.

La darrera guia d'IC de l'AHA del 2013 (2) ha fet recomanacions amb respecte a l'ús de biomarcadors emergents com els de fibrosi ST2 i la galectina-3 en la determinació del pronòstic de la IC aguda i crònica amb un grau de recomanació IIB.

3.4.2 Marcadors de lesió miocardíaca

Troponines I i T. Són proteïnes de l'aparell contràctil del miòcits que modulen la interacció entre l'actina i la miosina. Les isoformes cardíaques i del múscul esquelètic difereixen en la seva estructura. El complex de la troponina cardíaca està compost per la troponina I (inhibitòria), troponina C (unió calci), i troponina T (unió a la tropomiosina). Són els biomarcadors de primera elecció pel diagnòstic de la síndrome coronària aguda, ja que s'alliberen al torrent sanguini en context de la necrosi/isquèmia miocardíaca i són altament específics. En la IC s'han reportat consistentment nivells elevats de troponines independentment de l'etiologia isquèmica o no isquèmica, que es relacionen amb el pronòstic en la IC aguda (64-65) i crònica (66). Els mecanismes d'alliberament de troponina en la IC no estan ben establerts i probablement en convergeixen diversos (67). Alguns dels possibles mecanismes són: la isquèmia subendocàrdica per l'augment d'estrès de la paret transmural i la rigidesa del miocardi, la necrosi de miòcits (induïda per la isquèmia, inflamació i estrès oxidatiu), l'apoptosi dels miòcits, l'alliberament cel·lular de productes de degradació de la troponina citosòlics, i l'augment permeabilitat de la paret cel·lular a causa d'una lesió reversible. Amb l'aparició dels assaigs d'alta sensibilitat que milloren la precisió analítica en el límit inferior de detecció, la freqüència de nivells detectables o elevats de troponines en pacients amb IC ha augmentat notablement i han demostrat la seva superioritat pronòstica enfront els assaigs convencionals tant en la IC aguda (68,69) com crònica (70,71). En alguns dels treballs on s'analitzaren simultàniament diversos biomarcadors les troponines varen tenir significació pronòstica independent i inclús additiva al pèptids natriurètics (69,72).

3.4.3 Marcadors d'inflamació

La inflamació contribueix en la patogènesi i progressió de la IC, pel que s'ha fet molta recerca per d'identificar potencials biomarcadors com la proteïna C reactiva, el factor de necrosi tumoral alfa, les interleuquines 1 i 6 i la pentraxina 3 entre d'altres. Malgrat que diversos d'aquests marcadors aporten informació pronòstica, la seva inespecificitat limita l'aplicació en el maneig de la IC.

3.4.4 Marcadors estrès oxidatiu

L'estrès oxidatiu es defineix com el desequilibri entre la producció de substàncies reactives d'oxigen i els mecanismes de defensa antioxidants. L'augment de l'estrès oxidatiu pot estar implicat en la fisiopatologia i progressió de la IC a través de diversos mecanismes com l'apoptosi i necrosi a nivell miocardíac, activació de sistemes neurohormonals i augment d'inflamació (73).

La mieloperoxidasa és un enzim alliberat pels neutròfils i leucòcits i catalitza la formació d'espècies reactives d'oxigen. Es relaciona amb la mortalitat en IC aguda (74) i prediu el desenvolupament d'IC en població sana entre 65 i 75 anys sense factors de risc cardiovascular (75). Amb la tecnologia actual és difícil de mesurar en plasma.

3.4.5 Marcadors de remodelat matriu extracel·lular/fibrosi.

Marcadors del metabolisme del col·lagen. Les metaloproteïnases de la matriu (MMPs) són una família d'enzims que degraden el col·lagen i altres proteïnes de la matriu extracel·lular i la seva activitat s'inhibeix per unes proteïnes conegudes com inhibidors tissulars de MMPs (TIMPs). Aquestes proteïnes se sintetitzen als fibroblasts i un desequilibri entre la síntesi i degradació de col·lagen promou el remodelatge ventricular implicat en la progressió de la IC, ja sigui per excés de producció de col·lagen i fibrosi com per destrucció del mateix. La mesura de productes de degradació del col·lagen també s'està utilitzant com un subrogat de remodelatge de la matriu. S'han avaluat diverses MMPs amb resultats mixtes en quant a la seva capacitat pronòstica, sent les més estudiades la MMP2, MMP3, i la MMP 9. Alguns estudis apunten a que ofereix més bon resultats la determinació combinada de diversos marcadors de remodelatge (76). A títol d'exemple, una estratègia multimarcador de paràmetres de remodelatge de la matriu preservada en pacients amb hipertròfia ventricular esquerra prediu l'evolució a IC amb FE (77). S'han explorat altres aplicacions, com la identificació de pacients amb IC i fibrosi que es podrien beneficiar de certs tractaments com un desfibril·lador automàtic implantable (DAI) (78) o antagonistes del receptors mineralcorticoides (79).

Galectina-3. És considera un marcador de fibrosi i inflamació. És una glicoproteïna secretada per diverses cèl·lules com els macròfags actius i neutròfils i estimula la proliferació dels miofibroblasts i la producció de procol·lagen I (80). Els seus nivells es correlacionen amb altres marcadors de fibrosi com les MMP i la TIMP1 (81). S'ha mostrat

com bon marcador pronòstic en la IC aguda i crònica (82-84). En un estudi de 232 pacients Lok et al. van observar que la galectina-3 aporta valor complementari pèptids natriurètics (85) però això no s'ha pogut confirmar en treballs posteriors (86,87). La galectina-3 és predictiva d'aparició d'IC en pacients amb síndrome coronària aguda (88) i en població sana (89). No és útil pel diagnòstic de la IC ja que és menys sensible que els pèptids natriurètics. A les darreres guies de l'AHA 2013 (2) s'accepta el seu ús com a marcador pronòstic de la IC amb un amb un nivell de recomanació IIB.

3.4.6 Neurohormones

Tot i que no s'utilitzen de forma rutinària per la seva complexitat i costosa determinació val la pena recordar que el descobriment de l'augment de catecolamines i de l'activació de l'eix renina-angiotensina-aldosterona van sorgir el fàrmacs que constitueixen el tractament bàsic de la IC (b-blocadors, IECAS i antagonistes dels receptors de l'angiotensina –II [ARA-II]).

Endotelina-1. És produeix a l'endoteli i produeix vasoconstricció i remodelat ventricular. Els seus nivells s'associen amb el pronòstic i severitat de la IC (90,91). Els assaigs amb antagonistes de l'endoteli na 1 no ha mostrat una milloria del pronòstic.

Vasopressina. Es secreta a l'hipotàlem en resposta a canvis de l'osmolaritat i hipovolèmia i és un potent vasoconstrictor. Els seus nivells estan augmentats en la IC i comporten un pitjor pronòstic (92). El tractament amb antagonistes dels receptors de la vasopressina ha mostrat una milloria dels nivells de sodi, augment de diüresi i pèrdua de pes en pacients amb signes congestius però no ha s'ha relacionat amb una milloria de la supervivència (93). La **copeptina** és un fragment del precursor de la vasopressina (C-terminal pro-vasopressina) més estable a nivell analític que la vasopressina. En un model multimarcador la copeptina va millorar l'estratificació del risc en pacients amb IC crònica sobre un model predictiu que ja contenia l'NT-proBNP i la troponina T d'alta sensibilitat (72).

3.4.7 Marcadors extracardiològics

La IC afecta en el seu curs a altres òrgans i sistemes i sovint els pacients estan afectats d'altres comorbiditats. Destaquen per la seva importància pronòstica l'anèmia i la insuficiència renal.

Marcadors insuficiència renal. La relació bidireccional entre la IC i la insuficiència renal ha derivat en la denominació de la síndrome cardiorenal. Ambdues patologies comparteixen mecanismes fisiopatològics comuns i els canvis hemodinàmics i neurohumorals de la IC així com alguns del tractaments utilitzats poden produir un deteriorament de la funció renal. La insuficiència renal és molt prevalent en la IC i és un dels marcadors independents de mortalitat més potents (94). Els biomarcadors de la funció renal també han mostrat la seva capacitat predictiva. A més de la urea, la creatinina i el filtrat glomerular renal s'han descrit altres substàncies.

Cistatina-C. És una proteïna que pertany al grup de inhibidors de les cisteïna-proteases. És sintetitza en totes les cèl·lules nucleades de l'organisme, per la qual cosa té una amplia distribució cel·lular i es detecta als fluids biològics. Per la seva petita mida la seva eliminació depèn únicament del filtrat glomerular. Per aquest motiu La cistatina-C s'ha proposat com un estimador més precís del filtrat glomerular que la creatina (95). Altrament, és predictiu de mortalitat en la cardiopatia isquèmica i en la IC aguda (96) i crònica (97). La producció de la cistatina-C augment en situacions hipermetabòliques com l'hipertiroïdisme o el tractament amb corticoides.

Gelatinasa associada amb la lipocalina del neutròfil (NGAL). És una proteïna que es localitza a les cèl·lules endotelials del túbul renal entre d'altres. És presenta com un biomarcador de detecció precoç de lesió aguda renal perquè se sobreexpresa en fases inicials de lesió renal i es detecta ràpidament a plasma i orina. En pacients amb IC aguda, la determinació de NGAL urinari es relaciona amb empitjorament de la funció renal independentment del filtrat glomerular renal i amb esdeveniments adversos relacionats amb la IC (98). El nivells urinaris de NGAL i no els plasmàtics es van correlacionar amb un empitjorament de la natriuresi i diüresi, mentre que els nivells plasmàtics elevats es van associar a una reducció del filtrat glomerular. En la IC crònica s'han reportat nivells basals més alts de NGAL (99). En un estudi per valorar el dany tubular en la IC crònica, els nivells de NGAL no es trobaren augmentats en els pacients amb insuficiència renal en comparació amb els que no en tenien, a diferència d'altres marcadors de dany tubular com NAG i KIM-1, pel que és possible que no sigui un bon marcador d'injúria renal en el pacient crònic (100). En un subestudi del GISSI-HF (*Effect of rosuvastatin in patients with chronic heart failure*) l'augment de NGAL es va associar amb la mortalitat i el risc d'hospitalització, independentment del filtrat glomerular (101).

3.4.8 Estratègia multimarcador.

Tal i com s'ha exposat, hi ha múltiples biomarcadors que han mostrat el seu valor pronòstic de forma individual. Atesa la complexitat de la fisiopatologia de la IC, s'està explorant si la combinació de marcadors que reflecteixen diferents aspectes de la fisiopatologia de la IC podria millorar la precisió diagnòstica i pronòstica i per monitoritzar la resposta al tractament. Això es coneix com estratègia multimarcador (73). La majoria d'estudis en IC han valorat el valor addicional sobre els pèptids natriurètics i un model clínic (102, 103). Hi ha diversos factors que dificulten la comparació entre estudis: Tant les poblacions d'estudi com els models clínics i la metodologia estadística emprada són heterogenis. Així mateix, en molts articles no s'aporta informació clara sobre el valor complementari del biomarcador. en tots els estudis. Com tot biomarcador tenen un cost econòmic, l'addició de qualsevol biomarcardor hauria de justificar-se amb anàlisis estadístiques adequades de discriminació, calibratge i reclassificació que s'ampliaran a l'apartat de mètodes. El nombre i la millor combinació de marcadors resta encara per determinar, però alguns estudis han mostrat que no sempre la combinació de més millora la precisió pronòstica (105).

Taula 4. Biomarcadors IC

Estirament/Estrès miocardíac	Possibles aplicacions
Pèptids natriurètics: BNP, NT-proBNP, MR-proANP, adrenomodulina	Diagnòstic, pronòstic, monitorització i diana tractament
ST2	Pronòstic
GDF-15	Pronòstic
Lesió miocàrdica	
Troponines T i I	Diagnòstic, pronòstic
Cadena lleugera de la miosina 1	Pronòstic
Proteïna citoplasmàtica cardíaca transportadora d'àcids grassos (H-FABP)	Diagnòstic, pronòstic
Fas (Apo-1)	Pronòstic
Inflamació	
CRP	Pronòstic
Factor necrosi tumoral alfa	Pronòstic
IL-1, IL-6, IL-10, IL-18	
Pentraxina 3	Pronòstic
Procalcitonina	Pronòstic
Neopterina	Pronòstic
Osteoprotegerina	Pronòstic
Estrès oxidatiu	
Mieloperoxidasa	Pronòstic
Lipoproteïnes de baixa densitat oxidades	Pronòstic
Piopirrines urinàries	Pronòstic
GGT	Pronòstic
Àcid úric	Pronòstic
Remodelat/fibrosi	
MMPs, TIMP1, Propèptids col·lagen, Fragment N terminal col·lagen tipus III	Pronòstic,
Galectina-3	Pronòstic
ST2	Pronòstic
Osteopontina	Pronòstic
Neurohormones	
Catecolamines	Pronòstic-diana terapèutica
Sistema renina-angiotensina-aldosterona	Pronòstic-diana terapèutica
Vasopressina	Pronòstic
Copeptina	Pronòstic
Endotelina	Pronòstic
Adrenomodulina	Pronòstic
Extracardiològics	
Anèmia: hemoglobina, ample distribució eritrocitari, dèficit de ferro	Pronòstic-diana terapèutica
Renal: urea, creatinina, filtrat glomerular renal, quocient albúmina/creatinina orina, cistatina-C, NGAL, NAG, KIM-1, beta-traça proteïna	Pronòstic

3.5. Pronòstic. Models pronòstics

Tot i que el pronòstic de la IC ha millorat en els últims anys gràcies als avenços en el seu tractament, la mortalitat és elevada. Diverses causes poden explicar perquè no s'ha traslladat tot el benefici dels estudis a la pràctica clínica: les disparitats entre el perfil de pacients inclosos als assaigs i els pacients del món real (106), aplicabilitat limitada a un perfil de pacient relativament jove i amb disfunció sistòlica (107) i la baixa implementació del tractament òptim fora dels àmbits especialitzats (108). El curs clínic de la IC és molt heterogeni i es veu influenciat per múltiples factors com l'etiologia de la mateixa, el tractament rebut, les comorbiditats, factors ambientals i psicosocials i també factors genètics que podrien condicionar una resposta diferent al tractament (109-111)

Conèixer el pronòstic global de la malaltia és important per diverses raons com l'assignació de recursos sanitaris, per comparar diferents programes de salut o avaluar l'efecte de determinades intervencions en el temps entre d'altres.

S'han descrit nombrosos factors pronòstics clínics, demogràfics, funcionals, hemodinàmics, ecocardiogràfics i analítics. Per la seva disponibilitat a la pràctica clínica habitual s'enumeren alguns de les principals variables de mal pronòstic: edat avançada, sexe femení, classe funcional de la NYHA, freqüència cardíaca elevada, comorbiditats com la insuficiència renal, diabetis, anèmia, hiperuricèmia, hiponatrèmia i biomarcadors com els pèptids natriurètics. A la taula 5 es resumeixen alguns d'aquests factors pronòstics (1).

Taula 5. Factors pronòstics insuficiència cardíaca (adaptada guies ESC [1])

Demogràfics, història natural i exploració física	
Edat, sexe, raça, classe funcional NYHA, índex massa corporal	
Signes congestius, augment de pressió venosa jugular, tercer soroll, pressió arterial baixa, freqüència cardíaca elevada	
Diabetis mellitus, disfunció renal, depressió, malaltia pulmonar crònica obstructiva	
Etiologia isquèmica i antecedent d'infart de miocardi	
Probes de laboratori de rutina	
Sodi	
Enzims hepàtics i bilirubina	
Creatinina, aclariment de creatinina/filtrat glomerular, BUN/urea i marcadors de lesió tubular	
Albúmina sèrica	
Àcid úric	
Hemoglobina. Amplitud de distribució eritrocitària	
Troponina I/T	
Quocient albúmina/creatinina a orina	
Neurohormones, citocines i factors relacionats[a]	
Activitat de la renina plasmàtica	Vasopressina
Angiotensina II	Copeptina
Aldosterona	Citocines
Catecolamines	ST2
Endotelina-I	Galectina-3
Adrenomedulina	Marcadors del col·lagen
Pèptids natriurètics	
Variables elèctriques	
Amplada del QRS	
Hipertròfia del ventricle esquerre	
Fibril·lació auricular	
Arítmies ventriculars complexes	
Variabilitat del ritme cardíac	
Variables d'imatge	
Dimensions ventricle esquerre	
Fracció d'escurçament del ventricle esquerre	
Cardiomegàlia a la radiografia de tòrax	
Index de moviments de la paret ventricular[c]	
Fracció d'ejecció	
Mida de l'aurícula esquerra	
Patró d'ompliment restrictiu / temps de desacceleració breu	
Funció ventricular dreta[c]	
Inflamació (augment contrast c-RM), contingut de ferro (a la talassèmia)	
Amiloïdosi (cinètica de contrast c-RM)	
Isquèmia i viabilitat, substrats aritmogènics	
Prova d'esforç / variables hemodinàmiques	
VO2	
Pendent VE /VCO2	
Max / pic (normal> 20 ml / kg / min)	
Test dels 6 minuts (normal> 600 m[d])	
Índex cardíac (normal> 2,5 L / min / m2)	
Pressió telediastòlica del ventricle esquerre/pressió de l'artèria pulmonar arterial (normal <12 mmHg)	

Abreviatures: BUN= nitrogen ureic en sang; c-RM = ressonància magnètica cardíaca; MPOC = malaltia pulmonar obstructiva crònica; FG = Filtrat glomerular; IC = insuficiència cardíaca; VE = ventricle esquerre; NYHA = New York Heart Association; sST-2 = soluble ST-2; VO2 = Consum màxim d'oxigen.

a Aquesta llista no pretén ser exhaustiva i altres factors circulants també poden estar associats amb el pronòstic.

b Diversos pèptids incloent C-terminal, N-terminal, i regions mitges són predictius del pronòstic.

c Diverses mesures o classificacions poden ser útils.

d La capacitat funcional varia molt segons l'estat físic previ, l'edat i el sexe; Els valors indicats són una guia per a adults majors de 65 anys.

La predicció dels curs de la malaltia dels propis pacients i dels metges és poc precisa (112 i 113), el que resulta en un desequilibri entre l'estratificació de risc i la intensitat del tractament. Així mateix, el criteri clínic per identificar a Urgències pacients amb baix risc de mortalitat i/o complicacions a curt termini és poc homogeni i alguns estudis han mostrat que és poc acurat (114). Per bé que els factors de risc esmentats es correlacionen amb la supervivència en estudis estadístics, la seva capacitat de predicció la supervivència d'un pacient individual és limitada. Per tots aquests motius és important disposar d'instruments per millorar l'estimació del pronòstic. Els models pronòstics combinen múltiples variables per predir el risc d'esdeveniments com mort o ingrés hospitalari en pacients individuals de forma més o menys acurada. L'objectiu dels mateixos és complementar el judici clínic i ajudar en la presa de decisions clíniques que en gran mesura estan condicionades al pronòstic, com la intensificació del tractament, indicació de DAI i/o teràpia de resincronització (TRC) i el trasplantament. A més a més poden ser útils per donar informació pronòstica més acurada als pacients. Als darrers anys s'han desenvolupat diversos models multivariants pronòstics en IC, tant durant un ingrés hospitalari com en la fase estable, que difereixen en el tipus de variables utilitzades, la població de la que se'n deriven, la precisió, grau de validació en una altra cohort, calibratge i forma d'expressar el pronòstic. Els models pronòstics són també importants per millorar el disseny i anàlisi d'assaigs clínics aleatoritzats.

3.5.1 Models pronòstics IC aguda

D'entre els models per a pacients hospitalitzats amb IC aguda destaquem els dos desenvolupats pel grup **ADHERE (*Acute Decompensated Heart Failure National Registry)*.** La població d'estudi van ser pacients hospitalitzats per IC (N= 33.046) i es va avaluar la mortalitat (115). El primer model és de fàcil aplicació i estratifica els pacients en grups de mortalitat intrahospitalària que varien del 2.1 al 21.9%, segons la valoració seqüencial de tres variables a l'ingrés en forma d'arbre de decisió: urea sèrica, pressió arterial sistòlica i creatinina sèrica. El segon és un model logístic multivariant que inclou la urea sèrica, la pressió arterial sistòlica i la freqüència cardíaca. Tot i que no es pot calcular a la "capçalera del malalt" ha mostrat millor rendiment en un estudi de validació posterior (116) amb una AUC (àrea sota la corba ROC) de 075-0.77 que el model d'arbre de decisions (AUC de 0.65).

L'estudi **EFFECT (*Enhanced Feedback for Effective Cardiology Treatment*)** ha desenvolupat models de predicció a 30 dies i a un any post hospitalització per IC amb les

següents variables: edat, pressió arterial sistòlica, freqüència respiratòria, urea, sodi, hemoglobina, cirrosi hepàtica, càncer, demència, malaltia pulmonar obstructiva crònica i malaltia cerebrovascular (117). S'ha validat posteriorment i és accessible *online* (www.ccort.ca/Research/CHFRiskModel.aspx).

L'*Emergency Heart Failure Mortalit Risk Grade* (**EHMRG**) ha proposat un model per identificar els pacients de major risc de mortalitat als 7 dies posteriors a la valoració a Urgències, independentment de si varen ingressar (118). Les variables incloses foren l'edat, trasllat per equip sanitari, pressió arterial sistòlica, freqüència cardíaca, creatinina sèrica, saturació d'oxigen, potassi, troponina, càncer i tractament amb metalozona. Es pot calcular també *online* (https://ehmrg.ices.on.ca/#/). En base a aquest model s'ha proposat un algoritme de decisió a Urgències per valorar si cal ingrés hospitalari o bé pot ser donat d'alta (119).

3.5.2 Models pronòstics IC crònica

Hi ha diversos models predictius en IC crònica en pacients ambulatoris, amb diferents graus de validació i forma de presentar el pronòstic. A continuació s'exposen els més significatius.

El ***Heart Failure Survival Score*** **(HFSS)** va ser el primer model multivariant derivat d'una cohort (268 pacients) i validat de manera prospectiva en una altra i va analitzar l'objectiu combinat de mort, trasplantament urgent i implant de d'una assistència ventricular (120). Es va dissenyar per assistir en la decisió d'inclusió en llista de trasplantament. Els model estratifica els pacients en 3 categories de risc. Les variables incloses van ser l'etiologia isquèmica, la FE, la pressió arterial mitja, la freqüència cardíaca, QRS > 120 mseg, sodi i consum pic d'oxigen (VO2). La utilització del VO2 limita la seva aplicabilitat ja que només acostuma a estar disponible en centres amb trasplantament. La capacitat de discriminació del model valorada amb el l'estadístic C (equivalent a l'AUC) ha variat de 0.56 a 0.79 en les diferents cohorts de validació. La capacitat de discriminació ha estat pitjor en cohorts amb major percentatge de tractament amb beta-blocadors o DAI i en les cohorts més recents (posar refer article Alba i valorar si poso referències concretes).

El ***Seattle Heart Failure Model*** **(SFHM)** és probablement el més utilitzat. Es va derivar de l'estudi PRAISE (*Prospective Randomised Amlodipine Survival Evaluation*) que va incloure pacients (N=1125) en classe funcional IIIB i IV i FE <o = 30% i es va dissenyar per predir l'objectiu combinat de mort, trasplantament urgent i s'ha validat extensament en diverses cohorts d'estudis clínics. Consta de 20 variables: edat, sexe, pes, FE, pressió arterial sistòlica, classe funcional NYHA, dosi diària diürètic, limfòcits, hemoglobina, sodi plasmàtic, colesterol total, àcid úric, etiologia isquèmica, QRS > 120 mseg, tractament amb b-blocadors, IECAs, diürètics estalviadors de potassi, estatines, al·lopurinol i presència de DAI i TRC (121). Per cada pacient individual calcula l'expectativa de vida mitja o bé la supervivència a 1, 2 i 5 anys. A la cohort inicial de l'estudi cap dels pacients portava tractament amb b-blocadors, antagonistes de l'aldosterona o dispositius però aquestes variables es varen extrapolar d'assaigs clínics i sí que es varen incloure en les poblacions de validació. La capacitat de discriminació d'aquest model (calculada com AUC) es troba entre 0.63 i 0.81, amb una mitjana de 0.73. S'ha valorat també el calibratge del model en diverses cohorts amb una bona correlació (coeficient r > 0.97). En una de les cohorts de validació el SHFM sobreestimava la supervivència en pacients portadors de DAI/TRC i en pacients de raça negra. El SHFM és també predictiu de la causa de mort per IC (mort sobtada o progressió IC) (122) i pot identificar potencialment aquells pacients que compleixen criteris per implant d'un DAI però que no se'n beneficiarien pel mal pronòstic (123). La calculadora és pot utilitzar *online* (www.seattleheartfailuremodel.org).

El model ***Cardiac and Comorbid Conditions in Heart Failure Score*** **(3C-3HF)** ofereix un càlcul de la mortalitat a un any i incorpora comorbiditats freqüents (anèmia, diabetis mellitus complicada, hipertensió arterial, xifra de creatina) a més de les següents variables: classe funcional NYHA III-IV, FA, malaltia valvular severa, FE , edat, absència de tractament amb IECAS/ARA-II i b-blocadors (124). Fa un càlcul del risc de mort a 1 any. La cohort d'estudi (2016 pacients) prové d'un estudi multicèntric amb una població mixta de pacients ambulatoris i ingressats amb inclusió prospectiva i retrospectiva i es va validar en una cohort posterior. Les variables són d'àmplia disponibilitat i és també accessible online (www.3chf.org/site/index.php). L'AUC per la mortalitat a 1 any calculada amb regressió logística va ser de 0.83. Tanmateix, les AUC obtingudes amb aquest càlcul són més altes.

El model **HF-ACTION (*Heart Failure: A Controlled Trial Investigation Outcomes of Exercise Training*)** es va derivar d'una cohort recent de 2331 pacients amb FE < 35% inclosos en un assaig per valorar l'efecte de l'exercici físic (125). Es van construir 2 models diferents segons l'objectiu primari (mort i reingrés) i l'objectiu secundari (mortalitat) amb estimació del risc segons la puntuació de cada variable. Les variables incloses al model de l'objectiu primari foren: la duració del test cardiopulmonar (la variable amb més pes predictiu), l'estabilitat clínica segons el qüestionari de Kansas City, elevació urea i sexe masculí. Per l'objectiu secundari es substitueix el qüestionari per l'índex de massa corporal. La discriminació va ser pobre per l'objectiu primari (i moderada per l'objectiu secundari. No s'ha validat en una altra població. Un les avantatges d'aquest model és que la cohort de derivació té un alt percentatge de tractament reglat per la IC. La necessitat de fer un test cardiopulmonar limita la seva aplicabilitat.

4. JUSTIFICACIÓ I OBJECTIUS

Malgrat els avenços en el tractament i de la supervivència de la IC en les darreres dècades, la IC constitueix encara un problema de primera magnitud als països desenvolupats per la seva alta prevalença, morbi-mortalitat, impacte socio-econòmic i minva de la qualitat de vida. La importància d'aquesta patologia justifica la recerca de nous marcadors pronòstics per tal d'identificar els pacients d'alt risc tributaris d'un seguiment més estret per optimitzar al màxim el tractament farmacològic i no farmacològic, amb l'objectiu de millorar l'evolució, reduir ingressos i millorar la qualitat de vida.

Estimar el risc individual dels pacients amb IC segueix sent un repte pel clínic per l'heterogeneïtat del seu curs clínic, que es veu modulat per múltiples factors. A més a més, la precisió pronòstica dels clínics és baixa i l'aplicabilitat de factors pronòstics derivats d'estudis poblacionals o assaigs clínics a un pacient individual és sovint limitada. El models pronòstics combinen múltiples variables per fer una estimació del risc de patir un esdeveniment en un pacient individual, però alguns d'ells deriven de cohorts d'assaigs clínics que no rebien cap dels tractament bàsic de la IC i poden ser poc representatius de la població general. De manera molt destacable, cap d'ells inclou biomarcadors tret de les determinacions rutinàries de l'analítica.

La determinació senzilla en sang perifèrica de diferents biomarcadors que són reflex diferents mecanismes fisiopatològics de la IC està adquirint una major protagonisme en l'estratificació de risc dels pacients amb IC. Atesa la complexitat de la IC és factible que la combinació de diversos marcadors aporti més informació que la no pas una determinació única (73, 126). Com ja s'ha comentat, no hi ha cap model pronòstic que incorpori biomarcadors moderns, més enllà de dades analítiques rutinàries com l'hemoglobina, el sodi o la funció renal.

La tesi doctoral, que es presenta com a compendi de sis articles sorgeix de la necessitat de millorar l'estratificació pronòstica en termes de mortalitat global dels pacients amb IC crònica seguits a una Unitat d'Insuficiència Cardíaca multidisciplinària.

Els objectius foren:

1. Valorar el valor pronòstic addicional dels biomarcadors emergents ST2 i la troponina T d'alta sensibilitat sobre un model de variables clíniques establertes, incloent-hi l' NT-proBNP, tant de forma aïllada com combinada;

2. Comparar biomarcadors d'un mateix procés fisiopatològic en virtut de la seva capacitat predictiva de mortalitat, per una banda les troponines T i I (marcadors de dany miocardíac), per una altra banda l' ST2 i la galectina-3 (marcadors de fibrosi/remodelatge cardíac), i, finalment en un tercer treball, comparar cistatina-C amb el filtrat glomerular renal estimat (marcadors renals) i la seva combinació;

3. A raó de l'alta prevalença d'insuficiència renal en els pacients amb IC que pot alterar els nivells d'alguns biomarcadors, un altre objectiu fou comparar el valor pronòstic d'alguns biomarcadors segons l'estat de la funció renal;

4. Elaborar una calculadora de risc amb la incorporació de biomarcadors, a més a més de variables clíniques.

La població d'estudi, tot i tractar-se d'una Unitat multidisciplinària d'un centre terciari, reflecteix el perfil de pacients del nostre entorn sanitari i hauria de permetre una aplicabilitat dels resultats en la pràctica clínica habitual.

5. MÈTODES

5.1. Població d'estudi

Es van incloure de forma consecutiva pacients ambulatoris atesos a la Unitat d'IC de l'Hospital Universitari Germans Trias i Pujol. Tots els pacients van ser seguits en intervals predefinits regulars, amb visites addicionals si era necessari en cas de descompensació. El calendari regular de visites inclou un mínim de visites trimestrals amb les infermeres, visites bianuals amb els metges i visites electives amb geriatres, psiquiatres i metges rehabilitadors. Els que no va assistir a la visita regular van ser contactats per telèfon.

La mort per qualsevol causa va ser el l'objectiu principal en la majora dels treballs presentats i es va identificar a partir de la història clínica recollida a la Unitat, mitjançant la revisió de la història clínica electrònica de l'Institut Català de la Salut, o bé pels sistemes informàtics de l'INSALUD. El temps mitjà de seguiment ha estat entre 2.5 i 4.5 anys segons el treball.

5.2. Metodologia estadística

A cadascun dels articles està especificada la metodologia particular emprada. El cos de la tesi es fonamenta en la valorar la capacitat predictiva de diversos biomarcadors i models predictius i determinar si tenen valor afegit sobre un model de variables clíniques establertes amb l'objectiu amb final de valorar si tenen utilitat clínica (104, 127). Amb aquesta finalitat, cal practicar una anàlisi rigorosa amb mesures de rendiment de les prediccions. A continuació es revisen els conceptes bàsics.

Discriminació. És la capacitat de diferenciar als individus que presentaran un esdeveniment dels que no. L'àrea sota la corba (AUC) ROC (*receiver operating characteristic*) i l'índex de concordança (estadístic C) són les mesures de discriminació més freqüentment utilitzades (128, 129). Pels resultats binaris l'estadístic C és igual a l'AUC, que representa la relació entre la sensibilitat i 1 menys l'especificitat. De forma general a partir d'una AUC de 0,7 la discriminació del model es considera acceptable. Per comparar el valor additiu de diferents variables a un model mitjançant s'analitzen els canvis en l'estadístic C o l'AUC. Tanmateix, no hi ha un acord unànime en quin ha de ser el grau de modificació perquè es consideri clínicament rellevant. Entre les limitacions importants cal destacar que en models clínics molt robusts, l'addició d'una variable valuosa pot comportar mínims canvis en l'estadístic C. En els nostres estudis, l'estadístic C s'ha obtingut d'una generalització de la correlació de Somers 'Dxy' rank, que equipara

2×(c−1/2), on c es la probabilitat de concordança (discriminació), que incorpora per endavant informació de les dades censurades (130).

Calibratge. És una mesura que expressa la concordança entre els resultats observats i les prediccions del model. Mitjançant la proba de Hosmer-Lemeshow, que és una mesura de la bondat d'ajust, s'avalua la distància entre les dades observades i les esperades per grup amb el model. L'estadístic és un estadístic de la chi al quadrat. S'aporta un valor de P per valorar les diferències entre les dades que ha de ser superior a 0.05 perquè el model estigui ben calibrat. El criteri d'informació Bayesià (BIC), el criteri d'informació Akaike (AIC) i l'*score* de Brier per a observacions censurades també són mesures de calibratge. L'*score* de Brier és un quantificador de la precisió del model, avalua la distància entre les dades obtingudes i la probabilitat esperada pel model i els seus valors van de 0 a 1. Quan més petit el valor, millor prediu el model. Amb el BIC i l'AIC, el model millor calibrat serà també aquell que tingui un valor més baix. No hi ha cap test estadístic per comparar diferents valors d'aquestes mesures. Una altra mètode de valorar el calibratge és valorar la bondat global de l'ajust analitzada amb la raó de versemblança (*likelihood ratio test*). Un valor de P significatiu en aquest test indica que una variable nova, afegida a un model determinat, millora significativament la precisió d'aquest model.

Reclassificació. És una mesura per valorar la utilitat clínica de les variables (128,131). Algunes de les mesures de reclassificació són la "millora de la reclassificació neta" (net reclassification index-NRI) i la "millora de la discriminació integrada" (integrated discrimination improvement-IDI).

Pel càlcul de l'NRI s'han de predefinir categories de risc de l'esdeveniment que volem mesurar. És la suma balancejada dels canvis de categoria de risc pels esdeveniments i no esdeveniments quan afegim un marcador a un model. Els valors es poden expressar en taules de contingència, percentatges o bé amb el valor de la P, que quan és <0.05 i el NRI és positiu suggereix que un major nombre d'individus s'han reclassificat apropiadament que no pas de forma inapropiada.

L' IDI avalua els canvis de la predicció de mortalitat com a variable continua. Mesura si els individus "es mouen" molt de mitjana en el *contínuum* de la predicció de risc, És l'augment de la distància entre la mitjana de risc pels esdeveniments i no esdeveniments. Tal i com succeeix amb l'AUC, pot ser difícil valorar la magnitud de lDI, ja que depèn de la taxa d'incidència de l'esdeveniment que mesurem.

6. RESULTATS

6.1. Articles fonamentals

6.1.1: 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;14(1):32-8.

6.1.2: de Antonio M, Lupón 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;163(5):821-8.

6.1.3: Lupón J, de Antonio M, Galán A, Vila J, Zamora E, Urrutia A, Bayes-Genis A. Combined Use of the Novel Biomarkers High-Sensitivity Troponin T and ST2 for Heart Failure Risk Stratification vs Conventional Assessment. Mayo Clin Proc 2013;88(3):234-43. Erratum in: Mayo Clin Proc. 2013 May;88 (5):532.

6.1.4: de Antonio M, Lupón J, Galán A, Vila J, Zamora E, Urrutia A, Díez C, Coll R, Altimir S, Bayes-Genis. Head-to-head comparison of high-sensitivity troponin T and sensitive-contemporary troponin I regarding heart failure risk stratification. Clin Chim Acta 2013;426:18-24.

6.1.5: Lupón J, de Antonio M, Vila J, Peñafiel J, Galán A, Zamora E, Urrutia A, Bayes-Genis. Development of a novel heart failure risk tool: the barcelona bio-heart failure risk calculator (BCN bio-HF calculator). PLoS One 2014;9(1):e85466.

6.1.6: Bayes-Genis A, de Antonio M, Vila J, Peñafiel J, Galán A, Barallat J, Zamora E, Urrutia A, Lupón J. Head-to-head comparison of 2 myocardial fibrosis biomarkers for long-term heart failure risk stratification: ST2 versus galectin-3. J Am Coll Cardiol 2014;63(2):158-66.

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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.

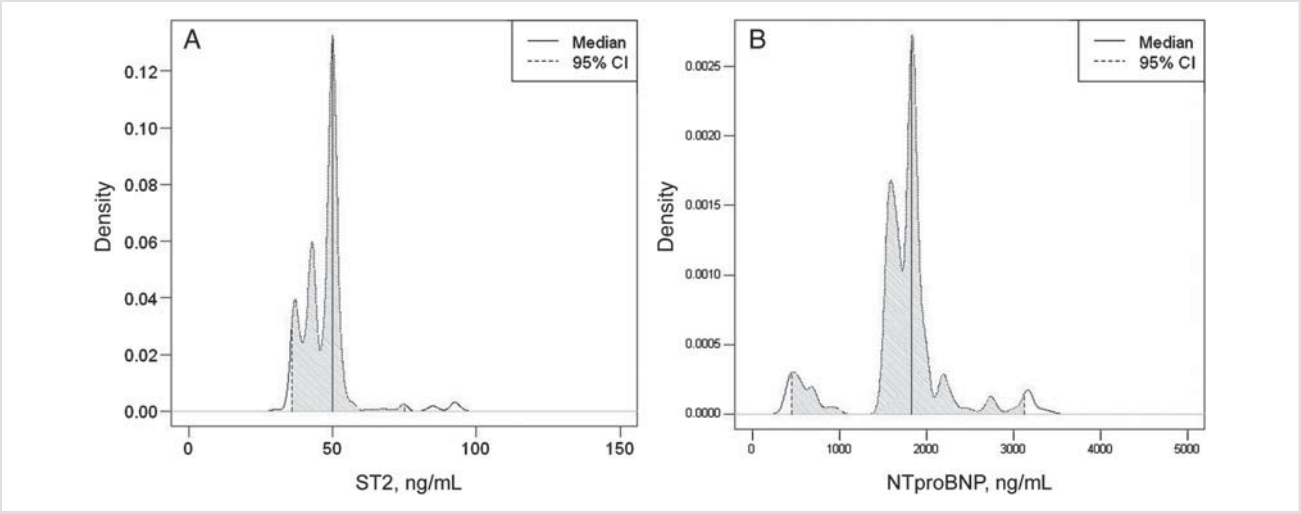

Figure 1 Bootstrap density plot of best cut-off points for ST2 (*A*) and N-terminal pro brain natriuretic peptide (NTproBNP) (*B*); values are expressed in ng/mL.

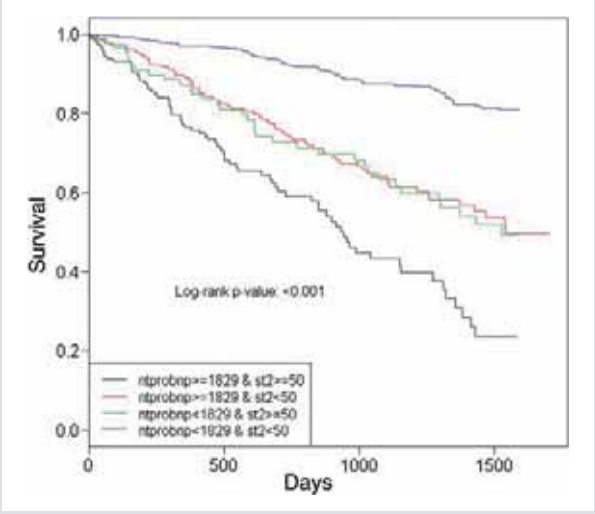

Figure 2 Kaplan–Meier survival curves according to ST2 and N-terminal pro brain natriuretic peptide (NT-proBNP) levels.

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.

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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:

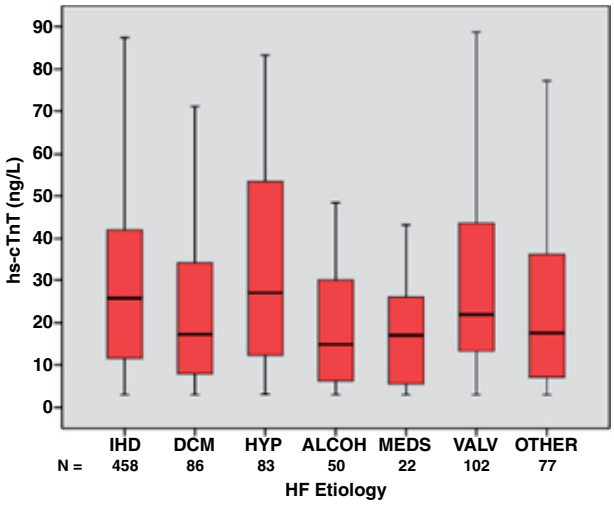
Discrimination. The improvement in the discrimination capacity of a model that included biomarkers compared with a model that did not was obtained by computing the concordance index (*C* statistic). The area under the receiver operating characteristic curve (AUC) summarized the diagnostic discrimination. 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.

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.

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.

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).

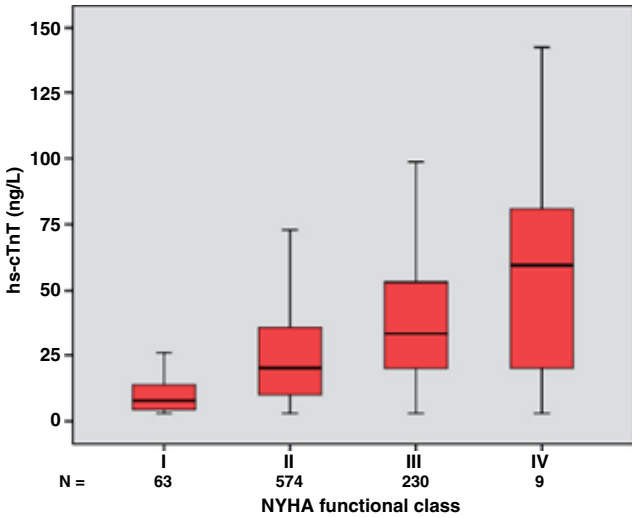
Results

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

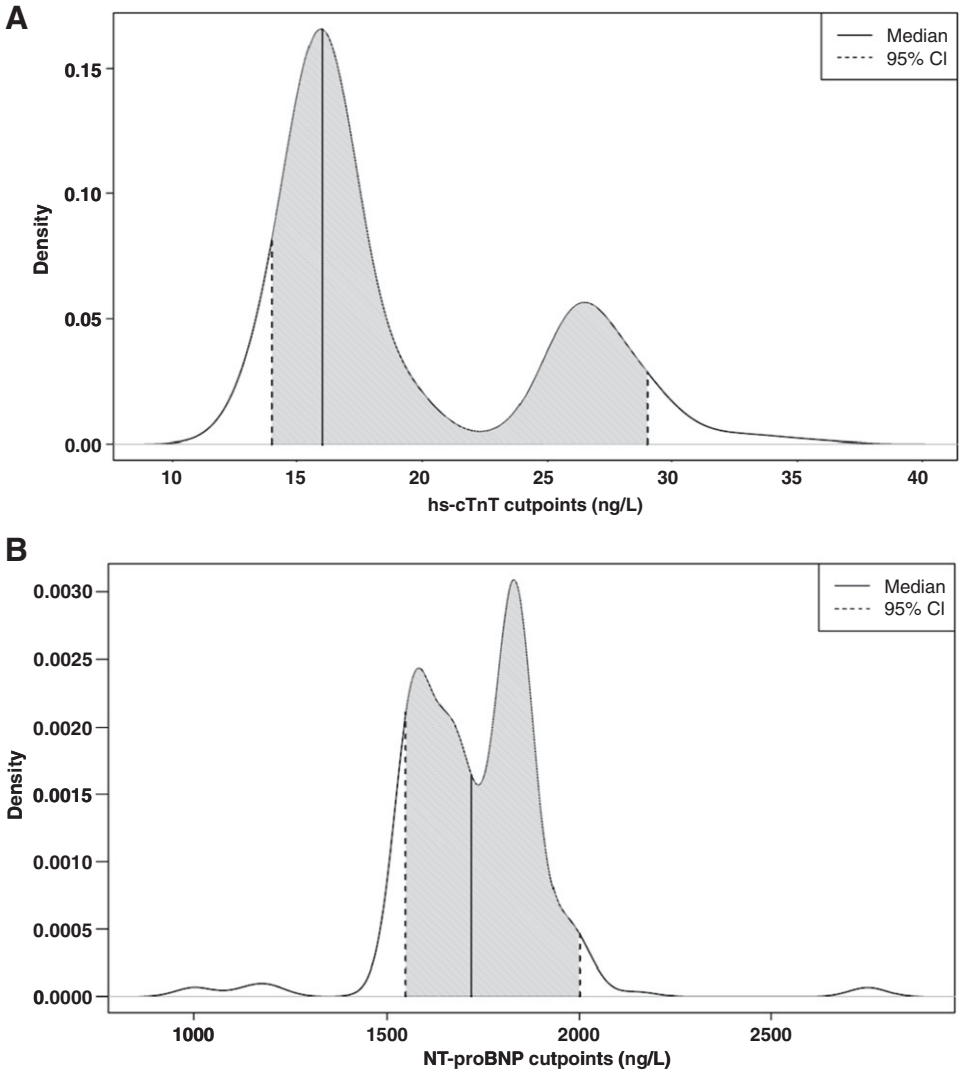
Hb, Plasma hemoglobin.

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.

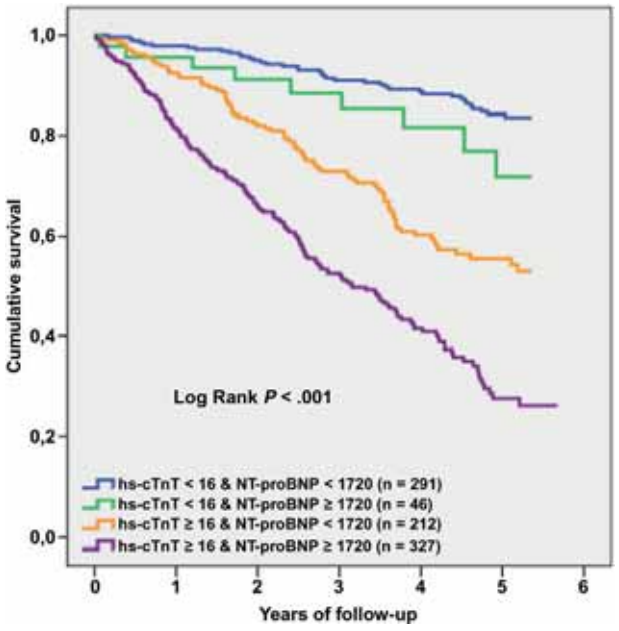
[95% CI, 0.84–3.58], $P = .136$], respectively). Patients with elevated levels of both hs-cTnT and NT-proBNP had a markedly increased risk (HR 7.42 [95% CI, 5.23-10.54], $P < .001$), indicating that assessment of both hs-cTnT and NT-proBNP is more effective at identifying a high-risk subgroup than individual 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	$\chi^2 = 8.6$ $P = .38$	$\chi^2 = 9.8$ $P = .28$	$\chi^2 = 2.2$ $P = .98$	$\chi^2 = 12.1$ $P = .14$
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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ORIGINAL ARTICLE

MAYO CLINIC

Combined Use of the Novel Biomarkers High-Sensitivity Troponin T and ST2 for Heart Failure Risk Stratification vs Conventional Assessment

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Abstract

Objective: To assess an innovative multimarker strategy for risk stratification of death in a real-life ambulatory heart failure (HF) cohort.

Patients and Methods: The study included 876 consecutive outpatients (median age, 70.3 years; left ventricular ejection fraction, 34%) between May 22, 2006, and July 7, 2010, prospectively followed up in a structured HF unit. A combination of biomarkers reflecting myocardial stretch (N-terminal pro-B-type natriuretic peptide [NT-proBNP]), myocyte injury (high-sensitivity cardiac troponin T [hs-cTnT]), and ventricular fibrosis and remodeling (high-sensitivity ST2 [hs-ST2]) were added to an assessment based on established mortality risk factors (age, sex, left ventricular ejection fraction, New York Heart Association functional class, diabetes mellitus, estimated glomerular filtration rate, ischemic etiology, sodium level, hemoglobin level, and pharmacologic treatment).

Results: During median follow-up of 41.4 months, 311 patients died. The combined addition of hs-cTnT and hs-ST2 to the model yielded good measurements of performance (C statistic, 0.789; Bayesian information criterion, 3611; integrated discrimination improvement, 4.1 [95% CI, 2.5-5.6]; and net reclassification index, 13.9% [95% CI, 6.2-21.6]). Reclassification did not significantly benefit after NT-proBNP addition into the full model; some indices even worsened with all 3 biomarkers. Separate addition of NT-proBNP provided prognostic discrimination only in the subgroup of patients with either hs-cTnT or hs-ST2 levels below the cutoff points (hazard ratio, 2.97; 95% CI, 2.24-9.39; $P<.001$).

Conclusion: A multimarker strategy seems useful for stratifying risk in chronic HF. However, NT-proBNP in addition to the new-generation biomarkers hs-cTnT and hs-ST2 had a limited effect on risk stratification.

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Chronic heart failure (HF) is a growing public epidemic with increasing incidence and prevalence.[1] Despite important progress in recent decades, mortality remains high for patients with HF. Moreover, established risk factors, such as New York Heart Association (NYHA) functional class, medication use, laboratory values, and left ventricular ejection fraction (LVEF) do not fully explain the mortality risk of patients with HF and do not estimate an individual's prognosis.[2-4] Risk stratification may be refined by the use of biomarkers of different pathophysiologic processes that established mortality risk factors do not necessarily directly reflect. However, despite a variety of recently identified novel biomarkers,[5,6] only natriuretic peptides have entered routine clinical practice and been included in clinical guidelines.[1,7] Natriuretic peptides are useful in a wide range of situations,[8] from screening asymptomatic individuals at risk for HF[9] or assessing patients with dyspnea[10,11] to stratifying prognosis in acute and chronic HF[12-14] and even for therapy guidance and monitoring.[15]

A single biomarker might not reflect all the facets of the HF syndrome, and a multimarker strategy may better characterize the complexity of HF.[5,6,16-18] To date, most multimarker strategies in HF have relied on the addition of one

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or more biomarkers to the well-established natriuretic peptides.[6] Whether biomarkers for other pathophysiologic pathways can take the place of natriuretic peptides remains unknown. Accordingly, in the present study, we investigated the value of combining N-terminal pro-B-type natriuretic peptide (NT-proBNP) (a marker of myocardial stretch), high-sensitivity cardiac troponin T (hs-cTnT) (a marker of myocyte injury), and high-sensitivity soluble ST2 (hs-ST2) (reflective of myocardial fibrosis and remodeling) in a large real-life cohort of ambulatory patients with HF. We examined different biomarker combinations in addition to performing an assessment based on established mortality risk factors to determine the relative role of each biomarker in risk stratification.

PATIENTS AND METHODS

Study Population

Between May 22, 2006, and July 7, 2010, ambulatory patients treated at a multidisciplinary HF unit were consecutively included in the study in an outpatient setting. Patients were referred to the unit by cardiology or internal medicine departments and, to a lesser extent, by 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, or a reduced LVEF.

Blood samples were obtained by venipuncture between 9 AM and noon during conventional ambulatory visits, and adequate centrifugation serum samples were stored at −80°C. The NT-proBNP, hs-cTnT, and hs-ST2 levels were analyzed from the same blood sample.

All the participants provided written informed consent, and the local ethics committee (Clinical Investigation Ethics Committee, Hospital Universitari Germans Trias i Pujol, Badalona, Spain) approved the study. All the study procedures were in accord with the ethical standards outlined in the 1975 Declaration of Helsinki, as revised in 1983.

Follow-up and Outcomes

All the patients were followed up at regular predefined intervals, with additional visits as required in cases of decompensation, need for up-titration, or other circumstances (such as renal function impairment and anemia) that required closer follow-up. 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.[19,20] 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 at the Catalan Institute of Health.

hs-cTnT Assay

Troponin levels were measured by electrochemiluminescence immunoassay using an hs-cTnT assay and the Modular Analytics E 170 system (Roche Diagnostics). The hs-cTnT assay had an analytic range of 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 ESC-ACCF-AHA-WHF Global Task Force for use in the diagnosis of myocardial necrosis.[21] The assays were run with reagents from lot 157123, which was unaffected by the analytical issues that emerged with Roche hs-cTnT assays.

hs-ST2 Assay

The level of ST2 was measured from plasma samples using a high-sensitivity sandwich monoclonal immunoassay (Presage ST2 assay; Critical Diagnostics). The antibodies used in the Presage assay were generated from recombinant protein based on the human complementary DNA clone for the complete soluble ST2 sequence.[22] The hs-ST2 assay had a within-run coefficient of less than 2.5% and a total coefficient of variation of 4%.

NT-proBNP Assay

The NT-proBNP levels were determined using an immunoelectrochemiluminescence assay and the Modular Analytics E 170 system. This assay has less than 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%.[12]

Statistical Analyses

Categorical variables are expressed as percentages. Continuous variables are expressed

as the mean ± SD or median (25th-75th percentiles [P_{25-75}]) according to normal or nonnormal distribution. Statistical differences between groups were compared using the χ^2 test for categorical variables.

The best cutoff points for hs-cTnT, hs-ST2, and NT-proBNP were found by bootstrapping the value that maximized the log-likelihood of the nonadjusted Cox models. Log-rank tests for Kaplan-Meier survival curves were performed for testing differences among the best NT-proBNP, hs-cTnT, and hs-ST2 cutoff points.

Survival analyses were performed using Cox regression models. To fulfill the assumption of linearity of the covariables hs-cTnT, hs-ST2, and NT-proBNP, the logarithmic functions of both NT-proBNP and hs-cTnT, the quadratic term of the logarithmic function of hs-cTnT and the quadratic term of hs-ST2 were used in the Cox models. The following variables were incorporated into the model: age, sex, LVEF, estimated glomerular filtration rate (eGFR), body mass index, NYHA functional class, diabetes mellitus, chronic obstructive lung disease, atrial fibrillation, ischemic etiology, plasma hemoglobin level, serum sodium level, β-blocker treatment, and angiotensin-converting enzyme inhibitor (ACEI) or angiotensin II receptor blocker (ARB) treatment. These variables were chosen because they were significant in the univariate analysis; if nonsignificant, they were considered to be of clinical importance (such as sex, LVEF, and ischemic etiology).

We used different measurements of performance to test the potential incremental prognostic value of these biomarkers, as follows.

Discrimination. The area under the receiver operating characteristic curve (AUC) summarized the diagnostic discrimination. Discrimination refers to a model's ability to correctly distinguish 2 classes of outcomes. We used the index of rank correlation, Somers D, which already incorporates information of censored data. The AUCs between models were compared using the *U* test for equality concordance.

Calibration. (1) The D'Agostino-Nam version of the Hosmer-Lemeshow calibration test was used to calculate a χ^2 value. 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 results). (2) The Bayesian information criterion (BIC), the Akaike information criterion (AIC), and the Brier score were calculated for each model. The AIC and BIC are measures of the relative goodness of fit of a statistical model. The BIC penalizes free parameters more strongly than does the AIC. The Brier score measures the average squared deviation between predicted probabilities for a set of events and their outcomes, so a lower score represents higher accuracy. It takes values between 0 and 1. Given any 2 estimated models, the model with the lower BIC, AIC, and Brier scores was preferred. No statistical tests compare different BIC, AIC, or Brier score estimations, and 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.

Reclassification. We used the method described by Pencina et al.[23] There are 2 main statistics to assess reclassification. Integrated discrimination improvement (IDI) considers the changes in the estimated mortality prediction probabilities as a continuous variable. Two-sided $P<.05$ was considered statistically significant. 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 the analyses were performed using the software R (version 2.11.1) statistical package (Foundation for Statistical Computing).

RESULTS

Among the 891 consecutive patients included between May 22, 2006, and July 7, 2010, the 3 biomarkers (hs-cTnT, hs-ST2, and NT-proBNP) were available in 876, who were finally included in this analysis. The median patient age was 70.3 years (P_{25-75}, 60.5-77.2 years). Table 1 shows the baseline characteristics of the entire sample. During median follow-up of 41.4 months (P_{25-75}, 22.1-60.5 months), 311 patients died. Of the cardiovascular causes of death (168), refractory HF was responsible in 91 patients (54.1%), sudden death in 30 (17.8%), and acute

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TABLE 1. Baseline Demographic and Clinical Characteristics of Patients With HF and Treatments During Follow-up[a,b]

Characteristic	Total (N=876)	Alive (n=565)	Deceased (n=311)	HR_{Cox} (95% CI)	*P* value
Age (y)[c]	70.3 (60.5-77.2)	66.1 (56.5-74.3)	75.6 (69.9-81.0)	1.07 (1.06-1.08)	<.001
Female, No. (%)	246 (28.1)	151 (26.7)	95 (30.5)	1.07 (0.84-1.36)	.58
White race, No. (%)	871 (99.4)	560 (99.1)	311 (100)	20 (0.04-11,902)	.34
Etiology, No. (%)					.005
Ischemic heart disease	456 (52.1)	288 (51.0)	168 (54.0)	1	
Dilated cardiomyopathy	86 (9.8)	67 (11.9)	19 (6.1)	0.57 (0.36-0.92)	.02
Hypertensive	83 (9.5)	44 (7.8)	39 (12.5)	1.29 (0.91-1.83)	.15
Alcoholic cardiomyopathy	50 (5.7)	40 (7.1)	10 (3.2)	0.53 (0.28-1.01)	.05
Toxic	22 (2.5)	15 (2.7)	7 (2.3)	0.88 (0.41-1.88)	.74
Valvular	102 (11.6)	56 (9.9)	46 (14.8)	1.37 (0.99-1.90)	.06
Other	77 (8.8)	55 (9.7)	22 (7.1)	0.79 (0.51-1.24)	.31
HF duration (mo)[c]	27.3 (4.9-73.8)	24.9 (3.6-67.7)	36 (9-88.1)	1.00 (1.00-1.00)	.02
HF hospitalizations in the previous mo, No. (%)	522 (59.6)	341 (60.4)	181 (58.2)	0.96 (0.77-1.20)	.71
LVEF (%)[c]	34 (26-43)	35 (26-43)	34 (25-45)	1.00 (0.99-1.01)	.79
LVEF ≥45%, No. (%)	202 (23.1)	124 (21.9)	78 (25.1)	0.98 (0.76-1.27)	.89
eGFR (mL/min/1.73 m^2)[c]	42.4 (29.3-59.5)	49.6 (34.7-66.1)	33.5 (23.4-44.5)	0.97 (0.96-0.97)	<.001
BMI[c]	26.9 (24.2-30.5)	27.1 (24.7-30.7)	26.4 (23.6-29.8)	0.95 (0.93-0.98)	<.001
NYHA functional class, No. (%)					<.001
I	63 (7.2)	59 (10.4)	4 (1.3)	1	
II	574 (65.5)	414 (73.3)	160 (51.4)	5.60 (2.08-15.11)	<.001
III	230 (26.3)	90 (15.9)	140 (45.0)	16.19 (5.98-43.77)	<.001
IV	9 (1.0)	2 (0.4)	7 (2.3)	25.30 (7.39-86.66)	<.001
Hypertension, No. (%)	536 (61.2)	330 (58.4)	206 (66.2)	1.35 (1.06-1.70)	.01
Diabetes mellitus, No. (%)	314 (35.8)	182 (32.2)	132 (42.4)	1.44 (1.15-1.81)	.001
COLD, No. (%)	148 (16.9)	73 (12.9)	75 (24.1)	1.67 (1.29-2.16)	<.001
SAHS, No. (%)	39 (4.5)	28 (5.0)	11 (3.5)	0.80 (0.44-1.46)	.46
Atrial fibrillation, No. (%)	146 (16.7)	83 (14.7)	63 (20.3)	1.45 (1.10-1.91)	.009
Treatments (follow-up), No. (%)					
ACEI or ARB	785 (89.6)	529 (93.6)	256 (82.3)	0.34 (0.26-0.46)	<.001
β-Blocker	767 (87.6)	528 (93.5)	239 (76.8)	0.36 (0.27-0.47)	<.001
Spironolactone/eplerenone	344 (39.3)	226 (40.0)	118 (37.9)	1.05 (0.84-1.32)	.66
Loop diuretic	743 (84.8)	458 (81.1)	285 (91.6)	2.25 (1.51-3.68)	<.001
Digoxin	269 (30.7)	158 (28.0)	111 (35.7)	1.33 (1.06-1.68)	.02
CRT, No. (%)	47 (5.4)	31 (5.5)	16 (5.1)	0.83 (0.50-1.38)	.47
ICD, No. (%)	92 (10.5)	66 (11.7)	26 (8.4)	0.69 (0.46-1.03)	.07
Sodium (mmol/L)[c]	139 (137-142)	140 (137-142)	139 (137-141)	0.93 (0.90-0.96)	<.001
Hemoglobin (g/dL)[d]	12.9±1.8	13.3±1.8	12.3±1.8	0.81 (0.77-0.85)	<.001
NT-proBNP (ng/L)[c,e]	1361 (510-3012)	965 (361-2376)	2215 (935-5193)	1.62 (1.49-1.76)	<.001
hs-cTnT (ng/L)[c,e]	22.6 (10.6-40.6)	15.7 (7.9-30.9)	34.2 (20.7-53.7)	11.68 (5.51-24.75)	<.001
hs-ST2 (ng/L)[c,e]	38.1 (30.8-50.9)	35.4 (29.3-45.5)	44.7 (34.1-61.0)	1.04 (1.03-1.05)	<.001

[a]ACEI = angiotensin-converting enzyme inhibitor; ARB = angiotensin II receptor blocker; BMI = body mass index (calculated as weight in kilograms divided by height in meters squared); COLD = chronic obstructive lung disease; CRT = cardiac resynchronization therapy; eGFR = estimated glomerular filtration rate; HF = heart failure; HR_{Cox} = Cox model hazard ratio; hs-cTnT = high-sensitivity circulating troponin T; hs-ST2 = high-sensitivity soluble ST2; ICD = implantable cardioverter-defibrillator; LVEF = left ventricular ejection fraction; NT-proBNP = N-terminal pro-B-type brain natriuretic peptide; NYHA = New York Heart Association; SAHS = sleep apnea–hypopnea syndrome.

[b]SI conversion factor: To convert hemoglobin values to g/L, multiply by 10.0.

[c]Data are expressed as median (25th-75th percentiles).

[d]Data are expressed as mean ± SD.

[e]The logarithmic functions of NT-proBNP and hs-cTnT, the quadratic term of the logarithmic function of hs-cTnT, and the quadratic term of hs-ST2 were used in the Cox models. hs-$ST2^2$: $P<.001$; log(hs-cTnT)^2: $P<.001$.

myocardial infarction in 15 (8.9%). Two patients were lost to follow-up and adequately censored.

Cox Regression and Modeling

In the bivariate analysis, the 3 biomarkers predicted death from all causes as continuous variables: log(NT-proBNP) (hazard ratio [HR], 1.62; 95% CI, 1.49-1.76; $P<.001$); log(hs-cTnT) (HR, 11.68; 95% CI, 5.51-24.75; $P<.001$); and hs-ST2 (HR, 1.04; 95% CI, 1.03-1.05; $P<.001$). In multivariate analysis, the 3 biomarkers remained independent predictors of mortality together with age, NYHA functional class, β-blocker treatment, and hemoglobin level (Table 2). When only cardiovascular death was analyzed, log(hs-cTnT) (HR, 7.07; 95% CI, 2.21-22.65; $P=.001$) and hs-ST2 (HR, 1.02; 95% CI, 1.0-1.04; $P=.04$) remained independently associated with cardiovascular mortality, whereas log(NT-proBNP) did not (HR, 1.15; 95% CI, 0.96-1.37; $P=.13$).

Density plots of the best cutoff points in nonadjusted Cox models were calculated using bootstrap methods to identify optimal prognostic cutoff points for NT-proBNP (1720 ng/L; 95% CI, 1550-2000 ng/L), hs-cTnT (16 ng/L; 95% CI, 14-29 ng/L), and hs-ST2 (50 ng/L; 95% CI, 37-85 ng/L). Two-by-two combinations of biomarkers showed that patients with hs-cTnT + hs-ST2 levels above the cutoff points had the highest risk (HR, 11.69; 95% CI, 7.81-17.49; $P<.001$). Kaplan-Meier survival curves according to hs-cTnT and hs-ST2 levels are shown in Figure 1, A. The separate addition of NT-proBNP provided prognostic discrimination only in patients with either hs-cTnT or hs-ST2 below the cutoff point (HR, 2.97; 95% CI, 2.24-9.39; $P<.001$). In patients whose hs-cTnT + hs-ST2 levels were above the cutoff points, NT-proBNP incorporation had a null effect (HR, 1.43; 95% CI, 0.92-2.29; $P=.11$) (Figure 1, B).

TABLE 2. Multivariate Cox Regression Analysis[a]

Variable	HR	95% CI	*P* value
Age	1.04	1.02-1.05	<.001
Female	0.76	0.58-1.01	.06
Ischemic etiology of HF	1.06	0.83-1.36	.65
LVEF	1.00	0.99-1.01	.60
NYHA functional class	1.69	1.32-2.17	<.001
eGFR	1.00	0.99-1.01	.81
BMI	1.00	0.98-1.03	.87
Diabetes mellitus	1.18	0.93-1.50	.17
COLD	1.10	0.98-1.03	.87
Atrial fibrillation	0.94	0.70-1.27	.68
ACEI or ARB treatment	0.81	0.57-1.15	.24
β-Blocker treatment	0.56	0.41-0.76	<.001
Sodium level	0.97	0.94-1.00	.07
Hemoglobin level	0.92	0.86-0.98	.02
log(NT-proBNP)[b]	1.15	1.01-1.31	.03
hs-ST2[b]	1.02	1.01-1.03	<.001
log(hs-cTnT)[b]	3.90	1.81-8.41	.001

[a]ACEI = angiotensin-converting enzyme inhibitor; ARB = angiotensin II receptor blocker; BMI = body mass index; COLD = chronic obstructive lung disease; eGFR = estimated glomerular filtration rate; HF = heart failure; HR = hazard ratio; hs-cTnT = high-sensitivity circulating troponin T; hs-ST2 = high-sensitivity soluble ST2; LVEF = left ventricular ejection fraction; NT-proBNP = N-terminal pro-B-type brain natriuretic peptide; NYHA = New York Heart Association.

[b]The logarithmic functions of NT-proBNP and hs-cTnT, the quadratic term of the logarithmic function of hs-cTnT, and the quadratic term of hs-ST2 were used in the Cox models. hs-ST2^2: $P=.001$; log(hs-cTnT)^2: $P=.004$.

Measurements of Performance

Discrimination. The AUC for the prediction of death increased significantly when any combination of 2 biomarkers, or all 3, was incorporated into the model with established mortality risk factors (age, sex, LVEF, NYHA functional class, diabetes mellitus, eGFR, ischemic etiology, sodium level, hemoglobin level, β-blocker treatment, and ACEI or ARB treatment) (models 2-5 in Table 3). The AUC for hs-cTnT + hs-ST2 was similar to that for NT-proBNP + hs-cTnT + hs-ST2 (model 4 vs model 5 in Table 4).

Calibration. The *P* values for the Hosmer-Lemeshow statistics indicated good calibration for all the models with and without biomarkers ($P>.12$ for all the comparisons) (Table 3). The BIC, AIC, and Brier scores were lower in the models that included hs-cTnT + hs-ST2 and the combination of the 3 biomarkers (Table 3). Global goodness of fit was better in models including biomarkers than in the model with only established mortality risk factors as evaluated by likelihood ratio tests ($P<.001$) (Table 3). The separate addition of NT-proBNP (model 4 vs model 5 in Table 4) improved global goodness of fit in the total population (likelihood ratio, $P=.04$). However, in the subgroup of patients whose hs-cTnT + hs-ST2 levels were equal to or above the cutoff points, the likelihood ratio was not significant ($P=.11$).

Reclassification. The IDI (risk as a continuous variable) increased significantly with

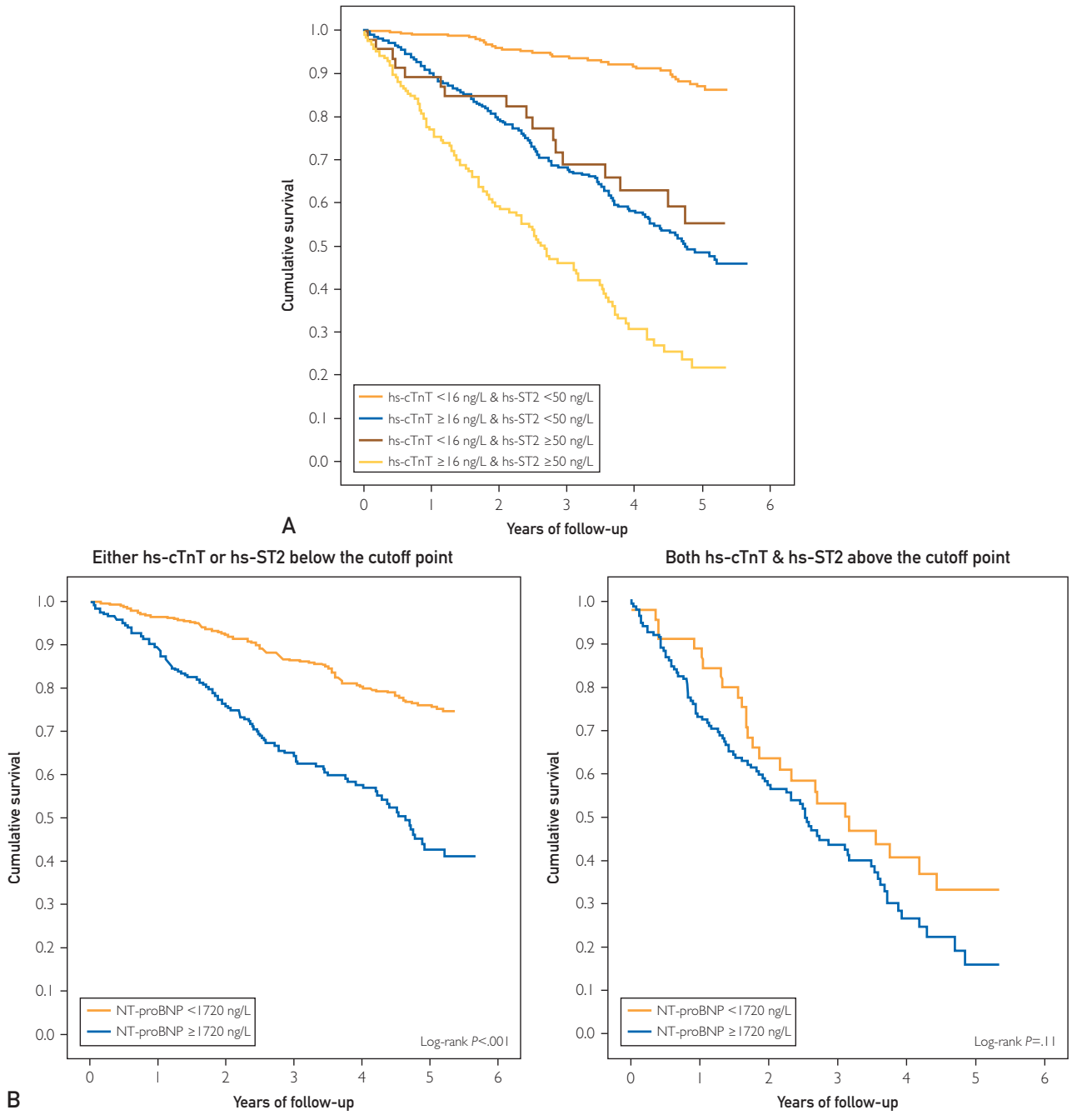

FIGURE 1. Kaplan-Meier survival curves according to biomarkers. A, Survival according to high-sensitivity cardiac troponin T (hs-cTnT) and high-sensitivity soluble ST2 (hs-ST2) levels (above or below the cutoff points). B, Survival according to N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels (above or below the cutoff point) in patients with either hs-cTnT or hs-ST2 levels below the cutoff points (left) and in patients with hs-cTnT and hs-ST2 levels above the cutoff points (right).

TABLE 3. Performance of the Models at 4 Years[a,b]

Variable	Model 1	Model 2	Model 3	Model 4	Model 5
Discrimination					
AUC	0.762	0.780	0.784	0.789	0.790
	(0.736 to 0.789)	(0.755 to 0.805)	(0.759 to 0.808)	(0.766 to 0.813)	(0.766 to 0.813)
	Reference	*P*=.004	*P*<.001	*P*<.001	*P*<.001
Calibration					
H-L	χ^2=8.6	χ^2=12.1	χ^2=12.1	χ^2=7.8	χ^2=12.7
	P=.38	*P*=.15	*P*=.15	*P*=.45	*P*=.12
Brier score	0.161	0.150	0.148	0.144	0.143
AIC	3591	3553	3554	3540	3538
BIC	3643	3619	3620	3611	3614
Likelihood ratio	Reference	*P*<.001	*P*<.001	*P*<.001	*P*<.001
Reclassification					
IDI		3.1 (1.7 to 4.5)	2.7 (1.3 to 4.9)	4.1 (2.5 to 5.6)	4.3 (2.7 to 5.9)
	Reference	*P*<.001	*P*<.001	*P*<.001	*P*<.001
NRI—all		4.2 (−3.0 to 11.3)	9.6 (2.5 to 16.8)	13.9 (6.2 to 21.6)	10.7 (2.6 to 18.7)
	Reference	*P*=.25	*P*=.008	*P*<.001	*P*=.009
NRI—deceased		3.6 (−1.8 to 9.18)	4.1 (−1.6 to 9.9)	7.8 (2.1 to 13.5)	5.4 (−0.8 to 11.6)
	Reference	*P*=.19	*P*=.15	*P*=.007	*P*=.09
NRI—alive		0.5 (−3.6 to 4.7)	5.5 (1.9 to 9.1)	6.1 (1.9 to 10.3)	5.3 (1.1 to 9.5)
	Reference	*P*=.8	*P*=.003	*P*=.005	*P*=.01

[a]AIC = Akaike information criterion; AUC = area under the receiver operating characteristic curve; BIC = Bayesian information criterion; H-L = Hosmer-Lemeshow test; hs-cTnT = high-sensitivity circulating troponin T; hs-ST2 = high-sensitivity soluble ST2; IDI = integrated discrimination improvement; NT-proBNP = N-terminal pro-B-type brain natriuretic peptide; NRI = net reclassification improvement.

[b]Model 1 = age, female sex, ischemic etiology of heart failure, left ventricular ejection fraction, New York Heart Association functional class, diabetes mellitus, estimated glomerular filtration rate, angiotensin-converting enzyme inhibitor or angiotensin II receptor blocker treatment, β-blocker treatment, sodium, hemoglobin; model 2 = model 1 + NT-proBNP + hs-cTnT; model 3 = model 1 + NT-proBNP + hs-ST2; model 4 = model 1 + hs-cTNT + hs-ST2; model 5 = model 1 + NT-proBNP + hs-cTNT + hs-ST2.

[c]All *P* values vs model 1.

any combination of biomarkers compared with the model with established mortality risk factors (*P*<.001), yet the benefit was highest for the combination hs-cTnT + hs-ST2 and with all 3 biomarkers (4.1 [95% CI, 2.5-5.6] and 4.3 [95% CI, 2.7-5.9], respectively; Table 3). Nevertheless, the separate addition of NT-proBNP into a model that already contained hs-cTnT + hs-ST2 did not significantly improve IDI reclassification (model 4 vs model 5; *P*=.34; Table 4).

The NRI (reclassification according to predefined risk categories) significantly improved after the inclusion of hs-ST2 (with either NT-proBNP or hs-cTnT) and with all 3 biomarkers but was not significantly better with NT-proBNP + hs-cTnT (Table 3). The best NRI values were obtained after the addition of hs-cTnT + hs-ST2 (13.9%; 95% CI, 6.2%-21.6%; model 4 in Table 4). Net reclassification indices even worsened after NT-proBNP addition into the full model (model 4 vs model 5 in Table 4).

Crude Mortality

Mortality during follow-up linearly increased from patients without any biomarker elevation (10%) to patients with 3 raised biomarkers above the cutoff points (63.7%) (Figure 2). Mortality for patients with high hs-cTnT + hs-ST2 (above the cutoff points) was 62.2%; however, this combination permitted the identification of 183 patients whereas only 138 patients had all 3 biomarkers above their cutoff points. Twenty-six additional deaths were detected with combining only hs-cTnT + hs-ST2 (114 vs 88). Moreover, if only cardiovascular death was analyzed, cardiovascular mortality for patients with high hs-cTnT + hs-ST2 levels was 34.4%, and this combination permitted the identification of 62 cardiovascular deaths; with all 3 biomarkers above their cutoff points, only 52 cardiovascular deaths were detected.

DISCUSSION

This study provides a comprehensive analysis of the prognostic value of the combination of

TABLE 4. Direct Comparison of Performance at 4 Years of Models Containing Biomarkers[a,b]

Variable	Model 2 vs model 4		Model 3 vs model 4		Model 4 vs model 5	
Discrimination						
AUC	0.780 (0.755 to 0.805)	0.789 (0.766 to 0.813)	0.784 (0.759 to 0.808)	0.789 (0.766 to 0.813)	0.789 (0.766 to 0.813)	0.790 (0.766 to 0.813)
	P=.003		*P*=.23		*P*=.71	
Calibration						
H-L	χ^2=12.1 *P*=.15	χ^2=7.8 *P*=.45	χ^2=12.1 *P*=.15	χ^2=7.8 *P*=.45	χ^2=7.8 *P*=.45	χ^2=12.7 *P*=.12
Brier score	0.150	0.144	0.148	0.144	0.144	0.143
AIC	3553	3540	3554	3540	3540	3538
BIC	3619	3611	3620	3611	3611	3614
Likelihood ratio	NA	NA	NA	NA	Reference	*P*=.04
Reclassification						
IDI		0.9 (−2 to 0.1)		1.4 (0.3 to 2.5)		0.2 (−0.2 to 0.7)
	Reference	*P*=.08	Reference	*P*=.009	Reference	*P*=.34
NRI—all		10.4 (5.1 to 15.7)		5.9 (−0.4 to 12.1)		−2.3 (−5.7 to 1.1)
	Reference	*P*<.001	Reference	*P*=.07	Reference	*P*=.18
NRI—deceased		4.7 (0.8 to 8.7)		4.6 (−0.3 to 9.5)		−2.2 (−4.7 to 0.4)
	Reference	*P*=.02	Reference	*P*=.06	Reference	*P*=.09
NRI—alive		5.7 (2.6 to 8.8)		1.3 (−2.3 to 4.8)		−0.1 (−2.0 to 1.8)
	Reference	*P*<.001	Reference	*P*=.48	Reference	*P*=.90

[a]AIC = Akaike information criterion; AUC = area under the receiver operating characteristic curve; BIC = Bayesian information criterion; hs-cTnT = high-sensitivity circulating troponin T; hs-ST2 = high-sensitivity soluble ST2; H-L = Hosmer-Lemeshow test; IDI = integrated discrimination improvement; NA = not applicable; NT-proBNP = N-terminal pro-B-type brain natriuretic peptide; NRI = net reclassification improvement.

[b]Model 2 = model 1 + NT-proBNP + hs-cTnT; model 3 = model 1 + NT-proBNP + hs-ST2; model 4 = model 1 + hs-cTNT + hs-ST2; model 5 = model 1 + NT-proBNP + hs-cTNT + hs-ST2.

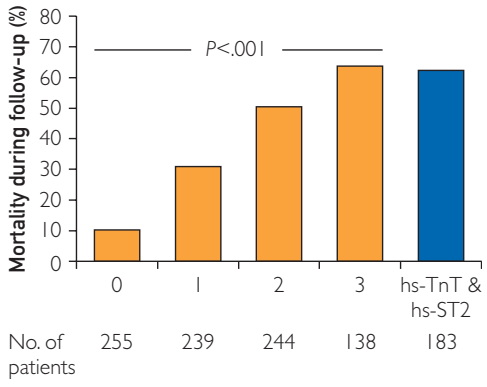

FIGURE 2. Crude mortality rates during follow-up. The presence of any biomarker above the cutoff point (from 0 to 3) is shown on the x-axis. The blue bar illustrates the high-sensitivity cardiac troponin T (hs-TnT) + high-sensitivity soluble ST2 (hs-ST2) combination above the cutoff points.

NT-proBNP (a marker of myocardial stretch), hs-cTnT (a marker of myocardial damage), and hs-ST2 (a marker of myocardial fibrosis and remodeling) in a real-life cohort of patients with chronic HF. All 3 biomarkers were incorporated on top of a model with 11 well-established risk factors (age, sex, ischemic etiology, LVEF, NYHA functional class, diabetes mellitus, eGFR, sodium level, hemoglobin level, β-blocker, and ACEI and ARB treatments). The AUC for this model was 0.76, which compares favorably with other proposed models in HF (ie, the community validation of the Seattle Heart Failure Model had an overall AUC of 0.73).[24]

The addition of 2 or more biomarkers from different pathophysiologic pathways into a multimarker analysis to obtain additive prognostic information in HF seems to be a rational and reliable strategy for identifying patients who need to be followed up more closely.[5,6,16-18] Most multimarker strategies have involved adding the combination of other biomarkers to the well-established BNP or NT-proBNP, given the usefulness of these peptides in a wide range of HF situations.[6] Second-generation biomarkers include hs-cTnT and hs-ST2. Cardiac troponin

is a marker of myocyte injury, and the hs-cTnT assay has recently become available for detecting extremely low troponin concentrations and improving precision at the lower limit of detection.[21] ST2 is a biomarker for myocardial fibrosis and remodeling; under the induction of separate promoters, the ST2 gene expresses two unique proteins: soluble ST2, which is the circulating form of ST2 (as assessed in this study), and ST2 ligand, which is the transmembrane form of the protein that signals through a complex involving interleukin 33.[25]

The additional prognostic information gained by any marker over a clinical risk model plus other biomarkers needs to be determined using adequate statistical tools.[26] A major problem in selecting a biomarker profile is the proportional increase in economic burden,[6] so the addition of any biomarker to a profile should be justified by adequate discrimination, calibration, reclassification, and likelihood analyses. According to the present results, the combination of an increasing number of biomarkers did not necessarily improve risk stratification in HF.

The usefulness of combining natriuretic peptides with either hs-ST2 or hs-cTnT has been reported previously,[18,27,28] but not the combination of the 3 in the setting of chronic HF. In this study, we found that hs-cTnT + hs-ST2 performed as well as or better than the combination of all 3 added biomarkers (NT-proBNP + hs-cTnT + hs-ST2). The different analysis yielded 3 relevant findings. First, NT-proBNP added to hs-cTnT + hs-ST2 did not improve prognostic accuracy or reclassification indices. Second, NT-proBNP increased prognostic discrimination only in patients with either hs-cTnT or hs-ST2 levels below the cutoff point. Third, the combination of hs-cTnT + hs-ST2 identified more decedents during follow-up than did the combination of the 3 biomarkers. The latter was observed even when only cardiovascular deaths were taken into account. Together, these main findings suggest that the pathways identified by hs-ST2 and hs-cTnT profoundly affect mortality in the context of chronic HF, whereas the information provided in their presence by natriuretic peptides might be redundant. However, as shown in this study and depicted in Figure 1, B the separate addition of NT-proBNP provided prognostic discrimination in patients with either hs-cTnT or hs-ST2 levels below the cutoff point.

Although modification of some mortality risk factors may decrease the risk of HF hospitalizations and death, evidence is lacking that reducing the levels of hs-cTnT and hs-ST2 will reduce risk. Therefore, these data should not be construed as implying a direct benefit from reducing biomarker levels. A better risk assessment is clinically of great value as it more accurately identifies patients with HF at increased risk of death who could then be targeted for more intensive monitoring and treatment.

In this study, we analyzed only 1 blood sample and cannot comment on the prognostic value of serial determinations. The population was a general HF population treated at a specific and multidisciplinary HF unit in a tertiary care hospital; most patients were referred from the cardiology department and, thus, were relatively young men with HF of ischemic etiology and reduced LVEF. As such, these results cannot necessarily be extrapolated to a global HF population.

CONCLUSION

A new generation of biomarkers (hs-cTnT and hs-ST2) for different pathophysiologic processes from those of natriuretic peptides perform as well as or better for risk stratification in chronic HF. If these results are validated, the incorporation of these biomarkers into clinical practice for the prediction of death could be accomplished quickly. Further studies should confirm whether natriuretic peptides might be nonmandatory for HF risk stratification.

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Abbreviations and Acronyms: **ACEI** = angiotensin-converting enzyme inhibitor; **AIC** = Akaike information criterion; **ARB** = angiotensin II receptor blocker; **AUC** = area under the receiver operating characteristic curve; **BIC** = Bayesian information criterion; **eGFR** = estimated glomerular filtration rate; **HF** = heart failure; **HR** = hazard ratio; **hs-cTnT** = high-sensitivity cardiac troponin T; **hs-ST2** = high-sensitivity soluble ST2; **IDI** = integrated discrimination improvement; **LVEF** = left ventricular ejection fraction; **NRI** = net reclassification improvement; **NT-proBNP** = N-terminal pro-B-type natriuretic peptide; **NYHA** = New York Heart Association; **P_{25-75}** = 25th-75th percentiles

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Potential Competing Interests: Dr Bayes-Genis has received lecture honoraria from Roche Diagnostics and Critical Diagnostics.

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Head-to-head comparison of high-sensitivity troponin T and sensitive-contemporary troponin I regarding heart failure risk stratification

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ABSTRACT

Background: High-sensitivity assays for cardiac troponins have recently become available, increasing the value of troponins in heart failure (HF) prognostication. We head-to-head compared the prognostic significance of high-sensitivity cardiac troponin T (hs-cTnT) and sensitive-contemporary cardiac troponin I (sc-cTnI) in an outpatient HF population.

Methods: We studied 876 patients, mainly of ischemic etiology (52.1%). Median left ventricular ejection fraction was 34%. Median follow-up was 3.45 years. Comprehensive statistical measurements of performance (discrimination, calibration, and reclassification) were obtained.

Results: hs-cTnT was ubiquitous in the patient cohort; sc-cTnI was detected in 276 patients (31.5%). During follow-up 311 patients died. According to multivariable Cox regression analysis, both hs-cTnT (HR 2.09, 95% CI 1.46–2.99, $P < 0.001$) and sc-cTnI (HR 1.61, 95% CI 1.24–2.08, $P < 0.001$) remained independent predictors of all cause and cardiovascular mortality. Using the best predictive cut-off point for both troponins calibration was better for hs-cTnT, which also reclassified a larger number of patients (NRI 9.0 [2.5;15.5] $P = 0.007$). The higher sensitivity of hs-cTnT permitted the identification of almost the double of deaths.

Conclusion: Both hs-cTnT and sc-cTnI predict mortality in a real-life cohort of ambulatory HF patients. However, hs-cTnT showed globally better measures of performance and identified a higher proportion of decedents during follow-up.

1. Introduction

Chronic HF is a real public epidemic [1]. Despite important progress in recent decades, mortality remains high for patients with HF. Risk stratification using clinical variables is often insufficient to estimate individual prognosis and requires the evaluation of circulating biomarkers to aid in clinical decision making.

Cardiac troponins, biomarkers of myocyte injury, are crucial in the diagnosis and prognosis of acute coronary syndromes and also predict adverse clinical outcomes in acute [2–4] and chronic HF [5,6]. High-sensitivity assays for cardiac troponin T and more efficient sensitive-contemporary assays for troponin I have become commercially available. These assays detect low troponin concentrations and improve precision at the lower limit of detection. Several clinical series have already suggested that both troponins provide useful prognostic information in different HF populations [7–15]. However, there has been limited head-to-head comparison of both assays in a long-term follow-up HF cohort. Accordingly, our aim was to compare the prognostic significance of commercially available sensitive troponin T and I assays in an outpatient HF population.

Abbreviations: hs-cTnT, high-sensitivity cardiac troponin T; sc-cTnI, sensitive-contemporary cardiac troponin I; HF, heart failure; LVEF, left ventricular ejection fraction; eGFR, estimated glomerular filtration rate; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; AUC, area under the receiver-operating characteristic curve; AIC, Akaike information criterion; BIC, Bayesian information criterion; IDI, integrated discrimination improvement; NRI, net reclassification improvement; NTproBNP, N-terminal pro-brain natriuretic peptide.

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2. Material and methods

2.1. 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, as well as (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 left ventricular ejection fraction (LVEF).

Blood samples were obtained by venipuncture between 9:00 a.m. and 12:00 p.m. during conventional ambulatory visits. After centrifugation, serum samples were stored at −80 °C; Both troponins were analyzed from the same blood sample.

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

2.2. Follow-up and outcomes

All patients were followed at regular predefined intervals, with additional visits as required in cases 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 [15–17]. 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 HF unit clinical records or by reviewing the electronic clinical history at the Catalan Institute of Health, as well as by contacting the patients' relatives. Furthermore, data was verified from databases of the regional and national health systems.

2.3. sc-cTnI assay

Troponin I levels were measured using a sandwich chemiluminescence immunoassay based on LOCI® technology (Troponin I LOCI Siemens, RF621) and processed on the automatic analyzer Dimension® EXL™ Integrated Chemistry System (Siemens Diagnostics). As described by the manufacturer (RF 621, 2009-04-22 Siemens Healthcare Diagnostics Inc.), the 99th percentile for normal is 0–56 ng/L and the functional sensitivity (limit of quantification with coefficient of variation <10%) is 50 ng/L. The analytic measurement range for LOCI® Troponin I measured in Dimension® EXL™ is 17–40,000 ng/L without any dilution or pretreatment. In the insert this assay is considered as high-sensitivity based on imprecision and other performance characteristics, but in a recent population study [18] it is classified as sensitive-contemporary troponin I assay (sc-cTnI) and the 99th percentile for normal was found to be 34 ng/L (39 ng/L for men and 22 ng/L for women).

2.4. hs-cTnT assay

Troponin T levels were measured using an electrochemiluminescence immunoassay (ultra-sensitive troponin T method, ref 05092744 190 Roche Diagnostics) with a Modular Analytics E170 system (Roche Diagnostics). The analytic performance of this assay has been validated [19]. As described by the manufacturer (ref 05092744 190 Roche Diagnostics) the 99th percentile for normal is 14 ng/L and the functional sensitivity (limit of quantification with coefficient of variation <10%) is 13 ng/L. The cTnT assay analytic range is 3–10,000 ng/L. According to the recent population study [18] it is classified as high-sensitivity troponin T (hs-cTnT) assay and the 99th percentile for normal was found to be 15 ng/L (20 ng/L for men and 13 ng/L for women). The assays were run with reagents from lot 157123, not affected by the analytical issues emerged with Roche hs-cTnT assays.

Both methods demonstrate analytic performance in accordance with the recommendations of the Task Force for use in the diagnosis of myocardial necrosis [20].

2.5. Statistical analysis

Categorical variables were expressed as percentages. Continuous variables were expressed as the mean (standard deviation) or median (25th–75th percentiles, $P_{25–75}$) according to normal or non-normal distribution. Statistical differences between groups were compared using the chi-squared test for categorical variables. Correlation between the levels of sc-cTnI and hs-cTnT was analyzed using Spearman's rho.

Survival analyses were performed using Cox regression models. Density plots of the best cut-off point in non-adjusted Cox models were calculated using bootstrap methodology to identify the optimal prognostic cut-off points for both troponins.

The following variables were incorporated into the model: age, sex, LVEF (in percent), estimated glomerular filtration rate (eGFR calculated with the CKD-EPI equation after standardization of creatinine values according to IDMS reference method, in mL/min/1.73 m^2), New York Heart Association functional class, presence of diabetes mellitus, ischemic etiology, hemoglobin (g/dL), serum sodium (mmol/L), N-terminal pro-brain natriuretic peptide (NTproBNP) (ng/L), β-blocker treatment, and angiotensin-converting enzyme inhibitor (ACEI) or angiotensin II receptor blocker (ARB) treatment. Log-rank tests for Kaplan–Meier survival curves were performed using the best cut-off points.

We used different measurements of performance to test the potential prognostic value of sc-cTnI and hs-cTnT (both as dichotomous variables; sc-cTnI distribution impeded its analysis as continuous variable), as follows:

a) *Discrimination*: The area under the receiver operating characteristic curve (AUC) summarized the diagnostic discrimination. Discrimination refers to a model's ability to correctly distinguish between two classes of outcomes. We used the index of rank correlation, Somers' D, which already incorporates information about censored data. To compare AUCs we used the method described by Pencina and D'Agostino [21].
b) *Calibration*: 1) The D'Agostino–Nam version of the Hosmer–Lemeshow calibration test was used to calculate a chi-squared value. 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. The AIC and BIC are measures of the relative goodness of fit of a statistical model. The BIC penalizes free parameters more strongly than does the AIC. Brier score measures the average squared deviation between predicted probabilities for a set of events and their outcomes, so a lower score represents higher accuracy. It takes values between 0 and 1. Given any two estimated models, the model with the lower BIC, AIC, and Brier scores was preferred. No statistical tests compare different BIC, AIC, or Brier estimations, and 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 model's accuracy.
c) *Reclassification*: We used the method described by Pencina et al. [22]. 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. $P < 0.05$ from two-sided tests was 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 at four years: <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.

Cut-off points used in the analysis were the optimal prognostic cut-off points obtained using bootstrap methodology and also the 99th percentiles recently published [18].

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

3. Results

Out of 891 consecutive patients included from May 22, 2006 to July 7, 2010, 876 had available hs-cTnT and sc-cTnI measurements and were finally included in this analysis. The median age of participants was 70.3 years ($P_{25–75}$ 60.5–77.2 years). Table 1 shows baseline characteristics of the studied population. During a median follow-up period of 3.45 years [$P_{25–75}$ 1.84–5.03], 311 patients died. Among cardiovascular causes of death (N = 168), mortality was attributed to refractory HF in 91 patients (54.1%), to sudden death in 30 patients (17.8%), and to acute myocardial infarction in 15 patients (8.9%). Two patients were lost to follow-up and adequately censored.

hs-cTnT was detected in 100% of patients and 607 patients (69.2%) had values ≥13 ng/L (limit of quantification with coefficient of variation <10%); in comparison, sc-cTnI was detected in 276 patients (31.5%), of which only 121 (13.7%) had values ≥50 ng/L (limit of quantification with coefficient of variation <10%). Online Supplementary Fig. 1 depicts the correlation between both biomarkers (rho 0.53, $P < 0.001$). We have already reported [15] a positive correlation of hs-cTnT levels with age (rho = 0.35, $P < 0.001$), eGFR (rho = 0.52, $P < 0.001$), and NTproBNP (rho = 0.62, $P < 0.001$). sc-cTnI also correlated, although more weakly with eGFR (rho = 0.10, $P = 0.004$) and NTproBNP (rho = 0.37, $P < 0.001$), but not with age (rho = 0.06, $P = 0.08$). Also hs-cTnT levels tended to be higher in men than in women (24 [$P_{25–75}$ 11–40.9] vs. 20.4 [$P_{25–75}$ 9.7–38.1]; $P = 0.086$), in diabetic patients (28.4 [$P_{25–75}$ 15.2–44.9] vs. 19 [$P_{25–75}$ 8.9–36.9], $P < 0.001$) and in patients with HF of ischemic etiology (25.9 [$P_{25–75}$ 11.5–42.1] vs. 20.1 [$P_{25–75}$ 9.3–36.5], $P = 0.004$). sc-cTnI was also higher in patients of ischemic etiology of HF (0 [$P_{25–75}$ 0–14.5] vs. 0 [$P_{25–75}$ 0–4], $P = 0.025$), but sc-cTnI levels were not statistically different for diabetics and non-diabetics ($P = 0.546$) and between sexes ($P = 0.747$). Finally, levels of hs-cTnT and sc-cTnI significantly correlated with NYHA functional class ($P < 0.001$ and $P = 0.001$, respectively) (Online Supplementary Fig. 3). Although the majority of patients had depressed systolic left ventricular function, 202 patients had a LVEF ≥ 45%. Both troponins were significantly higher in patients with reduced LVEF (<45%) than in those with LVEF ≥45% (Online Supplementary Fig. 4): hs-cTnT 25.2 [$P_{25–75}$ 11.6–41.4] vs. 19 [$P_{25–75}$ 8.9–36.9], respectively ($P < 0.001$); sc-cTnI 0 [$P_{25–75}$ 0–14.25] vs. 0 [$P_{25–75}$ 0–0], respectively ($P < 0.001$). Indeed, sc-cTnI was most often elevated in patients with reduced LVEF: 10% of patients with LVEF ≥45% had sc-cTnI levels above the 99th percentile vs. 17% in patients with reduced LVEF ($P = 0.03$), point that differed with hs-cTnT (54% vs. 61.1% respectively, $P = 0.07$).

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

	N = 876
Age, median ($P_{25–75}$), years	70.3 (60.5–77.2)
Males, n (%)	630 (71.9)
Etiology	
Ischemic heart disease, n (%)	456 (52.1)
Dilated cardiomyopathy, n (%)	86 (9.8)
Hypertensive, n (%)	83 (9.5)
Valvular, n (%)	102 (11.6)
Other, n (%)	149 (17.0)
Heart failure duration, median ($P_{25–75}$), months	27.3 (4.9–73.8)
LVEF, median ($P_{25–75}$), %	34 (26–43)
LVEF ≥ 45%	202 (23.1)
eGFR, median ($P_{25–75}$), mL/min/1.73 m^2	51.3 (32.6–71.6)
Sodium, median ($P_{25–75}$), mmol/L	139 (137–142)
Hemoglobin, mean ± SD, g/dL	12.9 ± 1.8
NYHA functional class, n (%)	
I	63 (7.2)
II	574 (65.5)
III	230 (26.3)
IV	9 (1.0)
Diabetes mellitus, n (%)	314 (35.8)
Treatments, n (%)	
ACEI or ARB	785 (89.6)
Beta-blocker	767 (87.6)
Spironolactone/eplerenone	344 (39.3)
Loop diuretic	743 (84.8)
Digoxin	269 (30.7)
ICD	92 (10.5)
CRT	47 (5.4)
sc-cTnI, median ($P_{25–75}$), ng/L	0.0 (0.0–9)
hs-cTnT, median ($P_{25–75}$), ng/L	22.6 (10.6–40.6)

LVEF = left ventricular ejection fraction; eGFR = estimated glomerular filtration rate; NYHA = New York Heart Association; HF = heart failure; ACEI = angiotensin converting enzyme inhibitor; ARB = angiotensin II receptor blocker; ICD = implantable cardiac defibrillator; CRT = cardiac resynchronization therapy; sc-cTnI = sensitive-contemporary circulating troponin I; hs-cTnT = high sensitive circulating troponin T; $P_{25–75}$: 25th–75th percentiles; SD: standard deviation.

3.1. Cox regression and modeling

The optimal prognostic cut-off points obtained using bootstrap methodology were 16 ng/L [95% CI, 14–29] for hs-cTnT and 1 ng/L [95% CI, 0.0–3] for sc-cTnI. Five-hundred thirty-nine (61.5%) patients had hs-cTnT values above the cutoff point whereas 265 (30.3%) had sc-cTnI levels above the cutoff point. In the bivariable analysis, both sc-cTnI and hs-cTnT were predictors of all-cause mortality (Fig. 1). Although a small number of patients with LVEF had elevated levels of sc-cTnI, these values had also prognostic implications (Online Supplementary Fig. 5).

In the multivariable analysis, which included 12 well-established mortality risk factors (age, sex, LVEF, New York Heart Association functional class, diabetes, eGFR, ischemic etiology, sodium, hemoglobin, NTproBNP, β-blocker treatment, and ACEI or ARB treatment), both sc-cTnI and hs-cTnT remained independent predictors of mortality (Table 2). Using the 99th percentile sc-cTnI lost statistical significance and hs-cTnT hazard ratio was lower (Table 2). On the other hand, patients with sc-cTnI ≤1 ng/L but with hs-cTnT levels >16 ng/L had better prognosis than those with both troponin levels above these cutoff points (Fig. 2). The same was observed using the 99th percentile: patients with sc-cTnI ≤99th percentile but with hs-cTnT levels >99th percentile had better prognosis than those with both troponin levels >99th percentile (Online Supplementary Fig. 2). Results on patients with LVEF ≥45% were less consistent than in the total population or in patients with depressed LVEF, in part probably due to the relatively small number of patients. Using bootstrapping best cut-off points only sc-cTnI remained independently associated with all-cause death (HR 2.02 [1.07–3.80], $P = 0.03$) whereas hs-cTnT was only of border-line significance (HR 1.84 [0.91–3.74], $P = 0.092$), but using the 99th percentile neither troponin remained significant.

3.2. Measurements of performance

3.2.1. Discrimination

The AUC for the prediction of death was significantly higher with the incorporation of sc-cTnI into the model with established mortality risk factors ($P = 0.018$). hs-cTnT showed a trend towards an increase of the AUC ($P = 0.057$; Table 3).

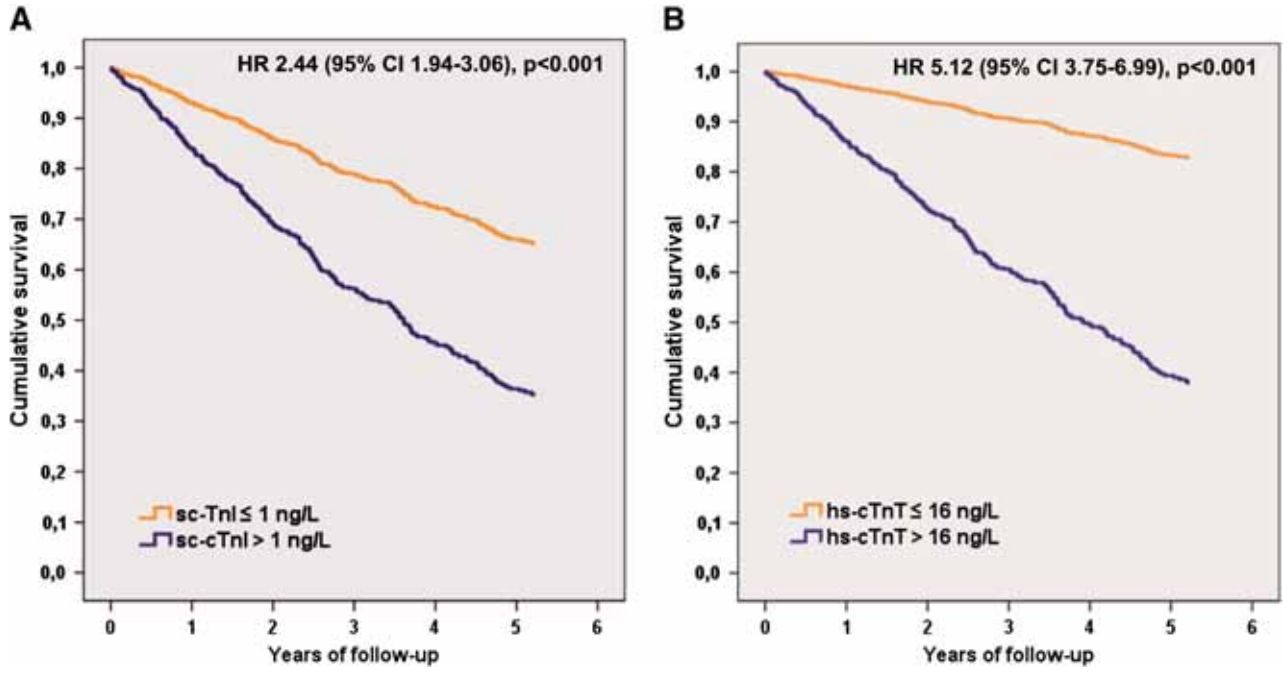

Fig. 1. Cox regression survival curves according to sc-cTnI and hs-cTnT. Panel A (left): Survival according to sc-cTnI levels ≤/> the cut-off point of 1 ng/L. Panel B (right): Survival according to hs-cTnT levels ≤/> the cut-off point of 16 ng/L.

3.2.2. Calibration

The *P*-values for the Hosmer–Lemeshow statistics indicated good calibration for all models ($P > 0.3$ for all comparisons; Table 3). The lowest BIC, AIC, and Brier scores were obtained in the model that included hs-cTnT, altogether an additional indication of better calibration with this biomarker. Global goodness-of-fit, as evaluated by the likelihood ratio test, was better in any model including cardiac troponins than in the reference (clinical + NTproBNP) model, except for sc-cTnI when using the 99th percentile as cut-off point (Table 3).

3.2.3. Reclassification

IDI (risk as a continuous variable) increased significantly with both troponins except for sc-cTnI when the 99th percentile as cut-off point was used (Table 3). By contrast, NRI (reclassification according to predefined risk categories) only improved significantly after adding hs-cTnT (bootstrapping best cut-off point) to the reference model (Model 3; Table 3).

Considering all measurements of performance together, models using bootstrapping-derived cut-off points showed better results (Table 3). When performance metrics were analyzed in the subset of patients with LVEF ≥45% no significant benefit was observed neither in discrimination nor in reclassification for all-cause death when troponins were added into the clinical model containing already NTproBNP.

3.3. Cardiovascular mortality

Focusing on cardiovascular mortality, both troponins remained again independent predictors of mortality in the multivariate Cox regression analysis using optimal bootstrapping cut-off points: sc-cTnI HR 1.32 [1.10–1.59], $P = 0.003$; hs-cTnT HR 1.29 [1.07–1.55], $P =$

Table 2
Multivariate Cox regression analysis for the four models.

	sc-cTnI at 1 ng/L			hs-cTnT at 16 ng/L			sc-cTnI at 99th percentile			hs-cTnT at 99th percentile		
	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
Age	1.05	[1.03; 1.06]	<0.001	1.04	[1.03; 1.06]	<0.001	1.05	[1.03; 1.06]	<0.001	1.04	[1.03; 1.06]	<0.001
Female	0.63	[0.48; 0.83]	0.001	0.70	[0.53; 0.92]	0.009	0.60	[0.46, 0.79]	<0.001	0.62	[0.48; 0.81]	<0.001
Ischemic etiology of HF	1.01	[0.79; 1.29]	0.950	1.06	[0.83; 1.36]	0.637	1.01	[0.79, 1.29]	0.967	1.03	[0.81; 1.32]	0.800
LVEF	1.00	[0.99; 1.01]	0.533	1.00	[0.99; 1.01]	0.653	1.00	[0.99; 1.01]	0.737	1.00	[0.99; 1.01]	0.738
NYHA functional class	1.89	[1.48; 2.41]	<0.001	1.81	[1.42; 2.30]	<0.001	1.85	[1.45; 2.36]	<0.001	1.79	[1.40; 2.29]	<0.001
eGFR, mL/min/1.73 m^2	1.00	[1.00; 1.00]	0.220	1.00	[1.00; 1.00]	0.828	1.00	[0.99; 1.00]	0.250	1.00	[0.99; 1.00]	0.607
Diabetes mellitus	1.26	[1.00; 1.59]	0.054	1.18	[0.93; 1.49]	0.179	1.27	[1.00; 1.60]	0.043	1.23	[0.98; 1.56]	0.079
ACEI or ARB treatment	0.74	[0.53; 1.03]	0.070	0.74	[0.53; 1.02]	0.067	0.73	[0.53; 1.02]	0.063	0.75	[0.54; 1.03]	0.078
β-Blocker treatment	0.56	[0.41; 0.76]	<0.001	0.54	[0.40; 0.72]	<0.001	0.52	[0.39; 0.71]	<0.001	0.52	[0.38; 0.70]	<0.001
Sodium	0.95	[0.92; 0.98]	0.003	0.95	[0.92; 0.99]	0.004	0.95	[0.92; 0.98]	0.002	0.95	[0.92; 0.98]	0.003
Hemoglobin	0.91	[0.85; 0.98]	0.011	0.93	[0.87; 1.00]	0.035	0.91	[0.85; 0.98]	0.008	0.92	[0.86; 0.99]	0.024
NTproBNP	1.26	[1.12;1.40]	<0.001	1.24	[1.11;1.38]	<0.001	1.32	[1.18; 1.47]	<0.001	1.27	[1.14; 1.42]	<0.001
sc-cTnI	1.59	[1.23; 2.05]	<0.001				1.22	[0.90; 1.65]	0.196			
hs-cTnT				2.15	[1.50; 3.07]	<0.001				1.61	[1.16; 2.22]	0.005

NTproBNP as log (NTproBNP); NYHA functional classes I–II vs. III–IV.
HF = heart failure; LVEF = left ventricular ejection fraction; NYHA = New York Heart Association; eGFR = estimated glomerular filtration rate; ACEI = angiotensin converting enzyme inhibitor; ARB = angiotensin II receptor blocker; sc-cTnI = sensitive-contemporary circulating troponin I; hs-cTnT = high sensitive circulating troponin T. NTproBNP = N-terminal pro-brain natriuretic peptide.
99th percentile for sc-cTnI: 39 ng/L for men and 22 ng/L for women.
99th percentile for hs-cTnT: 20 ng/L for men and 13 ng/L for women.

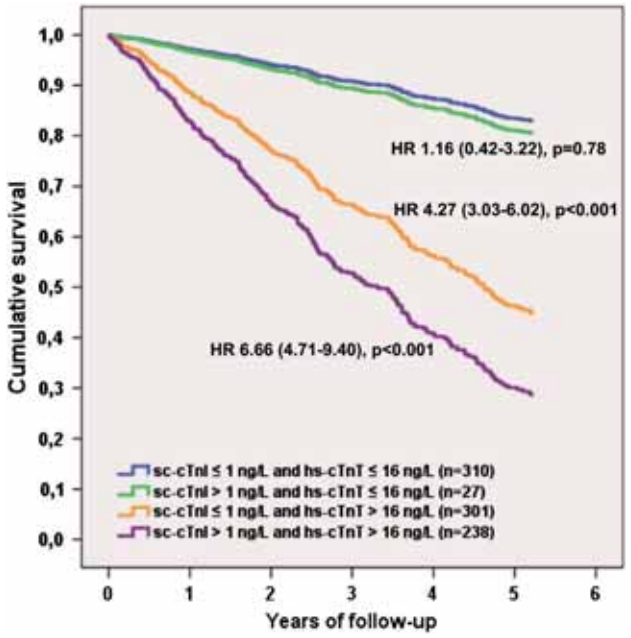

Fig. 2. Cox regression survival curves according to combined sc-cTnI and hs-cTnT levels using best bootstrapping cut-off levels. Groups: both troponin levels ≤cut-off point; sc-cTnI > cut-off + hs-cTnT ≤ cut-off; sc-cTnI ≤ cut-off + hs-cTnT > cut-off; and both troponins >cut-off point.

0.008, and also using 99th percentiles: sc-cTnI HR 1.25 [1.01–1.56], $P = 0.037$; hs-cTnT HR 1.30 [1.08–1.57], $P = 0.005$. Using bootstrapping cut-off point, performance of prediction models for cardiovascular mortality showed improved discrimination ($P = 0.04$) and NRI (7.8 [2.8–12.9], $P = 0.002$) for hs-cTnT; using the 99th percentile, NRI tended to improve for both troponins (3.7 [−0.1–7.6], $P = 0.057$ for sc-cTnI and 6.8 [1.4–12.2], $P = 0.013$ for hs-cTnT), although only statistically significant for hs-cTnT. In the subset of patients with LVEF ≥45% both troponins remained independently associated with cardiovascular death (sc-cTnI: HR 2.51 [1.48–4.25], $P = 0.001$; hs-cTnT: HR 1.88 [1.21–2.93], $P = 0.005$) using bootstrapping best cut-off points. When the 99th percentile was used, sc-cTnI remained independently associated with cardiovascular death (HR 1.96 [1.04–3.69], $P = 0.037$) whereas only a trend was found for hs-cTnT (HR 1.47 [0.94–2.30], $P = 0.092$). When performance metrics were analyzed in the subset of patients with LVEF ≥45% no significant benefit was observed neither in discrimination nor in reclassification for cardiovascular mortality when troponins were added into the clinical model already containing NTproBNP.

4. Discussion

In this study, we performed a head-to-head comparison of the prognostic value of a high-sensitivity commercial troponin assay for cTnT and a sensitive-contemporary assay for cTnI in a real-life cohort of patients with chronic HF. Both assays were predictors of mortality and added incremental prognostic information to a model with well-established risk factors. The presented data regarding prevalence, prognostic value, and comparison of both troponins in heart failure deserve some discussion.

First, hs-cTnT was detected in all patients, while only 31.5% of patients had detectable levels of sc-cTnI. According to recently published 99th percentiles for normal men and women, 59.5% of our patients had hs-cTnT levels >99th percentile while only 15.6% of them had sc-cTnI levels >99th percentile. Due to this higher sensitivity, more deaths could be detected using hs-cTnT. This discrepancy in the incidence of detection has several potential explanations. On the one hand, and probably mostly important, it may be due to the differing analytical performance of the assays, owing to the greater sensitivity of the hs-cTnT assay used. On the other hand, it may be due to the fact that the epitopes recognized by the antibody used in the troponin I method (C: 27–32; D: 41–56) differ from those used in the troponin T method, which recognizes epitopes located in the central part and other amino acid positions (125–131 and 135–147). Indeed, Online Supplementary Fig. 1 clearly illustrates this discrepancy. Patients with high levels of hs-cTnT often had low levels of sc-cTnI; in

Table 3
Model performance at 4 years.

	Model 1 (clinical + NTproBNP)	Model 2 Model 1 + sc-cTnI (cut-off 1 ng/L)	Model 3 Model 1 + hs-cTnT (cut-off 16 ng/L)	Model 4 Model 1 + sc-cTnI (99th percentile)	Model 5 Model 1 + hs-cTnT (99th percentile)
AUC[a]	0.771 (0.746–0.797) Reference	0.779 (0.754–0.804) $P = 0.018$	0.779 (0.755–0.804) $P = 0.057$	0.772 (0.747–0.798) $P = 0.559$	0.774 (0.749–0.799) $P = 0.352$
HL	Chi-squared: 9.5 $P = 0.39$	Chi-squared: 7.5 $P = 0.58$	Chi-squared: 7.6 $P = 0.57$	Chi-squared: 8.1 $P = 0.53$	Chi-squared: 7.4 $P = 0.60$
Brier score	0.155	0.152	0.150	0.155	0.152
AIC	3621	3611	3604	3622	3615
BIC	3679	3673	3666	3684	3677
Likelihood ratio	Reference	$P < 0.001$	$P < 0.001$	$P = 0.203$	$P = 0.004$
IDI[a]	Reference	1.1 [0.4; 1.9] $P = 0.004$	1.8 [1.0; 2.7] $P = <0.001$	0.1 [−0.2; 0.4] $P = 0.549$	0.8 [0.2; 1.4] $P = 0.008$
NRI, all[a]	Reference	3.2 [−2.5; 8.9] $P = 0.276$	9.0 [2.2; 15.8] $P = 0.009$	1.4 [−1.2; 4.1] $P = 0.291$	2.2 [−3.6; 8.0] $P = 0.464$
NRI, decedents[a]	Reference	1.7 [−2.8; 6.2] $P = 0.455$	5.9 [1.0; 10.8] $P = 0.019$	0.6 [−1.5; 2.7] $P = 0.590$	2.1 [−2.2; 6.4] $P = 0.341$
NRI, survivors[a]	Reference	1.5 [−1.5; 4.5] $P = 0.335$	3.1 [−0.7; 7.0] $P = 0.113$	0.9 [−0.7; 2.4] $P = 0.264$	0.1 [−3.1; 3.3] $P = 0.958$

AIC = Akaike information criterion; AUC = area under receiver operating characteristic curve; BIC = Bayesian information criterion; HL = Hosmer–Lemeshow test; IDI = integrated discrimination improvement; NRI = net reclassification improvement.
Model 1: Age, female, ischemic etiology of heart failure, left ventricular ejection fraction, New York Heart Association functional class, diabetes mellitus, estimated glomerular filtration rate, sodium, hemoglobin, angiotensin converting enzyme inhibitor or angiotensin II receptor blocker treatment, β-blocker treatment, and N-terminal pro-brain natriuretic peptide.
Model 2: Model 1 + sc-cTnI (cut-off 1 ng/L).
Model 3: Model 1 + hs-cTnT (cut-off 16 ng/L).
Model 4: Model 1 + sc-cTnI (cut-off 39 ng/L for men and 22 ng/L for women).
Model 5: Model 1 + hs-cTnT (cut-off 20 ng/L for men and 13 ng/L for women).
[a] *P*-values vs. Model 1.

addition, some patients with high levels of sc-cTnI had relatively low levels of hs-cTnT. The mechanisms of troponin release in HF are still not well established, and several processes are likely to be involved, 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 [23]. Thus, an alternative mechanism that deserves consideration could be explained by a different pattern of troponin release and differences in the structural properties of cardiac troponins in HF; however, this proposition is currently only speculative.

Using conventional assays, Ilva et al. [24] reported that cTnI was elevated more often than cTnT (51% vs. 30%) in acute HF. Using high-sensitivity assays, a small study of patients with chronic HF referred for right-heart catheterization [25] compared both troponins and found that hs-cTnI was above the 99th percentile in a greater proportion of patients than hs-cTnT (47.6% vs. 35.9%). Comparison of different troponin assays has been difficult because of the lack of standardization of techniques for conventional cTnI, sc-cTnI and hs-cTnI (there are numerous assays for conventional cTnI, sc-cTnI and hs-cTnI, but there is only a single assay for cTnT and hs-cTnT), lack of an international material for hs-cTnI, and the differing analytical imprecision of the assays [26]. In addition, cTnI measurements can be affected by multiple factors, including posttranslational modification (e.g., proteolytic degradation and phosphorylation) and complexing with other molecules (e.g., troponin C, heparin, heterophile or human anti-mouse antibodies, and cTnI-specific autoantibodies). However, the sc-cTnI assay used in this study recognizes epitopes from the central part of the troponin molecule, which are more stable and unaffected by posttranslational modifications, and interference with heterophilic antibodies has been minimized.

Second, despite the differences in prevalence, both hs-cTnT and sc-cTnI were good predictors of all-cause mortality and remained independent predictors of mortality in the multivariable analysis. Our results are in accordance with previous studies showing that both sc-cTnI and hs-cTnT are associated with increased morbidity and mortality [7–15]. Moreover, the addition of sc-cTnI and hs-cTnT to a model with established mortality risk factors (including NTproBNP) improved measurements of performance, mostly when the best cut-off points were used. According to our results, hs-cTnT better predicts mortality in ambulatory HF patients than sc-cTnI and identified a higher proportion of decedents during follow-up and should be preferred for the stratification of the risk of death in these patients.

Third, we evaluated the best cut-off point for hs-cTnT and sc-cTnI prognosis by bootstrapping the value that maximized the log-likelihood of the non-adjusted Cox models. This novel approach provides a more precise statistical cut-off point for prognostic purposes. In this study, the best cut-off point for hs-cTnT was 16 ng/L, a value slightly superior to the referenced functional sensitivity (13 ng/L), and 1 ng/L for sc-cTnI. This approach showed global better results than the use of the 99th percentile, even despite the use of gender-specific 99th percentile in all the analyses. HF is characterized by low-level sustained loss of cardiomyocytes, opposed to the massive sudden myocyte necrosis caused by an acute coronary syndrome. Thus, from a clinical perspective and considering specifically the sc-cTnI assay, the simple detection of values >1 ng/L is prognostically meaningful in HF patients. However, as these values are far below the limit of detection, the use of the 99th percentile as cut-off may be clinically desired.

The Roche hs-cTnT used in this study has been classified as a high sensitivity assay in recent reviews as it fulfills the two criteria proposed in a scorecard concept [26]. The total imprecision at the 99th percentile value is <10% and more than 50% of healthy individuals have measurable cTnT concentrations below the 99th percentile. The second criteria is not provided in the manufacturer's package insert and it has been based on published literature [19]. However, recent studies have obtained lower rates of measurable levels of hs-cTnT in healthy individuals, as outlined by Apple [18]. In this article, it is also highlighted that the lack of standardization in the definition of a healthy population among studies can lead to different results, as many factors (age, sex, race, ethnicity and underlying cardiovascular disease) are likely to affect findings. Other issues such as use of different types of samples (plasma or serum) and potential use of lots affected by poor recovery could also affect results. This issue will not be resolved until a universal definition for reference population for studies is defined or a universal sample bank for normal range determination is created. Despite the differences of reported measurable levels in healthy population, the current assay is still considered as high-sensitivity in all reports based on the validation study [19].

Although our results in patients with LVEF ≥45% should be considered with caution, it seems that although useful from the prognostic point of view when used in an univariate manner, the capacity of troponins for improving discrimination and reclassification of this subset of HF patients is limited. In this subgroup of patients sc-cTnI is less frequently elevated, but it carries a worse prognosis.

5. Limitations

There is a risk that the measurement of the biomarkers from frozen rather than fresh samples may have affected the absolute levels of hs-cTnT and sc-cTnI. There is not enough information about the long-term stability of frozen high-sensitive cardiac troponins. We have analyzed only one blood sample per patient, and cannot comment on the prognostic value of serial determinations. We recognize that serial sampling may be more useful for patient monitoring. The use of a bootstrap method to determine the cut-off point of hs-cTnT allows us to optimize the prognostic prediction, but complicates any comparison with other analyses that use different cut-off points. Finally, 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 and the percentage of patients with preserved systolic function is underrepresented. As such, the obtained results cannot necessarily be extrapolated to a global HF population.

6. Conclusions

In a real-life cohort of patients with chronic HF, commercially available hs-cTnT was elevated more often than sc-cTnI. Both hs-cTnT and sc-cTnI predict mortality independently to other established risk factors. However, hs-cTnT showed globally better measures of performance and identified a higher proportion of decedents during follow-up.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.cca.2013.08.014.

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Development of a Novel Heart Failure Risk Tool: The Barcelona Bio-Heart Failure Risk Calculator (BCN Bio-HF Calculator)

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Abstract

Background: A combination of clinical and routine laboratory data with biomarkers reflecting different pathophysiological pathways may help to refine risk stratification in heart failure (HF). A novel calculator (BCN Bio-HF calculator) incorporating N-terminal pro B-type natriuretic peptide (NT-proBNP, a marker of myocardial stretch), high-sensitivity cardiac troponin T (hs-cTnT, a marker of myocyte injury), and high-sensitivity soluble ST2 (ST2), (reflective of myocardial fibrosis and remodeling) was developed.

Methods: Model performance was evaluated using discrimination, calibration, and reclassification tools for 1-, 2-, and 3-year mortality. Ten-fold cross-validation with 1000 bootstrapping was used.

Results: The BCN Bio-HF calculator was derived from 864 consecutive outpatients (72% men) with mean age 68.2±12 years (73%/27% New York Heart Association (NYHA) class I-II/III-IV, LVEF 36%, ischemic etiology 52.2%) and followed for a median of 3.4 years (305 deaths). After an initial evaluation of 23 variables, eight independent models were developed. The variables included in these models were age, sex, NYHA functional class, left ventricular ejection fraction, serum sodium, estimated glomerular filtration rate, hemoglobin, loop diuretic dose, β-blocker, Angiotensin converting enzyme inhibitor/Angiotensin-2 receptor blocker and statin treatments, and hs-cTnT, ST2, and NT-proBNP levels. The calculator may run with the availability of none, one, two, or the three biomarkers. The calculated risk of death was significantly changed by additive biomarker data. The average C-statistic in cross-validation analysis was 0.79.

Conclusions: A new HF risk-calculator that incorporates available biomarkers reflecting different pathophysiological pathways better allowed individual prediction of death at 1, 2, and 3 years.

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Competing Interests: For this study ST2 assays were performed by Critical Diagnostics; hs-cTnT and NT-proBNP assays were provided by Roche Diagnostics, which also provided a grant for statistical development and online application of the calculator. Dr. A. Bayes-Genis has received lecture honoraria from Roche Diagnostics and Critical Diagnostics and Dr. J. Lupón from Roche Diagnostics. Dr. A. Bayes-Genis and Dr. J. Lupón have acquired stock shares of Critical Diagnostics. The BCNBioHFCalculator has been registered by J. Lupón and A. Bayes-Genis. There are no further patents, products in development or marketed products to declare. This does not alter the authors' adherence to all the PLOS ONE policies on sharing data and materials, as detailed online in the guide for authors.

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Introduction

Risk stratification of heart failure (HF) is a challenge, and as guidelines acknowledge, new accurate scoring models are needed. Several models have been developed [1–10], of which the Seattle HF model [6] has had the most visibility. Nevertheless, this scoring model is derived from a cohort of patients carefully selected for a randomized clinical trial over 20 years ago, with its inclusion and exclusion criteria. Serum biomarkers for patient risk stratification were not available. However, in recent times a number of biomarkers reflective of different pathophysiological pathways have been identified in HF [11]. Therefore, we developed a calculator for HF stratification that, in addition to classical risk factors, includes N-terminal pro B-type natriuretic peptide (NT-proBNP), a marker of myocardial stretch; high-sensitivity cardiac troponin T (hs-cTnT), a marker of myocyte injury; and high-sensitivity soluble ST2 (ST2), which is reflective of myocardial fibrosis and remodeling.

The Barcelona Bio Heart Failure risk calculator (BCN Bio-HF calculator), which is derived from a real-life cohort of contemporary treated HF patients, is a web-based calculator allowing quick

and easy interactive calculations of individual mortality at 1, 2, and 3 years and life expectancy.

Methods

Derivation Study Population

The study population, samples, and biomarker assays were described elsewhere [12]. In summary ambulatory patients treated at a multidisciplinary HF unit were consecutively included in the study in an outpatient setting. 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. Etiologies of HF were: ischemic heart disease 52.2%, dilated cardiomyopathy 10%, hypertensive 9.4%, alcoholic cardiomyopathy 5.7%, drug-related cardiomyopathy 2.5%, valvular disease 11.4% and others 8.8%.

All participants provided written informed consent, and the local ethics committee approved the study. All study procedures were in accordance with the ethical standards outlined in the Helsinki Declaration of 1975 as revised in 1983. The regular visitation schedule was reported elsewhere [12–15]. Death from all causes was the main outcome. Fatal events were identified from clinical records, family contact or by reviewing the electronic clinical history at the Catalan and Spanish Institute of Health. Physicians and nurses of the HF Unit identified adverse events (JL, M de A, AU, BG, RC, LC).

Model Making

A selection of 23 well-known mortality-related variables from the literature and from previous own studies was first evaluated, and 11 of them were included in eight Cox proportional hazard regression models due to their significance in the multivariate analysis or because considered of clinical significant relevance: one model without biomarkers ('clinical model') and seven additional models with all possible combinations of the three biomarkers.

Proportional assumptions needed to use Cox proportional hazard regression models were tested for all variables. Variables in which the non-linear component achieved significance were transformed according to what the figure of time vs. hazard suggested until non-significance of the non-linear component was achieved, as reported elsewhere [12,14,15]. In summary, to fulfill the assumption of linearity for the co-variables hs-cTnT, ST2, and NT-proBNP, the logarithmic functions of both NT-proBNP and hs-cTnT, the quadratic term of the logarithmic function of hs-cTnT, and the quadratic term of ST2 were used in the Cox proportional hazard regression models. In the 'clinical model', variables were removed one-by-one in a backward manner to assess whether their exclusion significantly reduced the likelihood of the model. When two variables were collinear in predicting outcome, the one with the better likelihood was included. All two-variable interactions were also tested. Some variables were dichotomized (such as New York Heart Association (NYHA) functional class or left ventricular ejection fraction (LVEF) for better performance).

Model Performance

We used different measures of performance to test the potential incremental prognostic value of the three biomarkers as follows:

Discrimination. The ability of the model to discriminate between patients who will have and will not have the event along all follow-up was measured by means of the C-statistic obtained from a generalization of Somers 'Dxy' rank correlation, which equals $2\times(c-1/2)$, where c is the concordance (discrimination) probability [16], which already incorporates information from censored data.

Calibration. How well the observed incidence rate fit the predicted risk was measured by Nam-D'Agostino statistics using the Hosmer and Lemeshow test for censored survival [17]. Calibration using this method was calculated for one-, two- and three-year mortality.

Accuracy. The integrated Brier score for censored observations was used to measure the accuracy of probabilistic predictions [18]. A lower score represents higher accuracy. This score takes values between 0 and 1 and was calculated for one-, two- and three-year mortality.

Best prediction. The Bayesian information criterion (BIC) and the Akaike information criterion (AIC), measures of the relative goodness-of-fit of a statistical model, were used to compare non-nested models. Lower values indicate a better model along all follow-up. Both indicators take into account the events along all follow-up.

Reclassification. We used the method described by Pencina et al. [19]. Integrated discrimination improvement (IDI) considers changes in the estimated mortality prediction probabilities as a continuous variable. 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 $>41\%$. NRI considers changes in the predicted probabilities of estimated mortality that imply a change from one category to another. Reclassification was evaluated for one-, two- and three-year mortality.

Generalization or validation. To assess how the results of the models can be generalized to an independent data set, a 10-fold cross-validation technique was used [20]. Using a bootstrapping technique, we created 1000 samples (allowing repetition) equal in size to the present cohort. One by one, each of the 1000 samples was split into 10 distinct blocks roughly equal in size. We left out the first block (the testing set) and fit a model with the remaining blocks (the training set) to predict the held-out-block. We continued this process until the model predicted all 10 held-out-blocks. The mean C-statistic was calculated and the process repeated for all 1000 samples.

Calculator algorithms

Mortality. The calculator was designed to run with the availability of none, one, two, or three of the chosen biomarkers, using the best model for each available combination. To calculate the probability of developing an event at a specific time, the following formula was applied:

$$\hat{p}(T<t|\vec{x}_i)=1-\hat{S}(t)^{\exp(\vec{\beta}*\vec{x_i})-(\vec{\beta}*\vec{\bar{x}})}$$

Where:

- $\hat{p}(T<t|\vec{x}_i)$ = probability to develop the event at time "T" before time "t" (e.g., 3 years) giving a combination "$\vec{x}_i$" of patient characteristics (i.e., age, sex, etc.);
- $\hat{S}(t)$ = estimated survival at time "t";

Table 1. Demographic, clinical and analytical characteristics of patients.

	NO EVENT	EVENT	Univariate analysis		Multivariate analysis without biomarkers		Multivariate analysis with biomarkers	
	N = 559	N = 305						
			HR_{Cox}	P-value	HR_{Cox}	P-value	HR_{Cox}	P-value
Age, years	64.6 (12.3)	74.4 (9.23)	1.07 [1.06;1.08]	<0.001	1.04 [1.03;1.06]	<0.001	1.04 [1.02;1.06]	<0.001
Female Gender	149 (26.7%)	93 (30.5%)	1.07 [0.84;1.36]	0.590	0.66 [0.5;0.89]	0.005	0.77 [0.57;1.03]	0.078
BMI, Kg/m^2	28.0 (4.95)	27.0 (5.15)	0.96 [0.93;0.98]	<0.001	1.00 [0.97;1.02]	0.724	1.01 [0.98;1.04]	0.695
NYHA III–IV	91 (16.3)	144 (47.2)	3.25 [2.59;4.07]	<0.001	1.87 [1.45;2.42]	<0.001	1.67 [1.29;2.17]	<0.001
LVEF,%	35.6 (13.0)	36.5 (15.0)	1.00 [0.99;1.01]	0.801				
LVEF >45%	122 (21.8%)	76 (24.9%)	0.98 [0.75;1.28]	0.905	0.76 [0.54;1.06]	0.108	0.95 [0.67;1.34]	0.770
Ischemic etiology of HF	286 (51.2%)	165 (54.1%)	1.06 [0.84;1.32]	0.633	1.22 [0.92;1.6]	0.162	1.14 [0.86;1.5]	0.365
Systolic Blood Pressure, mmHg	127 (23.1)	127 (22.9)	1.00 [0.99;1.00]	0.775	1.00 [0.99;1.00]	0.605	1.00 [0.99;1.01]	0.872
Heart rate, bpm	68.9 (13.5)	70.7 (13.7)	1.01 [1.00;1.02]	0.004	1.00 [0.99;1.01]	0.469	0.99 [0.9;1]	0.115
Diabetes mellitus	181 (32.4%)	129 (42.3%)	1.42 [1.14;1.79]	0.002	1.26 [0.98;1.63]	0.070	1.2 [0.93;1.55]	0.166
Atrial fibrillation	82 (14.7%)	63 (20.7%)	1.49 [1.13;1.97]	0.004	1.09 [0.8;1.48]	0.583	0.96 [0.7;1.32]	0.796
Hypertension	327 (58.5%)	200 (65.6%)	1.31 [1.04;1.66]	0.024	0.97 [0.74;1.27]	0.836	0.89 [0.68;1.16]	0.388
Sodium, mmol/L	139 (3.22)	139 (3.79)	0.94 [0.90;0.97]	<0.001	0.94 [0.91;0.97]	<0.001	0.96 [0.93;1]	0.03
COPD	71 (12.7%)	73/23.9%)	1.69 [1.30;2.20]	<0.001	1.11 [0.83;1.5]	0.480	1.08 [0.79;1.47]	0.633
eGFR, ml/min/1.73m2	52.0 (24.2)	35.8 (16.7)	0.97 [0.96;0.97]	<0.001	0.99 [0.98;1]	0.019	1 [0.99;1.01]	0.800
Hemoglobin, g/dl	13.3 (1.72)	12.3 (1.81)	0.77 [0.72;0.82]	<0.001	0.90 [0.84;0.97]	0.008	0.92 [0.85;0.99]	0.035
Iron defficiency	260 (46.8%)	174 (57.2%)	1.42 [1.13;1.79]	0.002	1.06 [0.83;1.36]	0.627	0.98 [0.76;1.26]	0.847
ACEI or ARB	523 (93.6%)	251 (82.6%)	0.35 [0.26;0.47]	<0.001	0.69 [0.49;0.95]	0.024	0.91 [0.63;1.31]	0.601
Beta-Blocker	523 (93.6%)	233 (76.4%)	0.35 [0.27;0.45]	<0.001	0.58 [0.42;0.79]	<0.001	0.61 [0.44;0.84]	0.003
Statins	420 (75.1%)	172 (56.4%)	0.50 [0.40;0.63]	<0.001	0.57 [0.43;0.74]	<0.001	0.61 [0.46;0.81]	<0.001
Loop diuretic dose:								
0 (no loop diuretic)	107 (19.1%)	26 (8.52%)	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Dose 1†	361 (64.6%)	172 (56.4%)	1.81 [1.20;2.73]	0.005	1.27 [0.81;2]	0.291	1.26 [0.69;1.16]	0.384
Dose 2‡	91 (16.3%)	107 (35.1%)	3.73 [2.43;5.73]	<0.001	1.75 [1.07;2.97]	0.025	1.5 [0.9;2.48]	0.119
Spironolactone/eplerenone	225 (40.3%)	115 (37.7%)	1.03 [0.82;1.30]	0.780	1.02 [0.79;1.32]	0.864	0.89 [0.82;1.30]	0.780
CRT	31 (5.55%)	16 (5.25%)	0.84 [0.50;1.38]	0.483	0.92 [0.53;1.59]	0.761	0.85 [0.49;1.47]	0.558
ICD	66 (11.8%)	26 (8.52%)	0.69 [0.46;1.04]	0.074	1 [0.64;1.55]	0.988	0.96 [0.62;1.5]	0.867
Cystatin-C, mg/L	1.09 [0.90;1.39]	1.48 [1.15;1.93]	2.69 [2.21;3.28]	<0.001			1.15 [0.68;1.96]	0.602
Hs-cTnT, ng/L	15.7 [7.90;30.7]	34.1 [20.5;53.6]	11.6 [5.46;24.8]	<0.001			3.79 [1.62;8.85]	0.002
ST2, ng/mL	35.5 [29.4;45.5]	44.7 [33.9;60.0]	1.48 [1.34;1.65]	<0.001			1.23 [1.09;1.38]	<0.001
NTproBNP, ng/L	975 [361;2376]	2215 [935;5193]	1.61 [1.48;1.75]	<0.001			1.13 [0.99;1.29]	0.073

Data are expressed as mean (standard deviation), median [percentiles 25th–75th] or absolute number (percentage).
ACEI = angiotensin-converting enzyme inhibitor; ARB = angiotensin II receptor blocker; BMI = body mass index; COPD: Chronic obstructive pulmonary disease; eGFR = estimated glomerular filtration rate; HF, heart failure; hs-cTnT, high-sensitivity circulating troponin T; ST2 = high-sensitivity soluble ST2; LVEF = left ventricular ejection fraction; NT-proBNP = N-terminal pro-brain natriuretic peptide; NYHA = New York Heart Association. CRT = cardiac resynchronization therapy; ICD = implantable cardiac defibrillator.
Ref. = Reference.
†Loop diuretic dose 1: Furosemide-equivalent dose up to 40 mg/day or Torasemide up to 10 mg/day.
‡Loop diuretic dose 2: Furosemide-equivalent dose >40 mg/day or Torasemide>10 mg/day.
The logarithmic functions of NTproBNP, hs-cTnT and cystatin C, the quadratic term of the logarithmic function of hs-cTnT, ST2 as ST2/10 and the quadratic term of ST2/10 were used in the Cox models. P value for $(ST2/10)^2<0.001$ in the univariate analysis and 0.006 in the multivariate analysis; P value for $\log(\text{hs-cTnT})^2<0.001$ in the univariate analysis and 0.015 in the multivariate analysis.
doi:10.1371/journal.pone.0085466.t001

- $(\overrightarrow{\beta} * \overrightarrow{x_i})$ = the vector of beta coefficients (natural log of the hazard ratio (HR)) multiplied by the vector of patient characteristics;
- $(\overrightarrow{\beta} * \overrightarrow{\bar{x}})$ = the vector of beta coefficients multiplied by the vector of mean covariates;

Life expectancy. To get an estimate of life expectancy we refitted all Proportional Hazard Cox-Regression models in parametric Weibull models [21]. In those models the mean survival, "E(T)", is estimated by:

$$\mathrm{E}(T) = \exp(\hat{\mu} + \hat{\beta}' x)\Gamma(1+\hat{\sigma})$$

where,

$\hat{\mu}$ is the estimated intercept obtained from the model

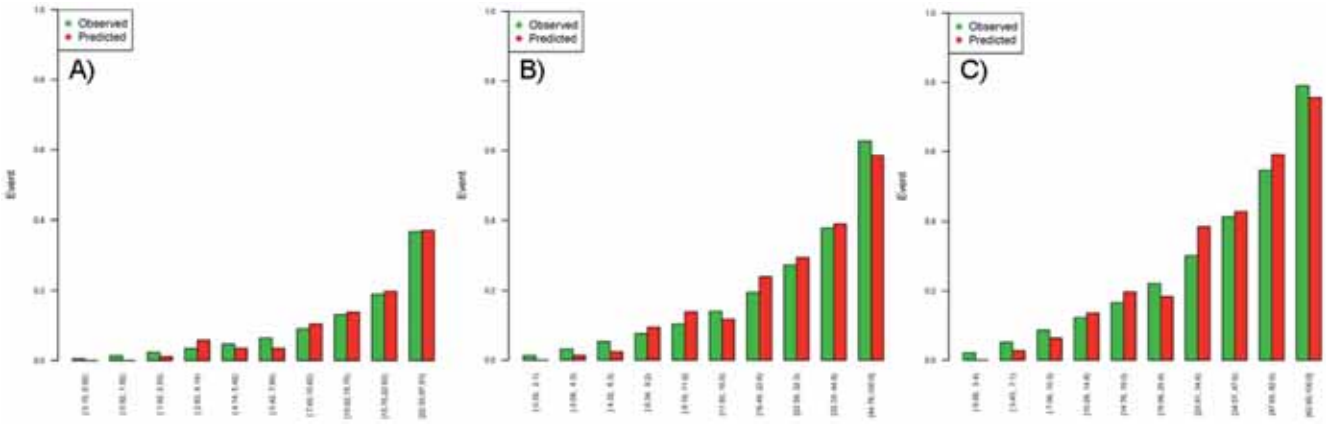

Figure 1. Observed and predicted mortality according to risk deciles (Hosmer and Lemeshow test) at 1-year (A), 2-year (B), and 3-year (C) follow-up for model 8 (with the three biomarkers).
doi:10.1371/journal.pone.0085466.g001

$\hat{\beta}'x$ is the product of the coefficients and patient's characteristics

$\Gamma(1+\hat{\sigma})$ is call the gamma function of the 1 plus the estimated "scale" parameter obtained from the model.

Statistical analyses were performed using R software version 2.15.2. (http://www.R-project.org/).

Results

After an initial evaluation of 23 variables, eight models (one without biomarkers and seven with combinations of the three studied biomarkers) were finally included in the risk calculator tool.

Table 1 provides the evaluated demographic, clinical, and biochemical characteristics of the studied patients with univariate and multivariate Cox regression analysis. During a median follow-up of 3.4 years (25^{th}-75^{th} percentiles 1.8–5.0 years) 305 deaths occurred. The follow-up for alive patients was 4.4 years (25^{th}–75^{th} percentiles 2.7–5.2). The following variables emerged as significant in at least one of the models: age, sex, NYHA functional class, LVEF, estimated glomerular filtration rate (eGFR), serum sodium, hemoglobin, daily loop diuretic dose, beta-blocker, angiotensin converting enzyme inhibitor (ACEI)/angiotensin-2 receptor blocker (ARB), statin treatment and hs-cTnT, ST2, and NTproBNP levels. No pair-wise interaction between variables achieved significance. Variables excluded from the models due to the lack of statistical improvement in the model were: ischemic etiology of HF, diabetes mellitus, body mass index, blood systolic pressure, heart rate, atrial fibrillation, chronic obstructive pulmonary disease, hypertension, iron deficiency, cystatin-C, spironolactone/eplerenone treatment, cardiac resynchronization therapy (CRT), and implantable cardiac defibrillator (ICD).

In the 'clinical model' (Model 1), LVEF (HR 0.69, P = 0.016), eGFR (HR 0.99, P = 0.017), and ACEI/ARB treatment (HR 0.67, P = 0.014) were significant outcome predictors. In the other models, these variables often lost significance after the addition of biomarkers. In Model 8, in which the predictors were adjusted for the three biomarkers, age (HR 1, P<0.001) and NYHA functional class (HR 1.67, P<0.001) remained strong risk factors, whereas female sex (HR 0.75, P = 0.029), statin treatment (HR 0.67, P = 0.001), serum sodium (HR 0.96, P = 0.036), plasma hemoglobin (HR 0.91, P = 0.006), and beta-blocker treatment (HR 0.60, P<0.001) showed a significant protective effect. The three biomarkers exhibited a relationship with mortality, but in model 8, NTproBNP only showed a prognostic trend (Log(hs-TnT): HR 3.38, P = 0.002; ST2/10: HR 1.23, P<0.001; and Log(NT-proBNP): HR 1.11, P = 0.078).

To calculate the probabilities to develop an event at specific time for a particular covariates combination, beta coefficients, survival at the mean of covariates and the sum of the product of coefficients per covariates mean are needed. Survival at the mean of covariates was 94.2% at 1 year, 87.5% at 2 years and 80.2% at 3 years. The remaining values are shown in Table S1 in File S1. When a covariate added no increased prognostic accuracy, it was not included in the risk calculation. An example of calculator functioning is shown in the appendix. Table 2 shows the C-statistic for the 'clinical model' and all of the models containing biomarkers (alone or in combination) in the derivation sample. The model with the three biomarkers had a C-statistic of 0.794 (95% CI 0.77;0.817). Calibration for 1-, 2-, and 3-year mortality was good (non-significant in the Hosmer and Lemeshow test) (Fig. 1).

Reclassification for 1-, 2-, and 3-year mortality was better in the models containing more than one biomarker, with the highest found using the combination of ST2 and hs-cTnT (Model 7; Table 2). The best overall performance was observed with models 7 and 8 (Table 2).

A web-based calculator (Fig. 2) (www.BCNBioHFcalculator.cat) has been developed, allowing interactive calculation of estimated individual probability. A graphic with monthly mortality probabilities is also available. Risk of death was found to be largely influenced by biomarkers' results. As a practical exemple a 68 year-old male in NYHA class III, LVEF 30%, sodium 130 mmol/L, eGFR 45 ml/min/m2, hemoglobin 12 g/dl, taking 60 mg of furosemide and on treatment with statins, ACEI and betablockers had a risk of death of 22%, 42% and 60% at 1,2 and 3 years, respectively. When adding the following biomarker levels: hs-cTnT 14 ng/L, ST2 40 ng/mL and NTproBNP 900 ng/L, the risk fell to 10%, 21% and 32%. However, if biomarker data had been hs-cTnT 70 ng/L, ST2 140 ng/mL, and NTproBNP 2500 ng/L the risk would rise to 35%, 62% and 80%, respectively (Fig. S1).

In the 10-fold cross-validation analysis with 1000 bootstrapping, the average C-statistic for the model with all combined biomarkers was 0.79 (Fig. 3), suggesting that the results may be generalized safely to independent data sets.

Mean (95% confidence interval) life expectancy for the entire cohort was 11.8 years (11.1−12.4) and expected mean age (95% confidence interval) for death was 80.2 years (79.7−80.7) using clinical model; and 11.4 years (10.7−12.0) and 79.8 years

Table 2. Performance of the models.

	Clinical Model	Clinical model + NTproBNP	Clinical model + hs-cTnT	Clinical model + ST2	Clinical model + NTproBNP + ST2	Clinical model + NTproBNP + hs-cTnT	Clinical model + hs-cTnT + ST2	Clinical model + all biomarkers
C-statistic	0.771	0. 777	0.782	0.779	0.785	0.784	0.792	0.793
	(0.744;0.797)	(0.751;0.803)	(0.758;0.807)	(0.754;0.804)	(0.76;0.809)	(0.759 0.809)	(0.768;0.815)	(0.77;0.817)
	Reference	P-value = 0.052	P-value = 0.061	P-value = 0.098	P-value = 0.015	P-value = 0.021	P-value = 0.003	P-value = 0.001
H-L								
1 year	χ^2: 8.3	χ^2: 4.23	χ^2: 5.54	χ^2: 5.34	χ^2: 7.51	χ^2: 4.46	χ^2: 10.6	χ^2: 8.67
	P-value = 0.50	P-value = 0.90	P-value = 0.79	P-value = 0.80	P-value = 0.58	P-value = 0.81	P-value = 0.23	P-value = 0.37
2 years	χ^2: 10.6	χ^2: 6.52	χ^2: 5.82	χ^2:10.03	χ^2:10.89	χ^2: 7.41	χ^2:8.03	χ^2:7.46
	P-value = 0.31	P-value = 0.69	P-value = 0.76	P-value = 0.35	P-value = 0.28	P-value = 0.49	P-value = 0.43	P-value = 0.49
3 years	χ^2:10.4	χ^2: 5.16	χ^2:7.63	χ^2:14.87	χ^2:11.20	χ^2:11.15	χ^2:7.53	χ^2:7.78
	P-value:0.32	P-value:0.82	P-value:0.57	P-value:0.094	P-value:0.26	P-value:0.19	P-value:0.48	P-value:0.46
Brier score								
1 year	0.252	0.251	0.250	0.250	0.249	0.249	0.247	0.247
2 years	0.198	0.195	0.193	0.193	0.192	0.191	0.188	0.188
3 years	0.169	0.166	0.163	0.164	0.161	0.161	0.157	0.157
AIC	3470	3455	3440	3455	3441	3435	3421	3421
BIC	3527	3517	3488	3512	3498	3492	3473	3478
IDI								
1 year		−0.02 [−0.62;0.57]	0.45 [−0.18; 1.08]	0.57 [−0.12;1.26]	0.54 [−0.20;1.28]	0.59 [−0.03; 1.20]	0.97 [0.15;1.80]	0.92 [0.11;1.72]
	Reference	P-value = 0.94	P-value = 0.16	P-value = 0.11	P-value = 0.15	P-value = 0.063	P-value = 0.021	P-value = 0.025
2 years		−0.02 [−0.92;0.88]	1.03 [0.03; 2.04]	0.79 [−0.22; 1.79]	0.85 [−0.27; 1.97]	1.32 [0.32; 2.31]	1.84 [0.56; 3.11]	1.80 [0.56; 3.04]
	Reference	P-value = 0.972	P-value = 0.044	P-value = 0.126	P-value = 0.136	P-value = 0.009	P-value = 0.005	P-value = 0.005
3 years		0.07 [−1.01;1.16],	1.52 [0.27; 2.78]	0.86 [−0.32; 2.04]	1.12 [−0.22; 2.45]	1.95 [0.70; 3.21]	2.55 [0.99; 4.10]	2.57 [1.05; 4.09]
	Reference	P-value = 0.89	P-value = 0.017	P-value = 0.15	P-value = 0.10	P-value = 0.002	P-value = 0.001	P-value = <0.001
NRI								
1 year		2.34 [−4.29;8.97]	7.03 [−0.77;14.84]	3.96 [−2.55;10.47]	11.36[4.09;18.63]	8.47 [0.95;15.99]	10.34[1.92;18.76]	9.58 [1.49;17.66]
	Reference	P-value = 0.489	P-value = 0.077	P-value = 0.233	P-value = 0.002	P-value = 0.027	P-value = 0.016	P-value = 0.02
2 years		2.35 [−4.24;8.95]	6.83 [−1.00;14.66]	3.96 [−2.55;10.47]	10.63[3.27;17.98]	7.96 [0.33;15.59]	10.09[1.64;18.53]	8.80 [0.77;16.82]
	Reference	P-value = 0.48	P-value = 0.087	P-value = 0.23	P-value = 0.005	P-value = 0.041	P-value = 0.019	P-value = 0.032
3 years		2.15 [−4.42;8.73]	7.09 [−0.80;14.97]	3.74 [−2.80;10.28]	10.92[3.63;18.21]	7.96 [0.35;15.57]	9.76 [1.23;18.28]	8.93 [0.95;16.92]
	Reference	P-value = 0.52	P-value = 0.078	P-value = 0.26	P-value = 0.003	P-value = 0.04	P-value = 0.025	P-value = 0.028

χ^2 = Chi-Square.
P values vs Model 1 (Clinical).
AIC = Akaike information criterion; BIC = Bayesian information criterion; HL = Hosmer–Lemeshow test; hs-cTnT, = high-sensitivity circulating troponin T;
ST2 = high-sensitivity soluble ST2; IDI = integrated discrimination improvement; NRI = net reclassification improvement. NT-proBNP = N-terminal pro-brain natriuretic peptide.
Model 1 (Clinical) = Age, Female, NYHA functional class (as I−II vs III−IV), LVEF (as ≥ vs <45%), eGFR (estimated glomerular filtration rate), Sodium,
Hemoglobin, Furosemide-equivalent doses treatment as 0, ≤40 mg/day and >40 mg/day or Torasemide as 0, ≤10 mg/day and >10 mg/day),
ACEI or ARB treatment, beta-blocker treatment, statin treatment.
Model 2 = Model 1+NT-proBNP.
Model 3 = Model 1+hs-cTnT.
Model 4 = Model 1+ST2.
Model 5 = Model 1+NT-proBNP+ST2.
Model 6 = Model 1+NT-proBNP+hs-cTnT.
Model 7 = Model 1+hs-cTNT+ST2.
Model 8 = Model 1+NT-proBNP+hs-cTNT.
doi:10.1371/journal.pone.0085466.t002

Figure 2. The Barcelona Bio-HF calculator has been implemented as an interactive program that employs the eight developed models to estimate 1-, 2-, and 3-year mortality for an individual patient. This tool is available at www.BCNBioHFcalculator.cat.
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(79.3−80.3) with Model 8 (containing the three biomarkers), respectively. Beta-coefficients of the different Weibull models used to calculate life expectancy are shown in Table S2 in File S1.

Discussion

HF risk prediction is a cornerstone of HF management. The development of an accurate HF risk calculator has the potential for tailored management. The BCN Bio-HF calculator reported here was derived from a real-life contemporarily treated consecutive cohort and includes, in addition to conventional prediction factors, three serum biomarkers (NTproBNP, ST2, and hs-cTnT) that are highly accurate for cardiac malfunction.

Mortality risk prediction models specific to the HF population have been developed with broad variation in the degree of validation and concretion of prognostic output, from classification into risk groups (low-high risk, low-medium-high risk, risk deciles) [2−5], to life expectancy [6] or individual mortality at a certain time point [3,5−7,10]. Most of these models have not included a substantial proportion of patients taking evidence-based treatments, including ACEI/ARBs, beta-blockers, and spironolactone/eplerenone, or were developed only for patients admitted to the hospital [8,9]. In the Seattle HF Model, the relative effect for HF medications could not be obtained from the derivation cohort and benefits were estimated from published trials or meta-analyses. In our cohort of ambulatory patients, 87% were on beta-blockers, 90% on ACEI/ARBs, and 40% on spironolactone/eplerenone. Independent of the causality of the risks and benefits of treatments, taking evidence-based HF drugs clearly reduces the risk of death, and they merit inclusion in a risk calculator. In fact, the estimated risk can be very significantly modified by treatment, both in the model without biomarkers and in the model containing biomarkers where treatment can also modify their level and the calculated risk of death would consequently change.

Some scores [4−6] have the advantage of a large derivation cohort. Their limitation is that all of the subjects in their derivation and validation samples were participating in a clinical trial and how well they represent those in routine clinical practice is unknown. In addition, all of the samples were obtained over a decade ago, and none included biomarker testing. The very recent 3C-HF score [7] did include contemporary treatment but not biomarkers in a mixed population of in- and outpatients included both prospectively and retrospectively. Also the even more recent MAGGIC score, derived from a metaanalysis of 30 studies [10]

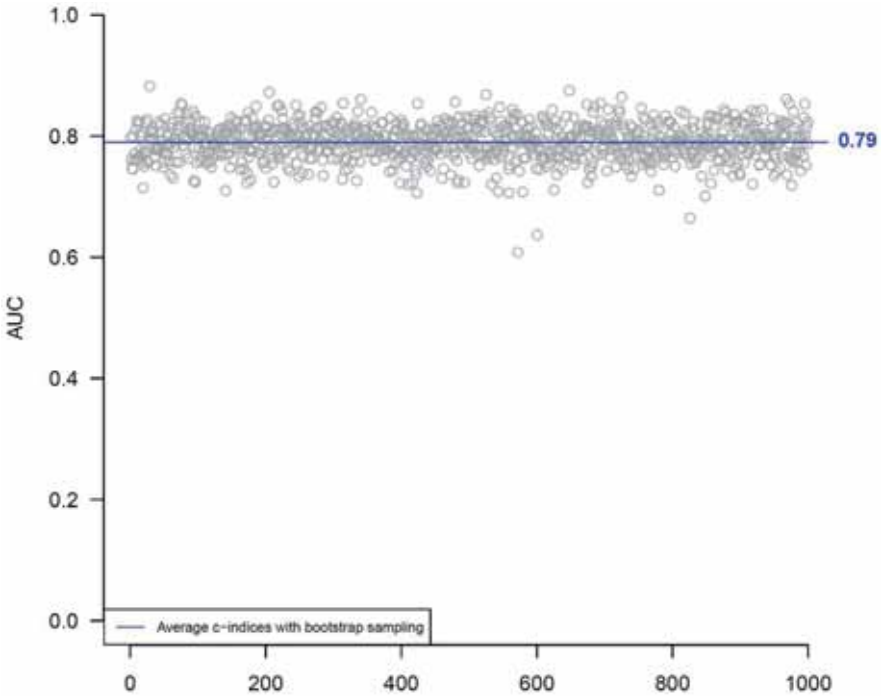

Figure 3. Ten-fold cross-validation with 1000 bootstrapping.
doi:10.1371/journal.pone.0085466.g003

did not include biomarkers. In the discussion, however, the authors of this score state: "Any new risk score's success depends on the patient variables available for inclusion. Current knowledge of biomarkers in HF is inevitably ahead of what data are available across multiple cohort studies… but could not be included in our model. In principle, its inclusion would enhance further the excellent prognostic discrimination we achieved with routinely collected longestablished predictors." The inclusion of B-type natriuretic peptide in the Valsartan-Heart Failure Trial to the Seattle HF Model increased AUC by ≈ 0.03 [6]. In the BCN Bio-HF Calculator, we included three commercially available complementary biomarkers that provide information about myocyte necrosis (hs-cTnT), fibrosis, remodeling, and inflammation (ST2), and chamber strain (NTproBNP). Other biomarkers are in the pipeline for the HF field, but some of them are not yet commercially available (i.e. growth differential factor-15) and others reflect pathways that overlap those used here. We and others previously reported on the prognostic utility of these three biomarkers [12,14,15]. This calculator was developed with eight models that include none, one, two, or the three biomarkers, allowing its use with any combination of biomarkers. This characteristic is unique to this new tool, which in combination with the use of state-of-the-art statistics for biomarker values, which include C-statistic, as well as calibration and reclassification, makes it more robust.

The Seattle HF Model [6] is probably the most extensively used model. It was prospectively validated in several trials. The validation AUC varied from 0.68 to 0.81 in these diverse populations, with an overall AUC of 0.73 and an AUC of less than 0.70 in the three biggest cohorts [6]. AUC from other studies rank from 0.75 (CHARM two-year mortality [4]) to 0.83 in the validation cohort of the 3C-HF score (one-year mortality with logistic regression analysis [5]). The use of Somers 'Dxy' rank correlation in the C-statistic analysis, which already incorporates information from censored data, is more correct from the survival point of view rather than determination of C-statistic for death at one fixed point with logistic regression models. The C-statistic analysis using logistic regression model in our population was 0.82 for 1-year, 0.82 for 2-year and 0.83 for 3-year mortality. We evaluated both the Seattle HF Model and the 3C-HF score in our population. Taking into account the inherent limitations (default values of Seattle HF for percentage of lymphocytes as well as "diabetes" instead of "diabetes with organ damage" for 3C-HF) the C-statistic using Somers 'Dxy' rank correlation in such models were 0.71 (95% CI 0.678-0.79) for the Seattle HF model and 0.73 (95% CI 0.68-0.73) for the 3C-HF score.

The validation obtained in our 10-fold cross-validation analysis with 1000 bootstrapping was substantially higher, averaging 0.79. Cross-validation is useful, especially when additional samples are hazardous, costly, or impossible to collect. The resulting average accuracy is likely somewhat of an underestimate for the true accuracy when the model is trained on all data and tested on external data (the optimal way for validation), but in most cases this estimate is reliable, particularly if the amount of available data is sufficiently large and the external data follows the same distribution as the available data [22]. Both the Seattle HF Model and the BCN Bio-HF calculator provide the individual risk of death at several points of time without the necessity of a physician calculating the score as an intermediate step. Also, as an added value to other scores, both allow predicting life expectancy, although using different statistical methods.

A number of the clinical variables in our calculator are also included in the Seattle HF Model, though the former has fewer variables. Some variables that may be considered clinically important, such as devices, aldosterone blockers, and systolic blood pressure, were excluded from our model due to the absence of significance in the multivariable model. In the case of devices, particularly ICD and CRT, the lack of significance could be influenced by the limited number of patients with such devices. Remarkably other variables such as blood pressure, ischemic

etiology and diabetes did not achieve statistical significance in the multivariate analysis and did not improve the model prediction of risk, and were not included in the calculator.

Recently, Ky et al.[23] showed that adding a more complex biomarker panel consisting of high-sensitivity C-reactive protein, myeloperoxidase, B-type natriuretic peptide, soluble fms-like tyrosine kinase receptor-1, troponin I, ST2, creatinine, and uric acid to the Seattle HF Model improves the predictive accuracy for 1-year all-cause death, with a C-statistic up to 0.8. In contrast, both of our clinical and biomarker additive models were less complex but performed similarly. Choosing the panel of biomarkers to deploy in clinical practice will depend on factors such as cost and ease of assay, among others.

Limitations

Our population was a general HF population treated at a HF unit in a tertiary hospital. Most patients were white and referred from the cardiology department and, thus, relatively young men with HF of ischemic etiology and reduced LVEF. As such, risk prediction is more accurate in these patients. The risk calculator is based on ambulatory patients with chronic HF and may require extensive adjustments when applied to an inpatient population, some of whom have acute decompensated HF. Absence of external validation represents an acknowledged limitation, although we overcame it using 10-fold cross-validation analysis with 1000 bootstrapping as already discussed.

Conclusion

We developed a new HF risk calculator that incorporates available biomarkers reflecting different pathophysiological pathways and allows quick and easy prediction of death at 1, 2, and 3 years.

Supporting Information

Figure S1 Graphic with monthly mortality probabilities in the same patient (68 year- old male in NYHA class III, LVEF 30%, sodium 130 mmol/L, eGFR 45 ml/min/m2, hemoglobin 12 g/dl, taking 60 mg of furosemide and on treatment with statins, ACEI and betablockers) according to model without biomarkers (continuous line) and with biomarkers (dashed line). Left panel biomarker values: hs-cTnT 14 ng/L and ST2 40 ng/mL and NTproBNP of 900 ng/L. Righ panel biomarker values: hs-cTnT 70 ng/L, ST2 140 ng/mL and NTproBNP 2500 ng/L.
(TIF)

File S1 Supporting tables.
(DOC)

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Author Contributions

Conceived and designed the experiments: JL AB. Performed the experiments: MdA JL AU AG EZ. Analyzed the data: JL AB JV JP. Contributed reagents/materials/analysis tools: AG JL AB JV JP. Wrote the paper: AB JL. Final approval of the version to be published: JL MdA JV JP AG EZ AU AB.

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Head-to-Head Comparison of 2 Myocardial Fibrosis Biomarkers for Long-Term Heart Failure Risk Stratification

ST2 Versus Galectin-3

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Objectives ST2 and galectin-3 (Gal-3) were compared head-to-head for long-term risk stratification in an ambulatory heart failure (HF) population on top of other risk factors including N-terminal pro–B-type natriuretic peptide.

Background ST2 and Gal-3 are promising biomarkers of myocardial fibrosis and remodeling in HF.

Methods This cohort study included 876 patients (median age: 70 years, median left ventricular ejection fraction: 34%). The 2 biomarkers were evaluated relative to conventional assessment (11 risk factors) plus N-terminal pro–B-type natriuretic peptide in terms of discrimination, calibration, and reclassification analysis. Endpoints were 5-year all-cause and cardiovascular mortality, and the combined all-cause death/HF hospitalization.

Results During a median follow-up of 4.2 years (5.9 for alive patients), 392 patients died. In bivariate analysis, Gal-3 and ST2 were independent variables for all endpoints. In multivariate analysis, only ST2 remained independently associated with cardiovascular mortality (hazard ratio: 1.27, 95% confidence interval [CI]: 1.05 to 1.53, $p = 0.014$). Incorporation of ST2 into a full-adjusted model for all-cause mortality (including clinical variables and N-terminal pro–B-type natriuretic peptide) improved discrimination (C-statistic: 0.77, $p = 0.004$) and calibration, and reclassified significantly better (integrated discrimination improvement: 1.5, 95% CI: 0.5 to 2.5, $p = 0.003$; net reclassification index: 9.4, 95% CI: 4.8 to 14.1, $p < 0.001$). Incorporation of Gal-3 showed no significant increase in discrimination or reclassification and worse calibration metrics. On direct model comparison, ST2 was superior to Gal-3.

Conclusions Head-to-head comparison of fibrosis biomarkers ST2 and Gal-3 in chronic HF revealed superiority of ST2 over Gal-3 in risk stratification. The incremental predictive contribution of Gal-3 to existing clinical risk factors was trivial. (J Am Coll Cardiol 2014;63:158–66) © 2014 by the American College of Cardiology Foundation

Heart failure (HF), a major epidemic in Western countries, is characterized by ventricular remodeling and variable degrees of myocardial fibrosis (1,2). The prognosis of HF patients, despite contemporary evidence-based treatment, remains poor (3). There is a need to refine the variables clinicians use to correctly classify patients at risk of developing adverse events. Assessment based on signs and symptoms together with echocardiography is valuable but insufficient, and some circulating biomarkers have been identified and developed for routine use. Among these are natriuretic peptides, which provide information about myocardial stretch, and are already acknowledged in HF guidelines (4–6). Novel biomarkers reflective of other pathophysiological pathways, such as ventricular remodeling and fibrosis, are promising, but their contribution must go beyond information available from conventional assessment, which already includes natriuretic peptides.

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Two such biomarkers are commercially available and approved by the U.S. Food and Drug Administration: soluble ST2 and galectin-3 (Gal-3). ST2 is a member of the interleukin-1 receptor family and exists in 2 forms, a transmembrane receptor (ST2L) as well as a soluble decoy receptor (ST2) (7). The ligand of ST2L is interleukin-33, which is involved in reducing fibrosis and hypertrophy in mechanically strained tissues. In in vitro and in vivo models, ST2L transduces the effects of interleukin-33, whereas excess soluble ST2 leads to cardiac fibrosis and ventricular dysfunction (8–10). Gal-3 is a soluble beta-galactosidase–binding glycoprotein released by activated cardiac macrophages (11,12). Released Gal-3 in the myocardium, via a paracrine effect, stimulates proliferation of myofibroblasts and procollagen 1 deposition (13). Both ST2 and Gal-3 are reflective of fibrosis and cardiac remodeling, key in HF pathophysiology, and strongly related to outcomes (14,15). A comparative prognostic analysis of both biomarkers using state-of-the-art statistics currently recommended for biomarker implementation has not been done. Accordingly, we performed a head-to-head evaluation of ST2 and Gal-3 in a large real-life cohort with a long-term follow-up. The value of the 2 biomarkers over conventional assessment was measured in terms of iscrimination, calibration, and reclassification analysis.

Methods

Study population. From May 2006 to July 2010, ambulatory patients treated at a multidisciplinary HF unit were consecutively included in the study in an outpatient setting, as previously reported (16). In summary, 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 1 HF hospitalization, or a reduced left ventricular ejection fraction (LVEF).

Blood samples were obtained by venipuncture between 9:00 AM and 12:00 AM during conventional ambulatory visits. After adequate centrifugation, the serum samples were stored at −80°C. ST2, Gal-3, and N-terminal pro–B-type natriuretic peptide (NT-proBNP) 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 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, need for up-titration, or other circumstances that necessitated closer follow-up. 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 (14,16). Those who did not attend the regular visit were contacted by telephone.

A death was considered to be from cardiovascular origin if it was caused by: HF (decompensated HF or treatment-resistant HF, in the absence of another cause); sudden death (unexpected death, witnessed or not, of a previously stable patient with no evidence of worsening HF or any other cause of death); acute myocardial infarction (directly related in time with acute myocardial infarction, whether due to mechanic, hemodynamic, or arrhythmic complications); stroke (associated with recently appearing acute neurologic deficit); procedural (post-diagnostic or post-therapeutic procedure death); and other cardiovascular causes (e.g., rupture of an aneurysm, peripheral ischemia, or aortic dissection).

Abbreviations and Acronyms

- **AIC** = Akaike information criterion
- **BIC** = Bayesian information criterion
- **CI** = confidence interval
- **Gal-3** = galectin-3
- **HF** = heart failure
- **HR** = hazard ratio
- **IDI** = integrated discrimination improvement
- **IQR** = interquartile range
- **LVEF** = left ventricular ejection fraction
- **NRI** = net reclassification improvement
- **NT-proBNP** = N-terminal pro–B-type natriuretic peptide
- **ST2** = high-sensitivity soluble ST2

Five-year all-cause and cardiovascular death and the combined all-cause death or HF hospitalization were the primary endpoints. Fatal events were identified from clinical records or by reviewing the electronic clinical history of the Catalan and Spanish Health databases.

ST2 assay. Soluble ST2 was measured from banked plasma samples using a high-sensitivity sandwich monoclonal immunoassay (Presage ST2 assay, Critical Diagnostics, San Diego, California). 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 complementary deoxyribonucleic acid clone for the complete soluble ST2 sequence (17). The ST2 assay had a within-run coefficient of <2.5%, a total coefficient of variation of 4%, and a limit of detection of 1.31 ng/ml.

Gal-3 assay. For Gal-3 measurement, we used an enzyme-linked fluorescent assay (BioMerieux ref. 411191) on a mini-VIDAS analyzer (BioMerieux, France). The coefficient of variation for the assay was <10%, the linearity 3.3 to 100.0 ng/ml, and the limit of detection 2.4 ng/ml.

NT-proBNP assay. NT-proBNP levels were determined using an immuno-electrochemiluminescence assay on the Modular Analytics E 170 (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% (18).

Statistical analysis. Categorical variables were expressed as percentages. Continuous variables were expressed as the mean ± SD or median (interquartile range) according to normal or skewed distribution. Survival analyses were

performed using Cox regression models. To fulfill the assumption of linearity of the covariables Gal-3, ST2, and NT-proBNP, the logarithmic function of Gal-3 and NT-proBNP, and ST2 plus the quadratic term of ST2 were used in the Cox models. Online Figure 1 shows the smoothing spline estimates for 5-year all-cause death for Gal-3 and ST2 nontransformed levels. ST2 analyses were performed per every 10 ng/ml change. The following variables were incorporated into the reference model: age; sex; LVEF (%); estimated glomerular filtration rate (ml/min/1.73 m^2); New York Heart Association functional class; presence of diabetes mellitus; ischemic etiology; hemoglobin (g/dl); serum sodium (mmol/l); beta-blocker treatment; angiotensin-converting enzyme inhibitor or angiotensin II receptor blocker treatment; and NT-proBNP level. Gal-3 and ST2 were subsequently added to this model. Log-rank tests for Kaplan-Meier survival curves were performed using Gal-3 and ST2 quartiles.

We used different measurements of performance to test the potential incremental prognostic value of these biomarkers, as follows.

DISCRIMINATION. C-statistics summarize the diagnostic discrimination. Discrimination refers to a model's ability to correctly distinguish 2 classes of outcomes. We used the index of rank correlation, Somers D, which already incorporates information of censored data. C-statistics between models were compared using the Mann-Whitney *U* test for equality concordance.

CALIBRATION. 1) The D'Agostino-Nam version of the Hosmer-Lemeshow calibration test was used to calculate a c^2 value. 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 results). 2) The Bayesian information criterion (BIC), the Akaike information criterion (AIC), and the Brier score were calculated for each model. The AIC and BIC are measures of the relative goodness of fit of a statistical model. The BIC penalizes free parameters more strongly than does the AIC. No statistical tests compare different BIC, AIC, or Brier score estimations, and 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.

RECLASSIFICATION. We used the method described by Pencina et al. (19). There are 2 main statistics to assess reclassification. Integrated discrimination improvement (IDI) considers the changes in the estimated mortality prediction probabilities as a continuous variable. Net reclassification improvement (NRI) requires a previous definition of meaningful risk categories (we used tertiles for the risk of death: <13.9%, 13.9% to 30.2%, and >30.2%). NRI considers changes in the estimated mortality prediction probabilities that imply a change from 1 category to another.

Values of $p < 0.05$ from 2-sided tests were considered to indicate statistical significance. The analyses were performed using the software R statistical package (version 2.11.1, Foundation for Statistical Computing, Vienna, Austria).

Role of the funding source. Funding sources did not have a role in study design; collection, analysis, and interpretation of data; writing of the report; or the decision to submit the paper for publication.

Results

Of the 891 consecutive patients included from May 2006 to July 2010, Gal-3 and ST2 were available for 876, the final number included in this analysis. Median age was 70.2 years (interquartile range [IQR]: 60.5 to 77.2 years). Table 1 shows the baseline characteristics of the entire sample. The median follow-up time was 4.2 years (IQR: 2.6 to 6.4 years), during which 392 patients died. Follow-up for alive patients was 5.9 years (IQR: 4.1 to 6.7 years). A total of 453 HF hospitalizations were registered from 198 patients.

Table 1 Demographic and Clinical Baseline Characteristics and Treatments During Follow-Up (N = 876)

Age, yrs	70.2 (60.5–77.2)
Female	249 (28.4)
Etiology	
Ischemic heart disease	457 (52.2)
Dilated cardiomyopathy	85 (9.7)
Hypertensive	81 (9. 2)
Valvular	103 (11.8)
Other	150 (17.1)
LVEF,%	34 (26–43)
eGFR, ml/min/1.73 m^2	43.2 (29.7–59.8)
Sodium, mmol/l	139 (137–142)
Hemoglobin, g/dl	12.9 ± 1.8
NYHA functional class	
I	64 (7.3)
II	576 (65.8)
III	227 (25.9)
IV	9 (1.0)
Hypertension	534 (61.0)
Diabetes mellitus	315 (36.0)
Treatments (follow-up)	
ACEI or ARB	786 (89.7)
Beta-blocker	767 (87.6)
Spironolactone/eplerenone	342 (39.0)
Loop diuretic	742 (84.7)
Digoxin	265 (30.3)
CRT	47 (5.4)
ICD	92 (10.5)
NT-proBNP, ng/l	1,398 (529–3,016)
Galectin 3, ng/ml	16.5 (12.6–22.7)
ST2, ng/ml	38.2 (30.8–50.9)

Values are median (IQR), n (%), or mean ± SD.

ACEI = angiotensin-converting enzyme inhibitor; ARB = angiotensin II receptor blocker; CRT = cardiac resynchronization therapy; eGFR = estimated glomerular filtration rate; ICD = implantable cardioverter-defibrillator; IQR = interquartile range; LVEF = left ventricular ejection fraction; NT-proBNP = N-terminal pro–B-type natriuretic peptide; NYHA = New York Heart Association; ST2 = high-sensitivity soluble ST2.

Among cardiovascular causes of death (n = 221), refractory HF was responsible in 113 (51.1%) patients, sudden death in 45 (20.4%) patients, and acute myocardial infarction in 20 (9%) patients. Five patients were lost to follow-up and adequately censored.

Cox regression and survival. In the bivariate analysis, both biomarkers were predictors of death from all cause as continuous variables (log(Gal-3) hazard ratio [HR]: 2.69, 95% confidence interval [CI]: 2.22 to 3.27, $p < 0.001$; ST2 HR: 1.45, 95% CI: 1.32 to 1.59, $p < 0.001$) and significantly predicted cardiovascular death (log(Gal-3) HR: 2.74, 95% CI: 2.12 to 3.54, $p < 0.001$; and ST2 HR: 1.55, 95% CI: 1.31 to 1.84, $p < 0.001$). For interpretation of these HR, Gal-3 values were normalized by log transformation, whereas ST2 was normalized by adding its quadratic transformation to nontransformed ST2 levels, and ST2 analyses were performed per every 10 ng/ml change (Online Appendix). Figure 1 shows Kaplan-Meier survival curves according to Gal-3 (Fig. 1A) and ST2 (Fig. 1B) quartiles. No interaction was found between mineralocorticoid antagonists and ST2 ($p = 0.778$) or Gal-3 ($p = 0.339$).

In multivariable analysis, log(Gal-3) was independently associated only with all-cause but not with cardiovascular death (Table 2), whereas ST2 remained strongly and independently associated with both all-cause and cardiovascular death (Table 2). When high-sensitivity cardiac troponin T was included in the multivariable analysis, log(Gal-3) lost the statistical significance even for all-cause death (Online Table 1).

Both biomarkers remained independently associated with the combined endpoint (all-cause death or HF hospitalization): log(Gal-3) HR: 1.39, 95% CI: 1.06 to 1.83, $p = 0.017$; and ST2 HR: 1.18, 95% CI: 1.08 to 1.29, $p < 0.001$. When high-sensitivity cardiac troponin T was included in the multivariable analysis, log(Gal-3) lost the statistical significance (HR: 1.28, 95% CI: 0.96 to 1.70, $p = 0.088$), whereas ST2 remained statistically associated with this combined endpoint (HR: 1.19, 95% CI: 1.08 to 1.32, $p < 0.001$). Figure 2 shows Kaplan-Meier curves for the combined endpoint according to Gal-3 (Fig. 2A) and ST2 (Fig. 2B) quartiles.

Performance metrics in risk prediction models.

DISCRIMINATION. C-statistics for the prediction of all-cause death and cardiovascular death significantly increased when ST2 was incorporated into the reference model with established mortality risk factors and NT-proBNP. It did not increase for either endpoint when Gal-3 was the added biomarker (Tables 3 and 4).

The same occurred for the combined end-point (all-cause death or HF hospitalization): C-statistic 0.735 [0.711 to 0.759] for reference model, 0.742 [0. 719 to 0.765], $p = 0.033$ for the ST2 model, and 0.737 [0.713 to 0.761], $p = 0.332$ for the Gal-3 model.

CALIBRATION. The p values for the Hosmer-Lemeshow statistics indicated good calibration for all the models except for the model containing Gal-3 for all-cause mortality ($p = 0.049$). Brier scores, AIC, and BIC were lower in the models that included ST2, both for all-cause mortality (Table 3) and for cardiovascular death (Table 4). Global goodness of fit was better in models including ST2 than in the model with only established mortality risk factors, as evaluated by likelihood ratio tests for both all-cause ($p < 0.001$) (Table 3) and cardiovascular death ($p = 0.007$) (Table 4). The likelihood ratio for models including Gal-3 was nonsignificant for cardiovascular mortality ($p = 0.127$) (Table 4).

RECLASSIFICATION. IDI (risk as a continuous variable) increased significantly with the addition of ST2 to the reference model, both for all-cause (IDI: 1.5, $p = 0.003$) (Table 3) and cardiovascular death (IDI: 1.3, $p = 0.004$) (Table 4), but not with the addition of Gal-3 in any case (Tables 3 and 4). NRI (reclassification according to predefined risk categories) for all-cause death improved only after inclusion of ST2 into the full-adjusted model

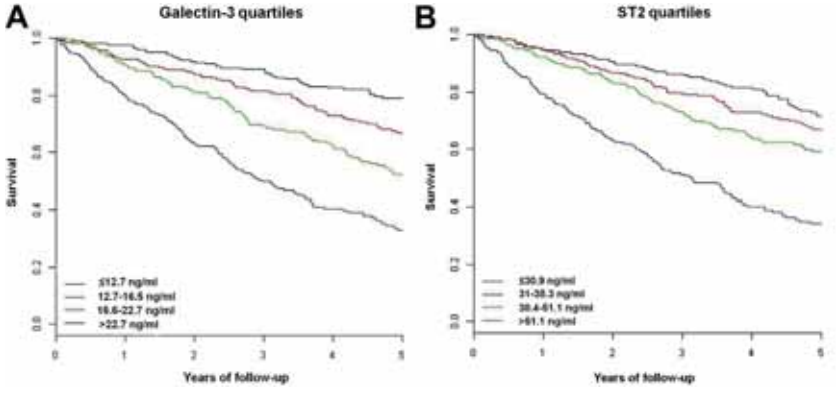

Figure 1 Kaplan-Meier Survival Curves According to Gal-3 and ST2

(A) Survival according to galectin-3 (Gal-3) quartiles. **(B)** Survival according to ST2 quartiles.

Table 2 Multivariate Cox Regression Analyses for All-Cause and Cardiovascular Mortality at 5 Years

	All-Cause Death						Cardiovascular Death					
	Gal-3			ST2			Gal-3			ST2		
	HR	95% CI	p Value	HR	95% CI	p Value	HR	95% CI	p Value	HR	95% CI	p Value
Age, yrs	1.04	1.03–1.06	<0.001	1.04	1.03–1.05	<0.001	1.04	1.03–1.06	<0.001	1.04	1.02–1.06	<0.001
Female	0.67	0.52–0.87	0.003	0.74	0.57–0.96	0.026	0.65	0.46–0.94	0.021	0.70	0.48–1.00	0.052
NYHA functional class	1.70	1.34–2.15	<0.001	1.64	1.29–2.08	<0.001	1.78	1.30–2.40	<0.001	1.83	1.34–2.50	<0.001
LVEF	1.00	1.00–1.01	0.320	1.01	1.00–1.01	0.316	1.01	1.00–1.02	0.229	1.01	1.00–1.02	0.199
Ischemic etiology of HF	1.12	0.88–1.41	0.357	1.14	0.9–1.45	0.265	1.34	0.97–1.84	0.074	1.32	0.96–1.81	0.092
Diabetes mellitus	1.17	0.93–1.46	0.179	1.16	0.93–1.45	0.187	1.28	0.95–1.7	0.102	1.25	0.93–1.69	0.143
eGFR, ml/min/1.73 m^2	1.00	1.00–1.01	0.927	1.00	0.99–1.00	0.245	1.00	0.99–1.01	0.650	1.00	0.99–1.01	0.464
Na, mmol/l	0.97	0.94–1.00	0.065	0.98	0.95–1.01	0.224	0.93	0.90–0.97	0.001	0.93	0.89–0.97	<0.001
Hb, g/dl	0.93	0.87–1.00	0.049	0.92	0.86–0.98	0.014	1.02	0.93–1.12	0.692	1.00	0.92–1.10	0.942
ACEI or ARB treatment	0.81	0.59–1.11	0.181	0.88	0.63–1.22	0.444	0.78	0.51–1.18	0.243	0.72	0.47–1.10	0.133
Beta-blocker treatment	0.52	0.39–0.69	<0.001	0.53	0.40–0.72	<0.001	0.48	0.32–0.72	<0.001	0.49	0.33–0.72	<0.001
Log(NT-proBNP)	1.12	1.15–1.41	<0.001	1.23	1.10–1.36	<0.001	1.29	1.13–1.48	<0.001	1.29	1.12–1.48	<0.001
Log(Gal-3)	1.37	1.03–1.83	0.032	—	—	—	1.35	0.92–1.98	0.127	—	—	—
ST2	—	—	—	1.23	1.12–1.36	<0.001	—	—	—	1.27	1.05–1.53	0.014

New York Heart Association as functional classes III to IV. The logarithmic functions of NT-proBNP and Gal-3 and the quadratic term of ST2 were used in the Cox models. ST2 per every 10 ng/ml change. The p value for $ST2^2$ = 0.001 for all-cause mortality and 0.024 for cardiovascular mortality. Dashes indicate non applicable.

CI = confidence interval; Gal-3 = galectin-3; Hb = hemoglobin; HF = heart failure; HR = hazard ratio; other abbreviations as in Table 1.

(ST2 NRI: 9.4, $p < 0.001$; Gal-3 NRI: 0.7, $p = 0.649$) (Table 3). For cardiovascular death, NRI improved mainly for alive patients when ST2 was added to the reference model (NRI alive: 4.6, $p < 0.001$). Gal-3 did not improve but worsened the NRI for deceased patients (NRI deceased: −4.2, $p = 0.047$) (Table 4). Direct comparison of ST2 and Gal-3 models revealed that ST2 significantly improved reclassification over Gal-3 (Table 5).

Again, when we considered the combined endpoint (all-cause death or HF hospitalization), Gal-3 did not improve reclassification (IDI: 0.3, 95% CI: −0.1 to 0.8, $p = 0.157$; NRI: 0.6, 95% CI: −3.1 to 4.3, $p = 0.739$), whereas ST2 significantly improved both reclassification metrics (IDI: 1.2, 95% CI: 0.4 to 1.9, $p = 0.002$; NRI: 5.4, 95% CI: 0.7 to 10.2, $p = 0.024$).

The addition of high-sensitivity cardiac troponin T in the baseline model did not change the significant value of ST2 in discrimination and reclassification metrics (Online Tables 2 to 4).

Discussion

This study highlights the importance of assessing the true value of emerging cardiac fibrosis biomarkers above and beyond clinical risk factors and natriuretic peptides particularly in light of the newly obtained ST2 and Gal-3 American College of Cardiology/American Heart Association class II recommendation for determination of prognosis in chronic HF (20). ST2 and Gal-3 were directly compared, and our findings demonstrate that: 1) both ST2 and Gal-3 were associated with an increased risk of all-cause mortality, but only ST2 with cardiovascular mortality; and 2) ST2 significantly refined discrimination and reclassification analysis, whereas Gal-3 had negligible effects on performance metrics in risk-prediction models.

The independent prognostic value of ST2 and Gal-3 was examined on top of 11 classical risk factors (age, sex, New York Heart Association functional class, estimated glomerular filtration rate, LVEF, diabetes mellitus, sodium, hemoglobin, ischemic etiology of HF, angiotensin-converting enzyme inhibitor/angiotensin II receptor blocker treatment, and beta-blocker treatment) plus NT-proBNP. Previously, results with Gal-3 concerning outcome prediction have been conflicting whenever natriuretic peptides are incorporated into the analysis. In a short series of 232 patients, Lok et al. (21) found that Gal-3 was a significant predictor of mortality even after adjusting for NT-proBNP. By contrast, Felker et al. (22) and Gullestad et al. (23), in large series of ambulatory HF patients with long-term follow-up, found that Gal-3 was significantly predictive of long-term outcomes only in univariate analysis; this association did not persist after adjustment for other predictors, especially NT-proBNP. On the side of ST2, in all studied cohorts with or without additional biomarkers, including natriuretic peptides, ST2 unambiguously emerged as a cardinal HF risk stratifier (14,24–29). In the current study, the 2 biomarkers remained

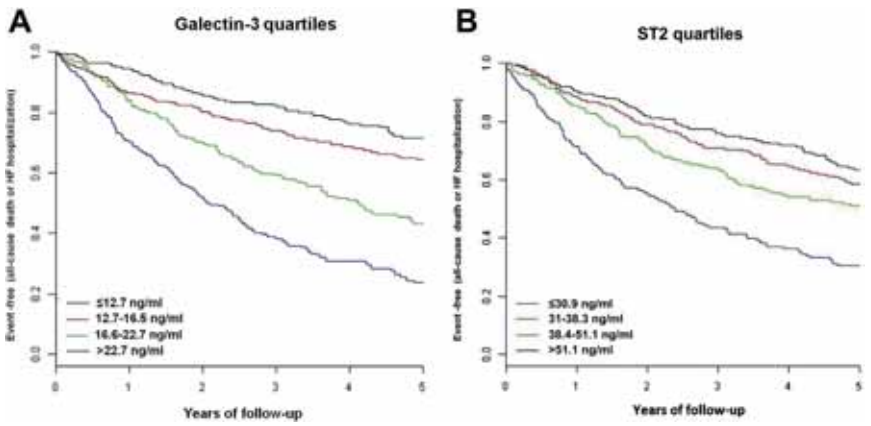

Figure 2 Kaplan-Meier Curves for the Combined Endpoint (All-Cause Mortality or HF Hospitalization) According to Gal-3 and ST2

(A) Event-free survival according to galectin-3 (Gal-3) quartiles. **(B)** Event-free survival according to ST2 quartiles. HF= heart failure.

as independent variables for all-cause mortality, but only ST2 was retained in the subgroup of cardiovascular mortality. Our data indicates that every 10 ng/ml increase in ST2 is associated to ~20% increase in risk.

The additional prognostic information gained by any biomarker over an established risk model needs to be determined using adequate statistical tools (30). At present, a major problem in selecting a biomarker is the proportional increase in economic burden, so any addition should be justified by adequate discrimination, calibration, and reclassification analyses (31). First, value of Gal-3 and ST2 on discrimination metrics: Gal-3 did not significantly increase discrimination (as assessed by the C-statistic) of the reference model. By contrast, incorporation of ST2 into a fully adjusted model significantly improved the C-statistic, which significantly rose up to 0.770 (p value relative to reference model, 0.004). Second, calibration of the models: The full set of calibration analyses used in this study to confirm correspondence of predicted and observed values indicated that overall, the model with ST2 is more accurate. In all models, the Hosmer-Lemeshow test was expected to be nonsignificant; yet, the model that incorporated Gal-3 was significant for all-cause mortality. The Brier score measures the average squared deviation between predicted probabilities for a set of events and their outcomes, so a lower score represents higher accuracy. Given any 2 estimated models, the model with the lower BIC, AIC, and Brier scores is preferred. In this study, the Brier score, the AIC, and the BIC were lower in the ST2 model. Third, value of the studied biomarkers on reclassification metrics: The model with ST2 significantly increased IDI and NRI for all-cause and cardiovascular mortality. Gal-3 had negligible or even deleterious effects on reclassification. Indeed, Gal-3 NRI for cardiovascular mortality reached significance in the opposite direction with a value of −4.2, which is indicative of worsening patient reclassification. Together, these main findings suggest that the pathways identified by ST2 profoundly affect risk stratification in the context of chronic HF and that the incremental predictive value of adding Gal-3 to existing clinical risk factors, particularly above and beyond NT-proBNP, is marginal.

Fibrosis is a fundamental component of the adverse structural remodeling of myocardium present in the failing heart (32). Replacement fibrosis appears at sites of previous cardiomyocyte necrosis to preserve the structural integrity of the myocardium, but not without adverse functional consequences. Increased stress or injury to the myocardium due to acute myocardial infarction, uncontrolled hypertension, and other forms of myocyte damage can contribute to fibrosis and cardiac remodeling. Responses to acute and chronic damage can involve recruitment of immune cells to the myocardium; production of cell signaling proteins from local perycites, mast cells, and macrophages, resulting in activation of resident fibroblasts and myofibroblasts; and the deposition of procollagen into the extracellular matrix, which is irreversibly cross linked to collagen-generating cardiac fibrosis. A multitude of regulators are involved in the pathophysiology of cardiac fibrosis and include ST2 and Gal-3. Given the limited benefit of Gal-3 observed in our cohort of ambulatory chronic HF patients, in which remodeling and fibrosis may be at an advanced stage, it is conceivable that Gal-3 could have a more prominent role in earlier stages of fibrosis pathobiology and ventricular remodeling. Indeed, recent studies found that higher levels of Gal-3 are associated with increased risk for new-onset HF in apparently healthy people (33); in addition, plasma Gal-3 is elevated in patients admitted with acute myocardial infarction and reduced ejection fraction at baseline (34). Gal-3 may be a modest complement to other HF biomarkers by providing an "upstream" signal of myocardial fibrotic state. Nevertheless, much remains to be clarified about Gal-3 at different stages of HF. Nativi et al. (35) recently reported

Table 3 Performance of the Models for All-Cause Mortality at 5 Years

	Reference Model	Model With Gal-3	Model With ST2
Discrimination			
C-statistic	0.757	0.760	0.770
	(0.733 to 0.782)	(0.735 to 0.785)	(0.746 to 0.793)
	Reference	p = 0.143	p = 0.004
Calibration			
H-L	Chi-square: 8.6	Chi-square: 16.9	Chi-square: 14.2
	p = 0.48	p = 0.049	p = 0.12
Brier score	0.171	0.170	0.165
AIC	4,020	4,016	4,003
BIC	4,077	4,078	4,070
Likelihood ratio	Reference	p = 0.032	p < 0.001
Reclassification			
IDI		0.2 (−0.2 to 0.6)	1.5 (0.5 to 2.5)
	Reference	p = 0.288	p = 0.003
NRI—all		0.7 (−2.4 to 3.9)	9.4 (4.8 to 14.1)
	Reference	p = 0.649	p < 0.001
NRI—deceased		−0.1 (−2.6 to 2.4)	4.4 (0.9 to 7.9)
	Reference	p = 0.929	p = 0.014
NRI—alive		0.8 (−1.2 to 2.9)	5.0 (2.0 to 8.1)
	Reference	p = 0.143	p = 0.001

Values are n or n (95% CI) unless otherwise indicated. Reference model includes age, female, ischemic etiology of heart failure, LVEF, NYHA functional class, diabetes mellitus, eGFR, ACEI or ARB treatment, beta-blocker treatment, sodium, hemoglobin, NT-proBNP. Model with Gal-3: Reference model + Gal-3. Model with ST2: Reference model + ST2. All p values versus the reference model.

AIC = Akaike information criterion; BIC = Bayesian information criterion; H-L = Hosmer-Lemeshow test; IDI = integrated discrimination improvement; NRI = net reclassification improvement; other abbreviations as in Tables 1 and 2.

that serum Gal-3 levels stay elevated despite replacement of diseased myocardium and reversal of HF state with heart transplant. These findings suggest that Gal-3 is a systemic biomarker rather than being specific to HF. By contrast, ST2 measurement provides a strong serologic overview of the cumulative myocardial fibrotic process and ultimately is a relevant addition to the predictive ability of the practicing clinician.

Because progressive cardiac fibrosis is a central aspect in the progression of cardiac dysfunction as well as the primary substrate for lethal arrhythmias and sudden death, it is intuitive that a blood marker of cardiac fibrosis would

Table 4 Performance of the Models for Cardiovascular Mortality at 5 Years

	Reference Model	Model With Gal-3	Model With ST2
Discrimination			
C-statistic	0.776	0.778	0.783
	(0.745 to 0.807)	(0.747 to 0.809)	(0.753 to 0.813)
	Reference	p = 0.288	p = 0.04
Calibration			
H-L	Chi-square: 10.2	Chi-square: 5.3	Chi-square: 14.7
	p = 0.33	p = 0.81	p = 0.1
Brier score	0.127	0.127	0.125
AIC	2,251	2,250	2,245
BIC	2,308	2,312	2,311
Likelihood ratio	Reference	p = 0.127	p = 0.007
Reclassification			
IDI		0.2 (−0.3 to 0.6)	1.3 (0.4 to 2.1)
	Reference	p = 0.447	p = 0.004
NRI—all		−4.2 (−8.8 to 0.5)	2.4 (−2.5 to 7.2)
	Reference	p = 0.078	p = 0.344
NRI—deceased		−4.2 (−8.3 to −0.1)	−2.3 (−6.2 to 1.6)
	Reference	p = 0.047	p = 0.254
NRI—alive		<0.1 (−1.8 to 1.8)	4.6 (2.1 to 7.2)
	Reference	p = 0.998	p < 0.001

Values are n or n (95% CI) unless otherwise indicated. Models as defined in Table 3. All p values versus the reference model.

Abbreviations as in Tables 1 to 3.

Table 5 Direct Comparison of Performance for All-Cause and Cardiovascular Mortality at 5 Years of Models Containing Gal-3 and ST2

	All-Cause Mortality		Cardiovascular Mortality	
	Gal-3 vs. ST2		Gal-3 vs. ST2	
Discrimination				
C-statistic	0.760 (0.735 to 0.785)	0.770 (0.746 to 0.793)	0.778 (0.747 to 0.809)	0.783 (0.753 to 0.833)
	p = 0.035		p = 0.254	
Calibration				
H-L	Chi-square: 16.9 p = 0.049	Chi-square: 14.2 p = 0.12	Chi-square: 5.3 p = 0.81	Chi-square: 14.7 p = 0.1
Brier score	0.170	0.165	0.127	0.125
AIC	4,016	4,003	2,250	2,245
BIC	4,078	4,070	2,312	2,311
Reclassification				
IDI	Reference	1.3 (0.2 to 2.4) p = 0.019	Reference	1.1 (0.1 to 2.1) p = 0.029
NRI—all	Reference	7.8 (2.5 to 13.1) p = 0.004	Reference	4.5 (–0.4 to 9.4) p = 0.074
NRI—deceased	Reference	3.4 (–0.9 to 7.6) p = 0.118	Reference	0.5 (–3.6 to 4.6) p = 0.800
NRI—alive	Reference	4.5 (1.3 to 7.7) p = 0.005	Reference	3.9 (1.4 to 6.5) p = 0.002

Values are n or n (95% CI) unless otherwise indicated. All models include age, female, ischemic etiology of heart failure, LVEF, NYHA functional class, Diabetes mellitus, eGFR, ACEI or ARB treatment, beta-blocker treatment, sodium, hemoglobin, NT-proBNP. All p values versus the reference model. Reference model = clinical factors + Gal-3; model with ST2 = clinical factors + ST2.

Abbreviations as in Tables 1 to 3.

be independently associated with cardiovascular mortality. This study shows that increased serum levels of ST2 were not only predictive of all-cause mortality but also of cardiovascular mortality. A previous study has already demonstrated the value of ST2 in predicting sudden cardiac death in ambulatory patients with mild-to-moderate chronic HF and left ventricular systolic dysfunction (36). Those authors found that the prognostic value of ST2 was independent of other clinical variables and, importantly, complementary to NT-proBNP. At present, no single test reliably predicts sudden death in patients with HF (37), but the combination of ST2 and NT-proBNP markedly improved risk stratification to identify high- and low-risk patients; this fact may have an important impact on clinical decision making, particularly for delineating optimal preventive strategies.

Study limitations. First, whether serial measurements of both biomarkers at pre-defined time points would have improved risk stratification was not incorporated into the design and is beyond the scope of the present report. Second, with regard to imaging techniques, ultrasounds were primarily used to characterize ventricular remodeling, and cardiac magnetic resonance imaging was not routinely performed or available to all patients. Finally, the population was a general HF population treated at a specific and multidisciplinary HF unit in a tertiary care hospital; most patients were referred from the cardiology department and thus were relatively young men with HF of ischemic etiology and reduced LVEF. As such, these results cannot necessarily be extrapolated to a global HF population. The low use of implantable cardioverter-defibrillators in this consecutive cohort is representative of HF management in Mediterranean countries. It is possible that more widespread use of implantable cardioverter-defibrillators might change our findings. We must also acknowledge that the estimation of effect size from adding biomarker measurements to the clinical model is limited.

Conclusions

The head-to-head comparison of 2 new-generation fibrosis biomarkers revealed that ST2 is an important addition to established risk factors, whereas the additive value of Gal-3 was trivial. The incorporation of ST2 into clinical practice for the prediction of all-cause and cardiovascular mortality should be readily contemplated by the practicing clinician. Further studies should confirm whether this superiority of ST2 is present at all stages of the HF continuum.

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Key Words: biomarkers ■ heart failure ■ myocardial fibrosis ■ remodeling ■ survival.

APPENDIX

For supplemental tables and a figure, please see the online version of this article.

6.2. Articles no fonamentals

6.2.1: Bayes-Genis A, Zamora E, de Antonio M, Galán A, Vila J, Urrutia A, Díez C, Coll R, Altimir S, Lupón J. Soluble ST2 serum concentration and renal function in heart failure. J Card Fail 2013;19(11):768-75.

6.2.2: Zamora E, Lupón J, de Antonio M, Vila J, Galán A, Gastelurrutia P, Urrutia A, Bayes-Genis A. Limited value of cystatin-C over estimated glomerular filtration rate for heart failure risk stratification. PLoS One 2012;7(12):e51234.

Soluble ST2 Serum Concentration and Renal Function in Heart Failure

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ABSTRACT

Background: Soluble ST2 (sST2) provides important prognostic information in patients with heart failure (HF). How sST2 serum concentration is related to renal function is uncertain. We evaluated the association between sST2 and renal function and compared its prognostic value in HF patients with renal insufficiency.

Methods and Results: Patients (n = 879; median age 70.4 years; 71.8% men) were divided into 3 subgroups according to estimated glomerular filtration rate (eGFR): ≥60 mL/min/1.73 m^2 (n = 337); 30−59 mL/min/1.73 m^2 (n = 352); and <30 mL/min/1.73 m^2 (n = 190). sST2 (rho = −0.16; $P < .001$), N-terminal pro−B-type natriuretic peptide (rho = −0.40; $P < .001$), and high-sensitivity cardiac troponin T (rho = −0.47; $P < .001$) inversely correlated with eGFR. All-cause mortality was the primary end point. During a median follow-up of 3.46 years, 312 patients (35%) died, 246 of them from the subgroup of 542 patients with eGFR <60 mL/min/1.73 m^2 (45%). Biomarker combination including sST2 showed best discrimination, calibration, and reclassification metrics in renal insufficiency patients (net reclassification improvement 16.6 [95% confidence interval (CI) 8.1−25; $P < .001$]; integrated discrimination improvement 4.2 [95% CI 2.2−6.2; $P < .001$]). Improvement in reclassification was higher in these patients than in the total cohort.

Conclusions: The prognostic value of sST2 was not influenced by renal function. On top of other biomarkers, sST2 improved long-term prediction in patients with renal insufficiency even more than in the total cohort. (*J Cardiac Fail 2013;19:768−775*)

Key Words: Heart failure, cardiac markers, prognosis, renal dysfunction, risk prediction.

Renal insufficiency is prevalent in patients with heart failure (HF), and the coexistence of both conditions results in a worse prognosis.[1,2] Although the causes of the relationship between renal insufficiency and HF are probably multiple and not absolutely defined, this interrelationship between HF and renal insufficiency is considered to be reciprocal and bidirectional, and the term ''cardiorenal syndrome'' has even been proposed to define the combined failure of both organs.[3] Although the definition of this term implies the joint and generally severe failure of both systems, it covers a very wide range of possible combinations in their severity, form of presentation, and evolution. A risk assessment approach in these patients with the use of a combination of biomarkers may be helpful to accurately identifying HF patients at increased risk of death, and who could then be targeted for more intensive treatment and monitoring. Natriuretic peptides are well recognized as important prognostic biomarkers in HF and are useful in a wide range of situations,[4] including risk stratification in acute and chronic HF.[5,6]

Soluble ST2 (sST2) has been identified as a novel biomarker for cardiac strain with important prognostic value in patients with HF.[7−10] It can be also considered as a marker of fibrosis and remodeling and of

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inflammation.[7,8,11,12] Under the induction of separate promoters, the ST2 gene expresses 2 unique proteins; sST2, the circulating form of ST2, and ST2L, the transmembrane form of the protein which signals through a complex involving interleukin (IL) 33.[11,13] sST2 acts as a decoy receptor that neutralizes the benefits of IL-33/ST2L related to antihypertrophic, antifibrotic, and antiapoptotic effects,[11] probably by binding IL-33.

We previously demonstrated that the addition of high-sensitivity sST2 and N-terminal pro-brain natriuretic peptide (NT-proBNP) improves the risk stratification for death beyond that of a model based only on established mortality risk factors.[14] We also previously evaluated the prognostic value of other biomarkers, such as high-sensitivity cardiac troponin T (hs-cTnT)[15] and its combination with NT-proBNP and sST2[16] as well as the relative added value of cystatin C.[17] Serum concentration of NT-proBNP is substantially influenced by the degree of renal insufficiency yet retains prognostic value.[18] Data on the influence of renal function on serum concentration of sST2 are scarce. Our objective was to analyze the relationship between sST2 serum concentration and renal function with the use of estimated glomerular filtration rate (eGFR), comparing the results obtained with those of NT-proBNP, and to analyze the prognostic value of sST2 in relation to other clinical parameters and biomarkers according to renal function in a HF outpatient population.

Methods

Study Population

From May 2006 to July 2010, ambulatory patients treated at a multidisciplinary HF unit were consecutively included in the study. Referral, inclusion criteria, and blood sampling have been described elsewhere.[14,15] Once obtained and after adequate centrifugation, serum samples were stored at −80°C. All biomarkers and creatinine were analyzed from the same blood sample. The present study is a post hoc analysis with expanded follow-up of the series reported elsewhere[14] that addresses the subgroup of patients with renal insufficiency (eGFR <60 mL/min/1.73 m^2).

Each of the participants provided written informed consent, and the local Ethics Committee approved the study. 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 predefined 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.[14−17,19] 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 HF unit clinical records, other hospital wards, the emergency room, general practitioners, and by contacting the patient's relatives. Furthermore, data was verified from databases of the Catalan and Spanish Health Systems.

sST2 Assay

sST2 serum concentrations were measured from samples with the use of a high-sensitivity sandwich monoclonal immunoassay (Presage sST2 assay; Critical Diagnostics, San Diego, California). The antibodies used in the Presage assay were generated from recombinant protein based on the human cDNA clone for the complete soluble sST2 sequence .[20] The sST2 assay had a within-run coefficient of <2.5% and total coefficient of variation of 4%.

NT-proBNP Assay

NT-proBNP serum concentrations were determined with the use of an immunoelectrochemiluminescence 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 interrun coefficients of variation ranging from 0.9% to 5.5%.[5]

hs-cTnT Assay

Troponin serum concentrations were measured with the use of an hs-cTnT electrochemiluminescence immunoassay on the Modular Analytics E 170 (Roche Diagnostics). This assay uses 2 monoclonal antibodies that recognize epitopes located in the central region of the cTnT protein. 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 (CV) was 9%.[15]

Cystatin C Assay

Cystatin C was measured with the use of a nephelometric technique that assesses immune complex formation between cystatin and anticystatin-C antiserum attached to latex particles (Cystatin C Radim, ref NPP42). Assays were processed with the use of a Delta nephelometer (Radim Group, Pomezia, Italy). The coefficient of variation between assays was 2.9%. To obtain standardized cystatin C values according to the International Federation of Clinical Chemistry Working Group for Standardization of Serum Cystatin C and the Institute for Reference Materials and Measurements, the Radim Cystatin C method (ref NPP42) was recalibrated to the IRP ERM-DA471/IFCC. According to the manufacturer's recommendations, standardized cystatin values (mg/dL) = 1.11 × Delta nephelometer cystatin values (mg/dL) + [0.00]. Normal values of the standardized method are 0.59−1.05 mg/L.

Estimated Glomerular Filtration Rate

eGFR was calculated with the use of the Chronic Kidney Disease Epidemiology Collaboration equation: men: $141 \times (\text{MIN}[(\text{creat} / 0.9, 1])^{-0.411} \times (\text{MAX}[\text{creat} / 0.9, 1])^{-1.209} \times (0.993^{\text{age}})$ (×1.159 if black); women: $141 \times (\text{MIN}[\text{creat} / 0.7, 1])^{-0.329} \times (\text{MAX}[(\text{creat} / 0.7, 1])^{-1.209} \times (0.993^{\text{age}}) \times 1.018$ (×1.159 if black).[21] Serum creatinine concentration were analyzed using the Siemens Crea method (ref FD33A) on a Dimension RxL Clinical Chemistry System (Siemens, Newark, New Jersey). Creatinine values were standardized according to the isotope dilution mass spectrometry method as recommended by the manufacturer. To obtain standardized creatinine values, the following equation was applied: standardized creatinine values (mg/dL) = 1.00 × Dimension RxL creatinine values (mg/dL) − [0.168].

Statistical Analysis

Categoric variables are expressed as percentages. Continuous variables are expressed as median (interquartile range [IQR]). Correlation between NT-proBNP, sST2, and eGFR values was analyzed with the use of the Spearman rho coefficient owing to skewed distribution. Loess-based approach was used for obtaining total adjustment curves. For multivariable adjustment of such correlation, all values were transformed into their logarithmic form. Statistical differences (P value for trend) in NT-proBNP and sST2 serum concentrations between eGFR groups also were computed with the use of Spearman test.

The best prognostic cutoff points for NT-proBNP, hs-cTnT, and sST2 were found by bootstrapping the value that maximized the log-likelihood of the nonadjusted Cox models as reported elsewhere.[14] Cox regression survival curves were plotted, testing the combination between the best NT-proBNP and sST2 cutoff points. Multivariate survival analyses were also performed using Cox regression models. To fulfill the assumption of linearity of the covariables sST2, hs-cTnT, cystatin C, and NT-proBNP, the logarithmic functions of NT-proBNP, cystatin C, and hs-cTnT and the quadratic term of sST2 and log(hs-cTnT) were used in the Cox models. For hazard ratio (HR) calculation in the 3 log-transformed variables, 1 SD increase was used and ST2 analyses were performed per every 10 ng/mL change. The following variables were incorporated into the model because of their significance in the univariate analysis or because they were considered to be of clinical significant relevance: age, sex, ischemic etiology of HF, left ventricular ejection fraction (LVEF), New York Heart Association (NYHA) functional class, presence of diabetes mellitus, hemoglobin (g/dL), serum sodium (mmol/L), β-blocker treatment, and angiotensin-converting enzyme inhibitor (ACEI) or angiotensin II receptor blocker (ARB) treatment, together with eGFR (in mL/min/1.73 m^2), NT-proBNP, cystatin C, hs-cTnT, and sST2.

We used different measurements of performance (calibration, discrimination, and reclassification) to test the potential incremental prognostic value of these biomarkers as described elsewhere.[14–16]

All analyses were performed using the software R (version 2.11.1) statistical package (Foundation for Statistical Computing, Vienna, Austria). Two-sided $P < .05$ was considered to be significant.

Results

Out of 891 consecutive patients included from May 22, 2006, to July 7, 2010, sST2, NT-proBNP, and eGFR values were available for 879, which were included in this subanalysis (age 70.4 years [IQR 60.5–77.2], 71.8% men). Most patients were in NYHA functional class II (65.8%) and III (25.9%). Table 1 shows the baseline characteristics of the entire sample. Comorbidities were recorded according to the documented medical history of patients. During a median follow-up period of 3.46 years (IQR 1.85–5.05), 312 of the total cohort of 879 patients died (35%). Of the subgroup of 542 patients with eGFR <60 mL/min/1.73 m^2, 246 died during follow-up (45%). Two patients were lost to follow-up and were adequately censored.

Table 1. Demographic and Clinical Baseline Characteristics and Treatments During Follow-Up (n = 879)

Age, y	70.4 (60.5–77.2)
Male	631 (71.8)
White	874 (99.6)
Etiology	
Ischemic heart disease	463 (52.7)
Dilated cardiomyopathy	87 (9.9)
Hypertensive	80 (9.1)
Alcohol	50 (5.7)
Toxic	23 (2.6)
Valvular	100 (11.4)
Other	76 (8.6)
HF duration, mo	26.9 (4–72)
LVEF, %	34 (26–43)
NYHA functional class	
I	65 (7.4)
II	578 (65.8)
III	228 (25.9)
IV	8 (0.9)
Body mass index, kg/m^2	26.9 (24.2–30.5)
Heart rate, beats/min	70 (60–78)
Sodium, mmol/L	139 (137–142)
Hemoglobin, g/dL	13 (11.75–14.2)
eGFR, mL/min/1.73 m^2	51.2 (32.7–71.6)
eGFR groups	
≥60 mL/min/1.73 m^2	337 (38.3)
30–59 mL/min/1.73 m^2	352 (40.0)
<30 mL/min/1.73 m^2	190 (21.6)
NT-proBNP, ng/L	1,355 (508-3,013)
sST2, ng/mL	37.8 (30.7–50.7)
hs-cTnT, ng/L*	22.4 (10.5–40.2)
Cystatin C, mg/L	1.34 (1.06–1.79)
Hypertension	537 (61.1)
Diabetes mellitus	314 (35.7)
COPD	146 (16.6)
Peripheral artery disease	128 (14.6)
Treatments (follow-up)	
ACEI or ARB	791 (90.0)
β-Blocker	771 (87.7)
Spironolactone/eplerenone	345 (39.2)
Loop diuretic	742 (84.4)
Digoxin	267 (30.4)

ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; COPD, chronic obstructive pulmonary disease; eGFR, estimated glomerular filtration rate; HF, heart failure; hs-cTnT, high-sensitivity cardiac troponin T; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro–B-type natriuretic peptide; NYHA, New York Heart Association; sST2, soluble ST2.

Values are presented as n (%) or median (interquartile range).

*Available in 867 patients.

Biomarkers and Renal Function Correlation

Both NT-proBNP (rho = −0.40; $P < .001$) and sST2 (rho = −0.16; $P < .001$) inversely correlated with eGFR (Fig. 1). Although statistically significant, the degree of correlation between sST2 and eGFR was in fact very weak. In the multivariate correlation analysis, adjusting by age, sex, NYHA functional class, and LVEF, correlation between sST2 and eGFR was much lower ($r = -0.09$; $P = .006$) than between NT-proBNP and eGFR ($r = -0.37$; $P < .001$). Remarkably, a significant slope in the NT-proBNP total adjustment curve at the lowest eGFR values was observed (Fig. 1). Hs-cTnT, as expected, significantly correlated with eGFR, both in the bivariate analysis (rho = −0.47; $P < .001$) and in the multivariate analysis ($r = -0.41$, $P < .001$). On the other hand, NT-proBNP

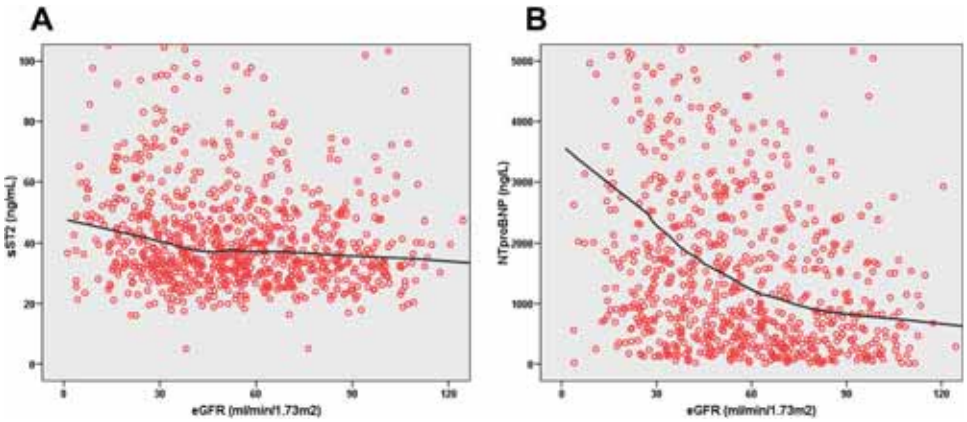

Fig. 1. (A) Scatter plot of soluble ST2 (sST2) serum concentrations according to estimated glomerular filtration rate (eGFR) values. Black curve line performed by Loess adjustment; at very low eGFR, sST2 serum concentrations tended to increase. Patients with sST2 serum concentration >100 ng/mL are not shown (10%). (B) Scatter plot of N-terminal pro-B-type natriuretic peptide (NT-proBNP) serum concentrations according of eGFR values. Black curve line performed by Loess adjustment. Patients with NT-proBNP serum concentration >5,000 ng/L are not shown (10%). n = 879.

and sST2 correlated significantly although modestly, both in the bivariate analysis (rho = 0.33; $P < .001$) and in the multivariate analysis ($r = 0.28$, $P < .001$) respectively.

Patients were divided into 3 subgroups according to eGFR: ≥60 mL/min/1.73 m^2; 30−59 mL/min/1.73 m^2; and <30 mL/min/1.73 m^2. Serum concentrations of NT-proBNP and sST2 significantly increased as eGFR worsened: NT-proBNP 769 ng/L (IQR 289-1,751), 1,591 (IQR 577-3,016), and 2,827.5 (IQR 1,042−7,751), respectively (P value for trend <.001); sST2 35.9 (IQR 29.4−48), 37.1 (IQR 30.2−48.4), and 43.5 ng/mL (IQR 33−61), respectively (P value for trend <.001). However, in the sickest patients (NYHA functional class III−IV), only NT-proBNP serum concentration were significantly increased with worsening eGFR status (P value for trend <.001; Fig. 2A), whereas sST2 serum concentrations remained similar in the 3 eGFR subgroups (P-value for trend=0.27; Fig. 2A) whereas NT-proBNP serum concentrations were significantly increased with worsening eGFR status (P-value for trend < 0.001; Fig. 2B).

Survival

In patients with renal insufficiency (eGFR <60 mL/min/1.73 m^2), sST2 remained as an independent prognostic marker when a comprehensive multivariable analysis including other biomarkers was performed (Table 2). NT-proBNP, eGFR, and cystatin C, with very significant prognostic value in the univariable analysis (Supplemental Table 1), lost their significance when hs-cTnT and sST2 were introduced into the multivariable analysis (Table 2). No interaction between sST2 and age, sex, LVEF, diabetes, or ischemic etiology was found in the multivariable analysis regarding prognostic value, but there was a significant interaction with NYHA functional class ($P = .016$; Supplemental Fig. 1). The combination of 2 biomarkers using best cutoff points significantly increased prognostication including either

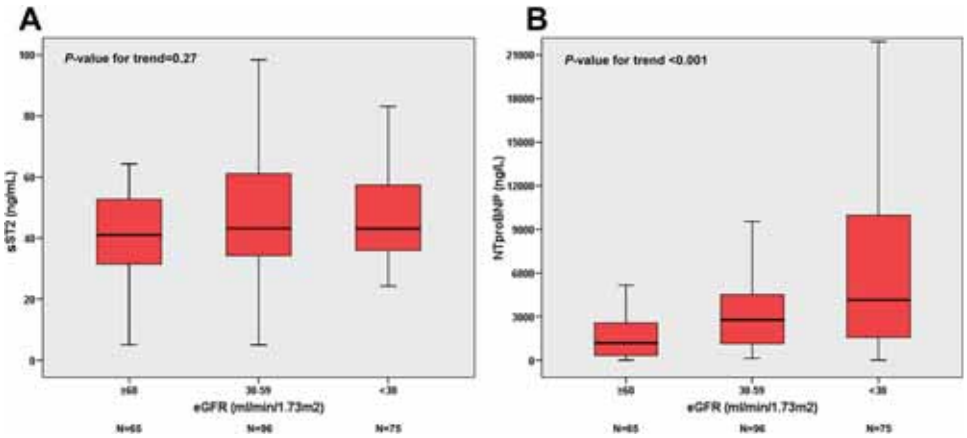

Fig. 2. (A) Box plot of sST2 serum concentrations according of eGFR-grouped values in patients with New York Heart Association (NYHA) functional class III or IV. (B) Box plot of NT-proBNP serum concentrations according to eGFR-grouped values in patients with NYHA functional class III or IV. The central box represents the values from the lower to the upper quartile, the middle line the median; the whiskers extend to the minimum and maximum values, excluding far out values which are not displayed. Abbreviations as in Figure 1.

Table 2. Multivariable Cox Regression Analysis for Risk of Death in the Total Cohort and in Patients With Renal Insufficiency (eGFR <60 mL/min/1.73 m^2)

	Total Cohort (n = 867)			Renal Insufficiency (n = 534)		
	HR	95% CI	*P* Value	HR	95% CI	*P* Value
Age	1.04	1.03−1.06	<.001	1.05	1.03−1.06	<.001
Female	0.77	0.59−1.02	.068	0.78	0.57−1.06	.114
Ischemic etiology of HF	1.08	0.84−1.38	.534	1.24	0.94−1.65	.129
LVEF	1.00	0.99−1.01	.76	1.00	0.99−1.02	.365
NYHA functional class*	1.65	1.29−2.11	<.001	1.77	1.34−2.33	<.001
eGFR	1	0.99−1.01	.636	1	0.99−1.02	.761
Diabetes mellitus	1.18	0.93−1.49	.173	1.39	1.07−1.82	.015
ACEI or ARB treatment	0.80	0.56−1.13	.204	0.75	0.52−1.08	.127
β-Blocker treatment	0.56	0.41−0.76	<.001	0.62	0.44−0.88	.008
Sodium	0.97	0.94−1.00	.057	0.96	0.93−1.00	.060
Hemoglobin	0.91	0.85−0.98	.013	0.93	0.85−1.01	.060
NT-proBNP	1.17	0.98−1.4	.084	1.12	0.91−1.37	.306
hs-cTnT†	1.58	1.27−1.97	<.001	1.65	1.24−2.2	<.001
Cystatin C	1.05	0.85−1.31	.644	0.98	0.76−1.25	.857
sST2‡	1.25	1.12−1.39	<.001	1.22	1.06−1.42	.007

CI, confidence interval; HR, hazard ratio; other abbreviations as in Table 1.

NT-proBNP as log(NT-proBNP); hs-cTnT as log(hs-cTnT); cystatin C as log(Cystatin C); for HR calculation in these 3 log-transformed variables, 1 SD increase was used. ST2 as ST2/10 ng/mL.

*NYHA functional class I-II vs III-IV.

†For $\log(\text{hs-cTnT})^2$, $P = .003$ for total population and $P = .157$ for patients with renal insufficiency.

‡For sST2^2, $P = .001$ for total population and $P = .051$ for patients with renal insufficiency.

NT-proBNP + sST2 (Fig. 3A) or hs-cTnT + sST2 (Fig. 3B). In both cases, however, if only 1 marker was abnormally elevated, sST2 tended to select a higher-risk population than NT-proBNP or hs-cTnT.

Measurements of Performance in the Renal Insufficiency Population

Calibration. The *P* values for the Hosmer-Lemeshow statistics indicated good calibration for all the models with and without biomarkers ($P > .17$ for all comparisons; Table 3). Brier scores, bayesian information criterion, and Akaike information criterion were lower in the model that included sST2 (Table 3). Global goodness of fit was better in models including biomarkers than the model with only established mortality risk factors, as evaluated by likelihood ratio tests ($P < .001$; Table 3).

Discrimination. The area under the receiver operating characteristic curve for the prediction of death increased significantly when sST2 was incorporated into the model with clinically established mortality risk factors as well as into the model already containing NT-proBNP, hs-cTnT, and cystatin C (Tables 3 and 4).

Reclassification. Integrated discrimination improvement (IDI; risk as a continuous variable) increased significantly with the inclusion of biomarkers compared with the baseline model, yet the benefit was highest when sST2 was added on top of the other biomarkers (Table 3). Furthermore, when the model containing sST2 was directly compared with the model with all the other biomarkers (NT-proBNP + hs-cTnT + cystatin C), IDI also significantly improved (Table 4).

Net reclassification improvement (NRI; reclassification according to predefined risk categories) also significantly improved after the inclusion of biomarkers. Again, the best data were obtained with the inclusion of sST2 into the model (Table 3), and direct comparison between the model containing NT-proBNP, hs-cTnT, and cystatin C with the model containing sST2 significantly favored the latter. Reclassification with the models containing sST2 was even better among patients with renal insufficiency than in the global population: NRI 16.6 (95% CI 8.1−25) versus 13.3 (95% CI 5.5−21) compared with model 1 (baseline model) and 8.6 (95% CI 2.8−14.4) versus 5.8 (95% CI 0.8−10.7) compared with model 3 (NT-proBNP + hs-cTnT + cystatin C), respectively.

Discussion

Two points are notable in this study. 1) We found a weak statistically significant but clinically irrelevant association of sST2 and eGFR in patients with HF. To our knowledge, few data about the influence of renal function in sST2 serum concentration have been previously reported. And 2) we demonstrated that sST2 provides independent and incremental prognostic information on top of clinical data as well as biomarkers such as hs-cTnT, cystatin C, and NT-proBNP in patients with HF and renal insufficiency.

Regarding the first point, Dieplinger et al[20] analyzed the analytic and clinical evaluation of the novel Presage sST2 assay for determining sST2 in human plasma. In that pilot study, sST2 plasma concentrations were similar in patients with renal insufficiency and healthy individuals. It was a small study with only 15 patients with HF and no renal dysfunction and 15 patients with renal dysfunction without HF. More recently, Bao et al[22] found that serum sST2 serum concentration correlated with chronic kidney severity in 69

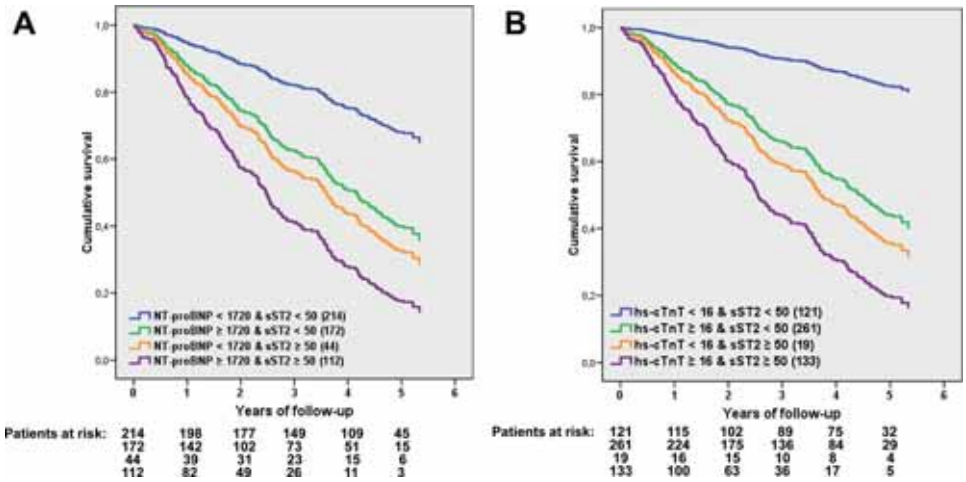

Fig. 3. (A) Survival curves according to the combination of NT-proBNP and sST2 best cutoff points in the cohort with renal insufficiency (eGFR <60 mL/min/1.73 m^2; n = 542). (B) Survival curves according to the combination of high-sensitivity cardiac troponin T (hs-cTnT) and sST2 best cut-off points in patients with renal insufficiency (n = 534, owing to missing values of hs-cTnT). Abbreviations as in Figure 1.

patients, but no data on cardiac function were reported. As far as we know, there are no data in a cohort with coexistence of HF and renal dysfunction.

We found that sST2 serum concentration modestly increased as eGFR worsened in the total population, but in the sickest patients (NYHA functional class III–IV) sST2 serum concentrations remained similar in the 3 eGFR strata. It is unclear why sST2 is similarly increased in more severe HF patients regardless of kidney function, but we may speculate that low cardiac output and the small size of sST2 (57 kD) may, at least partially, explain our results. Further studies in preclinical models are warranted to confirm this mechanistic relationship.

In contrast, much evidence on the relationship between NT-proBNP serum concentration and renal disease has been collected. Renal insufficiency affects the concentrations of both NT-proBNP and BNP, but it is not yet clear whether this increased release of the markers is due to the presence of cardiac dysfunction or due to reductions in their clearance. The clearance of NT-proBNP in humans is not well understood. NT-proBNP may be recovered in urine,[23] suggesting a degree of renal clearance. On the other hand, among hemodialysis patients also, NT-proBNP serum concentrations are markedly elevated.[24] Other results suggest that in patients with acute HF and concomitant renal insufficiency, the increase in circulating NT-proBNP may

Table 3. Performance of the Models at 4 Years in Patients With Renal Insufficiency (n = 534)

	Model 1	Model 2	Model 3	Model 4
Calibration				
H-L	χ^2 = 12.1	χ^2 = 5.3	χ^2 = 12.9	χ^2 = 7.9
	P = .21	*P* = .80	*P* = .17	*P* = .55
Brier score	0.180	0.169	0.168	0.159
AIC	2607	2588	2587	2579
BIC	2654	2648	2651	2651
Likelihood ratio	Reference	*P* < .001	*P* < .001	*P* < .001
Discrimination				
AUC	0.740 (0.709–0.772)	0.756 (0.727–0.786)	0.757 (0.727–0.787)	0.766 (0.738–0.794)
	Reference	*P* = .027	*P* = .024	*P* = .002
Reclassification				
IDI	Reference	2.8 (1.2–4.4)	3.0 (1.3–4.7)	4.2 (2.2–6.2)
		P < .001	*P* < .001	*P* < .001
NRI—all	Reference	10.3 (2.8–17.8)	6.2 (−0.9–13.4)	16.6 (8.1–25)
		P < .001	*P* = .089	*P* < .001
NRI—deceased	Reference	7.1 (1.8–12.5)	5.4 (0.2–10.5)	10.5 (4.2–16.7)
		P = .007	*P* = .04	*P* = .001
NRI—alive	Reference	3.2 (−1.8–8.1)	0.8 (−3.9–5.6)	6.1 (1.0–11.2)
		P = .209	*P* = .739	*P* = .02

AIC, Akaike information criterion; AUC, area under receiver operating characteristic curve; BIC, bayesian information criterion; HL, Hosmer-Lemeshow test; IDI, integrated discrimination improvement; NRI, net reclassification improvement. other abbreviations as in Table 1.

P values reflect comparisons with model 1. Model 1 = age, female, ischemic etiology of heart failure, LVEF, NYHA functional class, diabetes mellitus, eGFR, ACEI or ARB treatment, β-blocker treatment, sodium, hemoglobin. Model 2 = model 1 + hs-cTnT + cystatin C. Model 3 = model 2 + NT-proBNP. Model 4 = model 3 + sST2.

Table 4. Direct Comparison of the Model Containing sST2 with the Model Containing All the Other Biomarkers at 4 Years in Patients With Renal Insufficiency (n = 534)

	Model 3	Model 4
Calibration		
H-L	χ^2 = 12.9 *P* = .17	χ^2 = 7.9 *P* = .55
Brier score	0.168	0.159
AIC	2587	2579
BIC	2651	2651
Likelihood ratio	Reference	*P* = .002
Discrimination		
AUC	0.757 (0.727−0.787) Reference	0.766 (0.738−0.794) *P* = .002
Reclassification		
IDI	Reference	1.2 (0.2−2.3) *P* = .023
NRI—all	Reference	8.6 (2.8−14.4) *P* = .004
NRI—deceased	Reference	3.9 (−0.6−8.4) *P* = .086
NRI—alive	Reference	4.7 (1.3−8.1) *P* = .007

Model 3 = clinical model (model 1 [Table 3]) + NT-proBNP + hs-cTnT + cystatin C. Model 4 = model 3 + sST2. Abbreviations as in Table 3.

be mainly related to increased cardiac secretion and not to decreased renal clearance.[25] Other data suggest that urinary NT-proBNP excretion may be lower in patients with chronic HF compared with control subjects, and that it may be associated with renal plasma flow but not with eGFR.[26] Decreased renal plasma flow in these patients could be associated with a lower excretion of NT-proBNP.[27]

The usefulness of sST2 for risk stratification is another major issue derived from these results. In chronic HF, NT-proBNP usually provide independent prognostic information regardless of renal function.[27] In our study, the inverse correlation with eGFR was much higher for NT-proBNP than for sST2 for the total population. When patients were divided into 3 subgroups according to eGFR ≥60 mL/min/1.73 m^2, 30−59 mL/min/1.73 m^2, and <30 mL/min/1.73 m^2, serum concentration of both markers significantly increased as eGFR worsened. However, in the sickest patients (NYHA functional class III−IV), in which an accurate risk stratification is even more crucial, only NT-proBNP serum concentrations were significantly increased with worsening eGFR, whereas sST2 serum concentrations remained similar among the 3 eGFR subgroups. Thus, sST2 appeared to be less related than NT-proBNP to renal function in patients with more severe HF. From a prognostic point of view, when a multimarker approach that incorporates hs-cTnT and cystatin C is used, both biomarkers seemed to be useful for stratifying patients' risk of death, even in patients with renal insufficiency, as shown clearly in our analyses. In this population, however, adding sST2 into the model showed the best performance data. Furthermore, in the multivariable Cox regression analysis sST2 remained as independent prognostic marker whereas NT-proBNP lost statistical significance. Of interest, survival curves showed that when the combination of the 2 biomarkers was used, the prognostic discriminator capacity increased in the renal insufficiency cohort. Patients with elevated serum concentration of both sST2 and NT-proBNP had a markedly increased risk compared with those with elevated serum concentration of only 1 of these biomarkers. The same was observed when hs-cTnT and sST2 were combined. However, if only 1 marker was abnormally elevated, sST2 tended to select a population with higher risk than NT-proBNP or hs-cTnT, although differences were not significant. Considering the inflammatory status secondary to both HF and renal dysfunction, it is possible that sST2 was a stronger and independent outcome predictor in this setting because sST2 reflects both inflammation and cardiac strain. Considering these two findings (lower relationship of sST2 serum concentration with renal function and better stratification in long-term survival), use of sST2 in patients with renal insufficiency might be preferable when only 1 of the biomarkers is available, especially in NYHA functional class III−IV patients.

Study Limitations

The present study has some limitations. We analyzed only 1 blood sample and cannot comment on the prognostic value of serial NT-proBNP and sST2 determinations. We have no data regarding sST2 serum concentrations in urine samples. No other markers of inflammation were available (eg, C-reactive protein, IL-6, procalcitonin, growth differentiation factor 15) and we could not assess whether sST2 would be able to outperform other markers of inflammation regarding prognostic risk prediction. Our population was a general HF population treated at a specific multidisciplinary HF unit at a tertiary hospital; most patients were referred from the cardiology department and were relatively young men with HF of ischemic etiology and with reduced LVEF. As such, these results can not necessarily be extrapolated to a global HF population.

Conclusion

sST2 prognostic value was not significantly influenced by renal function in an ambulatory cohort of HF patients. In combination with NT-proBNP and other biomarkers, sST2 improved long-term prediction in patients with renal insufficiency even more than in the global sample population.

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Disclosures

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Supplementary Data

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.cardfail.2013.09.005.

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Limited Value of Cystatin-C over Estimated Glomerular Filtration Rate for Heart Failure Risk Stratification

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Abstract

Background: To compare the prognostic value of estimated glomerular filtration rate, cystatin-C, an alternative renal biomarker, and their combination, in an outpatient population with heart failure.Estimated glomerular filtration rate is routinely used to assess renal function in heart failure patients. We recently demonstrated that the Cockroft-Gault formula is the best among the most commonly used estimated glomerular filtration rate formulas for predicting heart failure prognosis.

Methodology/Principal Findings: A total of 879 consecutive patients (72% men, age 70.4 years [P_{25-75} 60.5–77.2]) were studied. The etiology of heart failure was mainly ischemic heart disease (52.7%). The left ventricular ejection fraction was 34% (P_{25-75} 26–43%). Most patients were New York Heart Association class II (65.8%) or III (25.9%). During a median follow-up of 3.46 years (P_{25-75} 1.85–5.05), 312 deaths were recorded. In an adjusted model, estimated glomerular filtration rate and cystatin-C showed similar prognostic value according to the area under the curve (0.763 and 0.765, respectively). In Cox regression, the multivariable analysis hazard ratios were 0.99 (95% CI: 0.98–1, $P = 0.006$) and 1.14 (95% CI: 1.02–1.28, $P = 0.02$) for estimated glomerular filtration rate and cystatin-C, respectively. Reclassification, assessed by the integration discrimination improvement and the net reclassification improvement indices, was poorer with cystatin-C (−0.5 [−1.0;−0.1], $P = 0.024$ and −4.9 [−8.8;−1.0], $P = 0.013$, respectively). The value of cystatin-C over estimated glomerular filtration rate for risk-stratification only emerged in patients with moderate renal dysfunction (eGFR 30–60 ml/min/1.73 m^2, chi-square 12.9, $P<0.001$).

Conclusions/Significance: Taken together, the results indicate that estimated glomerular filtration rate and cystatin-C have similar long-term predictive values in a real-life ambulatory heart failure population. Cystatin-C seems to offer improved prognostication in heart failure patients with moderate renal dysfunction.

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Introduction

Chronic heart failure (HF) is a growing public epidemic with increasing incidence and prevalence [1]. Despite important progress in recent decades, mortality remains high among patients with HF. Renal insufficiency is prevalent among patients with HF, and the coexistence of both conditions results in a worse prognosis [2–6]. The most precise methods for calculating kidney function, including the isotopic glomerular filtration rate and creatinine clearance in a 24-hour urine specimen, are not utilized in daily clinical practice [7]. Instead, several formulas based on creatinine clearance have been developed to determine the estimated glomerular filtration rate (eGFR), with the Cockroft-Gault formula [8], the simplified Modification of Diet in Renal Disease (MDRD-4) equation [9], and the Chronic Kidney Disease Epidemiology Collaboration equation [10] being the most commonly used in clinical practice. We recently demonstrated that the Cockroft-Gault formula is the best among these three eGFR formulas for predicting long-term prognosis in HF patients [11].

In the last few years, cystatin-C has emerged as a novel renal biomarker with prognostic implications in patients with HF [12–13]. However, to the best of our knowledge, no data have assessed the benefits of cystatin-C over eGFR in terms of prognosis in patients with chronic HF. The objective of the present study was to compare the long-term prognostic value of cystatin-C and eGFR using the Cockroft-Gault formula in an outpatient population with HF and to assess whether the simultaneous use of both markers is helpful in improving patient risk stratification.

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 criteria were HF according to the European Society of Cardiology guidelines irrespective of etiology, and at least one HF hospitalization and/or reduced left ventricular ejection fraction (LVEF). Blood samples were obtained by venipuncture between 9:00 a.m. and 12:00 p.m. during conventional ambulatory visits, and adequately centrifuged serum samples were stored at -80°C. Both cystatin-C and creatinine were analyzed from the same blood sample.

All participants provided written informed consent, and the local ethics committee approved the study. All study procedures were in accordance 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 predefined intervals with additional visits as required in the 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 [11,14]. 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 the clinical records of the HF unit, other hospital wards, the emergency room, general practitioners, and by contacting the patient's relatives. The data were verified using the databases of the regional and national health systems.

Glomerular Filtration Rate

The eGFR was calculated using the Cockroft-Gault formula: (140-age in years)×weight in kilograms/(72×serum creatinine level in mg/dl) adjusted by sex (×0.85 in women) [8], and then adjusted by body surface area [11]. Serum creatinine levels were analyzed using the CREA method with a Dimension® Clinical Chemistry System (Siemens, Newark, USA) and a modification of the kinetic Jaffe reaction described by Larsen with picrate as the reactant.

Cystatin-C

Cystatin-C was measured using a nephelometric technique that assesses immune complex formation between cystatin and antiserum anticystatin-C attached to latex particles. Assays were processed twice by a Delta nephelometer (ref. 010138; Radim SPA, Pomezia, Italy, ref NPP42). The coefficient of variation between assays was 2.9%. Normal values are 0.53–0.95 mg/L.

Statistical Analysis

Categorical variables were expressed as percentages. Continuous variables were expressed as the mean (standard deviation) or median (25^{th} and 75^{th} percentiles [P_{25-75}]) according to normal or non-normal distribution. Differences in cystatin-C levels between groups were compared using the Mann-Whitney and Kruskal Wallis tests, and correlations between cystatin-C and continuous variables were evaluated using the Rho Spearman coefficient. Colinearity between eGFR and Cystatin-C was assessed with Eigen-values analysis, Condition Index and Variance Inflation Factor.

Survival analyses were performed using Cox regression models incorporating the following variables: age, sex, New York Heart Association (NYHA) functional class, ischemic etiology of HF, LVEF (in %), HF duration, presence of diabetes mellitus, chronic obstructive lung disease and peripheral artery disease, plasma hemoglobin (g/dl), serum sodium (mmol/L), β-blocker treatment, and angiotensin-converting enzyme inhibitor (ACEI) or angiotensin II receptor blocker (ARB) treatment, together with eGFR (in ml/min/1.73 m^2) or cystatin-C. A Cox regression model with both renal markers was also performed. Kaplan-Meier survival curves were plotted for eGFR and cystatin-C quartiles and the groups compared using the log-rank test. In addition, Kaplan-Meier survival curves were plotted for cystatin-C levels below or above the median for each quartile of eGFR.

We used different measurements of performance to test the potential incremental prognostic value of the two renal biomarkers.

a) Discrimination: The area under the receiver operating characteristic curve (AUC) summarized the diagnostic discrimination. Discrimination refers to a model's ability to distinguish two classes of outcomes correctly. We used the index of rank correlation, Somers' D, which already incorporates information from censored data. AUCs between models were compared using the U-statistic test for equality concordance.

b) Calibration: The D'Agostino–Nam version of the Hosmer and Lemeshow calibration test was used to calculate a chi-square value. A model is well calibrated when predicted and observed values agree for any reasonable grouping of the observation (no significant differences in the Hosmer–Lemeshow test). In addition, 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. No statistical tests compare different BIC, AIC, or Brier estimations, and lower values indicate a better model. When a biomarker was added to another, the global goodness-of-fit of the model was evaluated by a likelihood ratio test.

c) Reclassification: We used the method described by Pencina et al [15]. Two main statistics are used to assess reclassification. The integrated discrimination improvement (IDI) considers changes in the estimated mortality prediction probabilities as a continuous variable. *P*-values less than 0.05 from two-sided tests were considered significant. 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

Out of 891 consecutive patients included from May 22, 2006 to July 7, 2010, eGFR and cystatin-C were available in 879, which were finally included in this analysis. Median age of 70.4 years (P_{25-75} 60.5–77.2 years). Table 1 shows the baseline characteristics of the entire sample. During a median follow-up period of 3.46 years (P_{25-75} 1.85–5.05), 312 patients died. Among the cardiovas-

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

	N = 879
Age, yr*	70.4 (60.5–77.2)
Males–no. (%)	631 (71.8)
White–no. (%)	874 (99.6)
Etiology–no. (%)	
Ischemic heart disease	463 (52.7)
Dilated cardiomyopathy	87 (9.9)
Hypertensive	80 (9.1)
Etoh	50 (5.7)
Toxic	23 (2.6)
Valvular	100 (11.4)
Other	76 (8.6)
HF duration, months*	26.9 (4–72)
LVEF, in %*	34 (26–43)
BMI, kg/m^2*	26.9 (24.2–30.5)
NYHA functional class–no. (%)	
I	65 (7.4)
II	578 (65.8)
III	228 (25.9)
IV	8 (0.9)
Hypertension–no. (%)	537 (61.1)
Diabetes mellitus–no. (%)	314 (35.7)
COLD–no. (%)	146 (16.6)
Treatments–no. (%)	
ACEI or ARB	791 (90.0)
β-blocker	771 (87.7)
Spironolactone/eplerenone	345 (39.2)
Loop diuretic	742 (84.4)
Digoxin	267 (30.4)
ICD	93 (10.6)
CRT	47 (5.3)
Sodium, mmol/L*	139 (137–142)
Hemoglobin, g/dl†	13.0±1.8
eGFR, ml/min/1.73 m^2*	42.4 (29.4–59.4)
Cystatin-C, mg/L*	1.21 (0.96–1.61)

*median (percentiles 25th and 75th).
†(mean ± standard deviation).
ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; BMI, body mass index; COLD, chronic obstructive lung disease; CRT, cardiac resynchronization therapy; eGFR, estimated glomerular filtration rate; Etoh, alcoholic cardiomyopathy; HF, heart failure; ICD, implantable cardiac defibrillator; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association.
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cular causes of death (167), refractory HF was responsible in 90 (53.9%) patients, sudden death in 31 (18.5%) patients, and acute myocardial infarction in 15 (9.0%) patients. Two patients were lost to follow-up and adequately censored.

Cystatin-C Levels

Cystatin-C levels correlated significantly with age (Rho 0.44, $P<0.001$) and eGFR (Rho −0.82, $P<0.001$), but not with LVEF (Rho 0.05, $P=0.12$). However, no consistent colinearity was found between Cystatin-C and eGFR. Cystatin-C levels were significantly higher in women ($P=0.005$), diabetic patients ($P<0.001$), hypertensive patients ($P<0.001$), and in patients not treated with β-blockers or ACEI-ARB. No relationship was found between cystatin-C levels and ischemic or non-ischemic HF etiology. In addition, cystatin-C levels progressively increased with worse NYHA functional class ($P<0.001$).

Cox Regression and Modeling

In the bivariable analysis, both eGFR and cystatin-C predicted death from all causes as continuous variables (eGFR hazard ratio [HR] 0.97 [95% confidence interval (CI) 0.96–0.97], $P<0.001$; cystatin-C HR 1.30 [95% CI, 1.21–1.40], $P<0.001$). In separate multivariable analyses, both biomarkers remained independent predictors of mortality (Table 2). When both variables were incorporated into the multivariable analysis, a significant interaction was found ($P=0.001$, Table 2), indicating that the effect of cystatin-C on prognosis differs according to eGFR.

Kaplan–Meier survival curves according to eGFR (Figure 1A) and cystatin-C levels (Figure 1B) and divided into quartiles showed significant predictive prognostic values (log rank test chi-square 105.8 and 107.2; $P<0.001$ for both). When cystatin-C was analyzed as an addition to eGFR, its value for risk stratification was only present in moderate renal dysfunction patients (quartiles 2 and 3, eGFR 30–60 ml/min/1.73 m^2, Figure 2).

Measurements of Performance

eGFR vs. cystatin-C. The AUC for the prediction of death was very similar for eGFR and cystatin-C in the adjusted model (Table 3). The *P*-values for the Hosmer–Lemeshow statistics indicated good calibration for both markers ($P>0.56$ for all comparisons). Also BIC, AIC, and Brier scores were very similar for both markers (Table 3). Taking the model with eGFR as a reference, IDI (risk as a continuous variable) and NRI (reclassification according to predefined risk categories) decreased significantly with cystatin-C (−0.5 [−1.0;−0.1], $P=0.024$ and −4.9 [−8.8;−1.0], $P=0.013$, respectively) (Table 3).

Combined addition of eGFR and cystatin-C. The combined addition of the two markers in the adjusted model did not improve discrimination, calibration, or reclassification according to IDI and NRI (NRI was significantly worse: −2.0 [−3.9;−0.21], $P=0.034$). However, when the variable interaction eGFR$^{\times}$cystatin-C was included in the model, the global goodness-of fit increased significantly (likelihood ratio *P*-value = 0.002) and reclassification using IDI significantly improved (1.0 [0.2;1.8], $P=0.01$) with respect to the model with eGFR alone (Table 3), suggesting that cystatin-C affects prognosis according to eGFR.

Discussion

Cystatin-C is a protein that belongs to a group of cysteine proteinase inhibitors, one of the four types of proteinases in mammalian cells. These types of proteins are encoded by the so-called housekeeping genes that regulate the factors necessary for global cell function, and all nucleated cells produce them at a stable production rate [16]. The protein is located extracellularly and detected mainly in biological fluids. Because of its small size, cystatin-C is freely filtered by the glomerulus and is not secreted, reabsorbed, or catabolized in the proximal tubules; it does not return to the blood and is not detected in urine [17]. Production depends on the metabolic rate and increases in hypermetabolic situations, such as hyperthyroidism and corticosteroid treatment [18]. Cystatin-C has been reported to provide a more accurate and precise estimate of GFR than serum creatinine [19–22]. In

Table 2. Multivariable Cox Regression analyses.

	Model with eGFR			Model with Cystatin-C			Model with eGFR, Cystatin-C and interaction eGFR×Cystatin-C		
	HR	**95% CI**	**p-value**	**HR**	**95% CI**	**p-value**	**HR**	**95% CI**	**p-value**
Age	1.05	[1.03;1.06]	<0.001	1.06	[1.04;1.07]	<0.001	1.05	[1.03;1.07]	<0.001
Female gender	0.75	[0.57;0.98]	0.036	0.74	[0.56;0.97]	0.031	0.75	[0.57;0.98]	0.037
NYHA functional class	1.74	[1.36;2.22]	<0.001	1.71	[1.33;2.18]	<0.001	1.65	[1.29;2.11]	<0.001
Diabetes mellitus	1.25	[0.99;1.57]	0.064	1.25	[0.99;1.58]	0.061	1.25	[0.99;1.58]	0.06
Beta-blocker treatment	0.5	[0.37;0.67]	<0.001	0.51	[0.38;0.7]	<0.001	0.49	[0.36;0.67]	<0.001
ACEI or ARB treatment	0.58	[0.42;0.79]	<0.001	0.59	[0.43;0.81]	0.001	0.58	[0.43;0.8]	<0.001
LVEF	0.99	[0.98;1]	0.057	0.99	[0.98;1]	0.032	0.99	[0.98;1]	0.05
Ischemic aetiology of HF	1.02	[0.8;1.3]	0.877	1.02	[0.8;1.3]	0.874	1.04	[0.81;1.33]	0.743
HF duration	1	[1;1]	0.044	1	[1;1]	0.043	1	[1;1]	0.062
COLD	1.14	[0.86;1.51]	0.349	1.16	[0.88;1.53]	0.303	1.1	[0.83;1.45]	0.524
Peripheral artery disease	1.52	[1.13;2.03]	0.005	1.51	[1.13;2.02]	0.006	1.48	[1.1;1.98]	0.009
Na, mmol/L	0.95	[0.92;0.98]	<0.001	0.95	[0.92;0.98]	0.001	0.95	[0.92;0.98]	<0.001
Hb, g/dL	0.88	[0.82;0.95]	<0.001	0.87	[0.81;0.93]	<0.001	0.9	[0.84;0.97]	0.005
eGFR, ml/min/1.73m^2	**0.99**	**[0.98;1]**	**0.006**	-	-	-	**0.97**	**[0.96;0.99]**	**<0.001**
Cystatin-C	-	-	-	**1.14**	**[1.02;1.28]**	**0.02**	**0.89**	**[0.72;1.09]**	**0.249**
Interaction eGFR×Cystatin-C	-	-	-	-	-	-	**1.02**	**[1.01;1.03]**	**<0.001**

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 hemoglobin; HF, heart failure; LVEF, left ventricular ejection fraction; Na, serum sodium; NYHA, New York Heart Association (I–II vs. III–IV).

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this study of a HF population, we found that cystatin-C levels were influenced by age, sex, NYHA functional class, eGFR, treatments, and comorbidities, such as diabetes and hypertension.

In recent years, cystatin-C has emerged as a marker of cardiovascular events and mortality in different situations. For example, in patients with ischemic heart disease, cystatin-C was found to be an independent risk factor together with traditional cardiovascular risk factors, renal function, or the presence of microalbuminuria [23]. The combined association of albuminuria and cystatin-C-based eGFR was associated with mortality, coronary heart disease, and HF outcomes in the ARIC community study [24]; and in the Cardiovascular Health Study it was a more powerful predictor of death and cardiovascular events in the elderly than creatinine [25]. Remarkably, the usefulness of cystatin-C as a cardiovascular-related prognostic biomarker has been linked not only to its ability to estimate renal function, but also to its relationship with ventricular remodeling and fibrosis and vascular wall stiffness [26–27].

In the specific setting of HF, most of the information on the prognostic usefulness of cystatin-C derive from acutely decompensated HF patients, and the data are encouraging. Lassus et al. [12] found that cystatin-C was a strong predictor of mortality in 480 patients hospitalized for acute HF, both in-hospital and during 1 year of follow-up, and was independent of other renal markers (serum creatinine and eGFR values estimated using the Cockroft-Gault formula). Interestingly, Naruse et al.[28] found the best relationship between high levels of cystatin-C and the risk of cardiac death in patients with acute HF and an eGFR calculated by the MDRD formula between 44 and 79 ml/min/1.73 m^2, independent of volemia and body weight. Cystatin-C was also an independent predictor of prognosis at 2 years of follow-up for the occurrence of death, heart transplantation, or readmission due to worsening HF in 138 systolic HF patients admitted for acute descompensation [29].

In contrast, little information exists on the value of cystatin-C in chronic HF. The first work that examined the ability of cystatin-C to predict mortality in these patients was published by Shlipack et al. [13], who analyzed a subgroup of 279 patients with prevalent HF from the Cardiovascular Health Study. Cystatin-C was exclusively assessed by Cox regression analysis and remained a better independent pronosticator than creatinine and eGFR calculated by the simplified MDRD equation. Arimoto et al. [30], analyzing 140 patients with HF and 64 control subjects, found that serum cystatin-C levels were higher in the patients with HF. Patients with high cystatin-C levels had a markedly higher cardiac event rate (cardiac death and HF hospitalization), independent of creatinine levels. A recent publication [31] in a small cohort of 102 young patients with chronic HF assessed creatinine, eGFR calculated by MDRD and simplified MDRD formulas, and cystatin-C as predictors of renal function using isotope glomerular filtration rate as the gold standard. Despite the small number of recorded events (8 deaths, 10 HF hospitalizations, and 3 heart transplantations), cystatin-C levels were similar to both eGFR formulas for predicting renal function and had similar prognostic properties as MDRD and simplified MDRD in ROC analysis and Cox regression analysis. Our study included a substantially larger cohort of patients (879 vs. 102) who were older (median 70 years vs. mean 58 years), had greater impairment of renal function (eGFR 42.4 ml/min/1.73 m^2 vs. 65 ml/min/1.73 m^2), and higher cystatin-C levels (1.21 vs 0.8 mg/L). The follow-up also differed significantly (3.5 years vs. 2 years) and the number of deaths was much higher (312 vs. 8). The additional prognostic information gained by any marker over a clinical risk model plus other biomarkers needs to be determined using adequate statistical tools [32]; therefore we performed a very

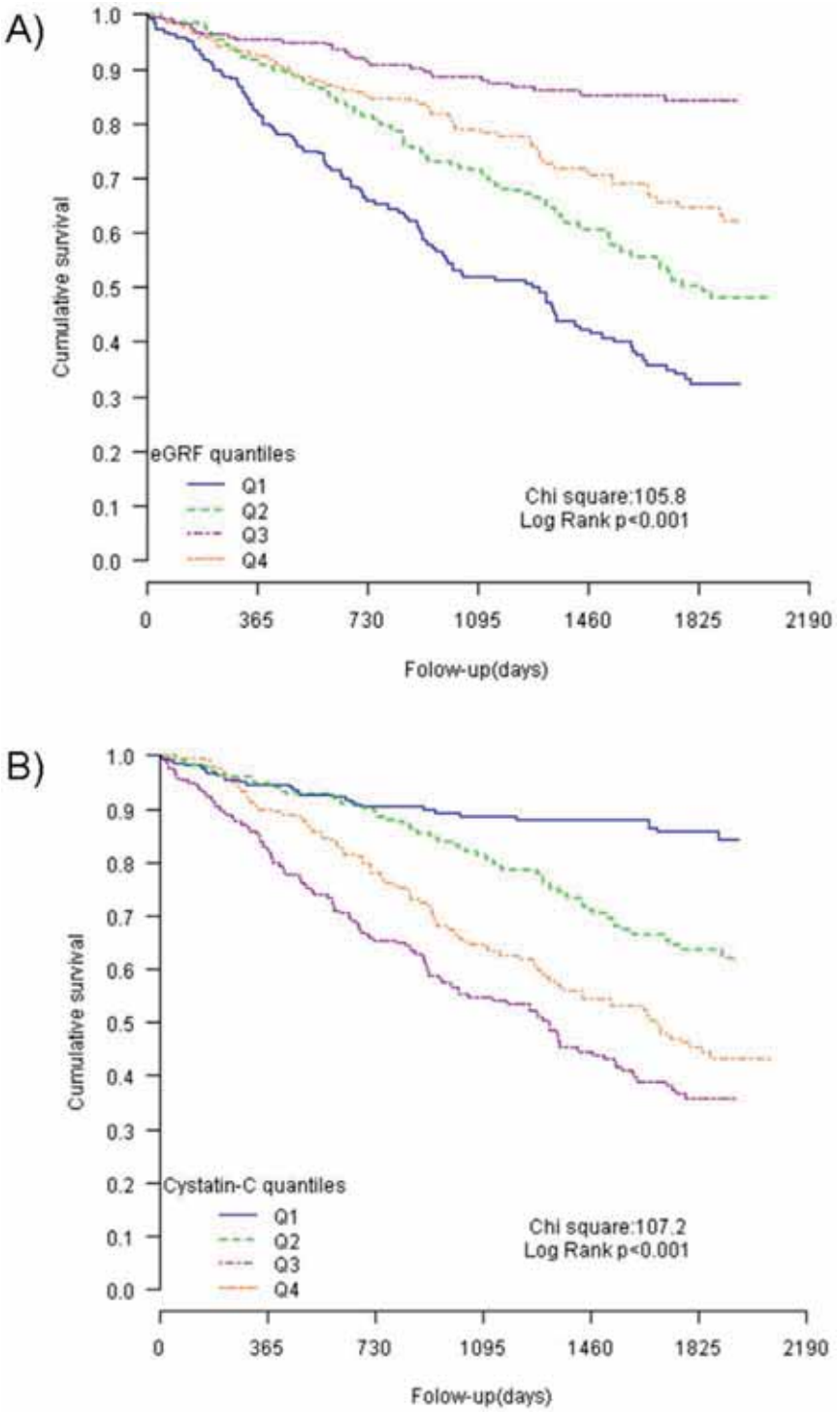

Figure 1. Kaplan–Meier survival curves according to eGFR and cystatin-C levels. Caption: Both eGFR levels (Panel A) and cystatin-C levels (Panel B) have been divided in quartiles.
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comprehensive state-of-the-art statistical analysis that included multivariate Cox regression, discrimination, calibration, and reclassification indices. In our study, cystatin-C had a similar predictive long-term prognostic value as eGFR estimated by the Cockroft-Gault formula after adjusting for some covariates according to discrimination, calibration, and Cox regression, though reclassication was poorer according to IDI and NRI.

Importantly, and not assessed in previous studies, we found that when both markers were used together, cystatin-C levels significantly affected prognosis, and differently according to eGFR. Remarkably, we found that cystatin-C improved risk stratification, mainly in patients with an eGFR between 30 and 60 ml/min/1.73 m^2. This finding is in agreement with a previous study [28] in

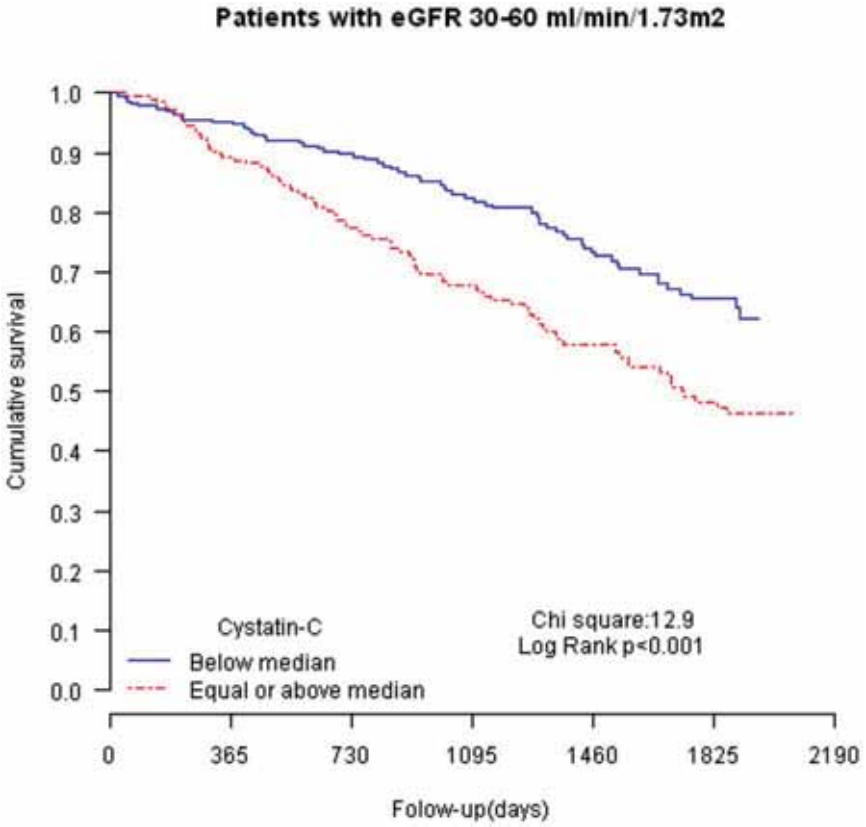

Figure 2. Kaplan–Meier survival curves according to cystatin-C levels in patients with an eGFR between 30 and 60 ml/min/1.73 m^2.
Caption: cystatin-C levels have been divided according to median values (below vs. equal/above). N = 443.
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Table 3. Performance of the adjusted models at 4 years.

	Model with eGFR	Model with Cystatin-C	Model with both eGFR and Cystatin-C	Model with eGFR, Cystatin-C and interaction eGFR×Cystatin-C
AUC	0.763 (0.737;0.79)	0.765 (0.739;0.791)	0.764 (0.738;0.79)	0.768 (0.742;0.794)
	reference	p-value: 0.577	p-value: 0.254	p-value: 0.159
H-L	Chi-square: 6.1	Chi-square: 7.7	Chi-square: 7.3	Chi-square: 4.4
	p.value = 0.73	p.value = 0.568	p.value = 0.608	p.value = 0.881
Brier score	0.16	0.16	0.159	0.157
AIC	3656.5	3659.4	3658.2	3648.5
BIC	3723.2	3726.1	3729.7	3724.8
Likelihood R	–	–	*p.value = 0.584	*p.value = 0.002
IDI		−0.5 [−1.0;−0.1],	−0.03 [−0.1;0.1],	1.0 [0.2;1.8],
	reference	*p-value = 0.024	*p-value = 0.619	*p-value = 0.01
NRI-All		−4.9 [−8.8;−1.0],	−2.0 [−3.9;−0.2],	2.4 [−2.6;7.5],
	reference	*p-value = 0.013	*p-value = 0.034	*p-value = 0.343
NRI-Cases		−3.1 [−5.9;−0.2],	−1.4 [−2.8;<0.1],	2.0 [−1.8;5.9],
	reference	*p-value = 0.033	*p-value = 0.045	*p-value = 0.298
NRI-Control		−1.8 [−4.2;0.6],	−0.6 [−1.8;0.6],	0.4 [−2.4;3.2],
	reference	*p-value = 0.133	*p-value = 0.336	*p-value = 0.783

*Versus model 1.
AUC, area under the ROC curve; AIC, Akaike information criterion; BIC, Bayesian information criterion; H-L, Hosmer and Lemeshow test; Likelihood R: Goodness-of-fit assessed by likelihood ratio; IDI, integrated discrimination improvement; NRI, net reclassification improvement.
Covariates included in models: Age, Female gender, NYHA functional class, Diabetes mellitus, Beta-blocker treatment, ACEI or ARB treatment, LVEF, Ischemic aetiology of HF, HF duration, COLD, Peripheral artery disease, Na, Hb.
doi:10.1371/journal.pone.0051234.t003

acutely decompensated HF patients (eGFR between 44 and 79 ml/min/1.73 m^2).

Doubts still exist about the exact mechanism by which cystatin-C has predictive value in HF and whether its prognostic capacity goes beyond renal function. Damman et al. [31] studied the relationship between cystatin-C and inflammation; though they could not exclude some relationship between cystatin-C levels and several inflammatory markers, this effect seemed small in relation to the strong association between cystatin-C and glomerular filtration rate. Furthermore, whether the relationship of cystatin-C with the mechanisms of ventricular remodeling may influence its predictive role is unknown. Taking into account the reduced availability of cystatin-C in routine laboratories and the cost, its usefulness as a prognostic factor should only be considered in patients with moderate degrees of renal dysfunction and it is advisable to continue using the classical eGFR formulas.

This study has some limitations. The optimal time for determining cystatin-C in regards to the clinical situation and if it is better to make serial or a single determination are unknown. We have no data about the presence of hyperthyroidism, inflammatory parameters, or the use of corticosteroids, which may be related to metabolism and protease levels. Our population was a general HF population treated at a specific and multidisciplinary HF unit in a tertiary hospital; most patients were referred from the cardiology department and, accordingly, mainly experienced HF of ischemic etiology with reduced LVEF. As such, these results cannot necessarily be extrapolated to a global HF population.

Conclusions

The eGFR and cystatin-C have a similar long-term prognostic value in ambulatory HF patients when analyzed in a model adjusted by several established mortality risk factors. Cystatin-C seems to offer improved prognostication in heart failure patients with moderate renal dysfunction.

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Author Contributions

Conceived and designed the experiments: EZ AB JL. Performed the experiments: MDA JL AG AU. Analyzed the data: EZ JL JV PG AB. Contributed reagents/materials/analysis tools: AG JV. Wrote the paper: EZ JL AB. Final approval of the version to be published: EZ JL MDA JV AG PG AU AB Critical revision of the manuscript for important intellectual content: EZ JL MDA JV AG PG AU AB.

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7. DISCUSSIÓ CONJUNTA

7.1. Exploració de nous biomarcadors

7.1.1 ST2 i NT-proBNP

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;14(1):32-8.

En el primer treball es va avaluar si la incorporació d'ST2 mesurat amb un assaig d'alta sensitibilitat (marcador de fibrosi miocàrdica i remodelatge, erò també d'estirament miocardíac i d'inflamació) i NT-proBNP (indicatiu d'estirament miocardíac) en un model amb factors de risc de mortalitat establerts millorava l'estratificació de risc de mort pacients atesos a una Unitat d'IC multidisciplinària. Les variables del model foren: edat, sexe, FE, filtrat glomerular renal, classe funcional NYHA, diabetis mellitus, etiologia isquèmica, hemoglobina, sodi, tractament amb b-blocadors, tractament amb IECAs/ARA-II. Aquest model clínic és el que s'ha seguit a la resta d'articles.

L'ST2 és un biomarcador emergent, membre de la família dels receptors de la interleuquina 1 i s'ha postulat que exerceix un efecte antifibròtic, antiapoptòtic i anthipertròfic a nivell miocàrdic. En pacients amb una descompensació aguda d'IC té utilitat pronòstica (58) així com en la IC crònica (60). En un estudi cas-control niuat a registre va indentificar un subgrup de pacients amb risc de mort sobtada (62).

Es van avaluar 891 pacients consecutius, amb una mediana d'edat de 70.2 anys, FE mediana 34% i amb etiologia principalment isquèmica. Durant una mediana de seguiment de 33,4 mesos es van registrar 244 defuncions. A l'anàlisi de regressió de Cox ambdós marcadors van predir el risc de mort. A l'anàlisi multivariant, l'associació va romandre estadísticament significativa juntament amb l'edat, sexe, classe funcional NYHA, tractament b-blocador, sodi i hemoglobina.

Mitjançant *bootstraping* es van calcular els punts de tall òptims per estimar el pronòstic. En dividir la població en 4 grups segons si presentaven valors per sobre o per sota del punt de tall dels marcadors, la combinació dels dos biomarcadors amb valors per sobre del punt de tall va indentificar el subgroup de major risc de mort (HR 6.38).

La inclusió individual de l'NT-proBNP i ST2 al model de factors de risc establerts va millorar l'estadístic C (per la inclusió conjunta 0.79). La reclassificació mesurada per l'NRI

després de l'addició separada de ST2 al model amb factors de risc establerts i NT-proBNP es va estimar en 9,90% i la millora de la discriminació integrada en 1,54 (95% CI , 0,29-2,78], p = 0.015).

Un estudi previ per Ky et al (60) també va practicar una valoració multimarcador en una població amb IC crònica. Aquests autors van trobar que la combinació d'ST2 i NT-proBNP oferia una millora moderada en l'estratificació del risc, però, a diferència dels nostres resultats, no van trobar una millora substancial en l'estratificació de risc després de l'addició d'ST2 a un model de clínica amb NT-proBNP (segons l'avaluació de l'NRI i l'estadístic C). Aquesta discrepència es podria explicar en part per les diferències de les dues poblacions d'estudi (més jove i menys greu per valors dels marcadors més baixos en l'estudi de Ky) i en el temps de seguiment (més llarg en el nostre treball). Cal destacar que en el model clínic de Ky et al. l'AUC va ser més alta (0.81 vs 0.76), fet que podria dificultar millorar la predicció de risc a l'afegir variables al model.

Així, en la nostra població l'ST2 afegeix informació pronòstica independent i addicional al NT-proBNP per predir el risc de mortalitat global sobre un model variables pronòstiques que inclou l'NT-proBNP . La millora en l'avaluació del risc es va mantenir quan es van utilitzar mesures de rendiment que avaluen la discriminació, reclassificació i el calibratge del model, i l'ajust del model global.

Aquest treball aporta noves dades sobre el valor pronòstic d'ST2 i del valor complementari a l'NT-proBNP en el medi ambulatori i de confirmar-se els nostres resultats en altres sèries podria aplicar-se a la pràctica clínica habitual per predir el risc de mortalitat. Aquesta és una cohort de grandària raonable de pacients amb IC amb una taxa de mortalitat elevada, i en aquest sentint ofereix una informació incremental clínicament significativa.

7.1.2 Troponina T d'alta sensibilitat (*high sensitivity cardiac troponin* [hs-cTnT]) i NT-proBNP

de Antonio M, Lupón 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;163(5):821-8.

Seguint el model del treball anterior, en aquest s'analitzaren els canvis en l'estratificació pronòstica amb la incorporació de la troponina T (marcador de lesió miocàrdica)

mesurada amb un assaig d'alta sensibilitat i l'NT-proBNP sobre un model amb les mateixes variables pronòstiques descrites.

La troponina T mesurada amb l'assaig convencional (c-TnT) és un bon predictor de mort en la IC aguda (66-65) i crònica (66). Amb els assaigs d'alta sensibilitat (hs-cTnT), la gran majoria de pacients amb IC presenten nivells detectables i/o elevats i s'han associat també amb el risc de mort, però en la IC crònica l'evidència prové d'estudis petits (72,133,134) amb poc seguiment i d'un assaig randomitzat (70). Els mecanismes d'elevació de troponina en la IC no estan aclarits i probablement intervenen múltiples factors com la isquèmia subendocàrdica per l'augment d'estrès transmural i rigidesa del miocardi, la necrosi i apoptosi de miòcits, l'alliberament de productes de degradació de la troponina i l'augment permeabilitat de la paret cel·lular a causa d'una lesió reversible.

Es van incloure 876 pacients consecutius (edat mediana 70.3 anys, fracció d'ejecció ventricular esquerra mediana 34%) amb IC crònica. Durant un seguiment mitjà de 41.4 mesos 311 pacients van morir.

Tots els pacients tenien nivells detectables de hs-cTnT (mediana 22.6 ng/L) i els seus nivells es correlacionaren amb diverses variables clíniques com l'edat, el filtrat glomerular renal i els nivells d'NT-prBNP i de forma inversa i dèbil amb la FE i el temps d'evolució de la IC. En l'anàlisi de regressió multivariat les variables associades amb nivells de hs-cTnT per sobre del punt de tall foren el sexe, classe funcional, diabetis mellitus, filtrat glomerular renal i l'NT-proBNP.

Entre l'associació dels nivells de hs-cTnT cal destacar la classe funcional NYHA. Els pacients més simptomàtics presentaven nivells més alts de hs-cTnT, el que suggereix que en formes avançades de la malaltia hi ha més lesió miocàrdica persistent, que condiciona la progressió de la mateixa. D'entre la resta de cardiopaties d'etiologia no isquèmica, la miocardiopatia hipertensiva va ser la que va presentar nivells més alts de hs-cTnT. Tot i tractar-se d'una anàlisi per subgrups aquesta troballa és remarcable i podria explicar-se perquè hipertrofia ventricular secundària a la hipertensió arterial condiciona isquèmia subendocàrdica, resultant en nivells més alts de marcadors de lesió miocàrdica i progressió del remodelat cardíac. En pacients hipertensos sense IC s'ha descrit que els nivells de cTnT són predictors d'events cardiovasculars futurs (134) i en la població general asimptomàtica (135-137), els nivells de troponina elevats es correlacionen amb cardiopatia estructural i esdeveniments cardiovasculars. Els pacients

diabètics també presentaren nivells més alts de hs-cTnT, en línia amb altres estudis en població general sense cardiopatia coneguda (138). No es coneix el mecanisme exacte però es postula que podria estar en relació amb malaltia microvascular i mecanismes lipotòxics (139,140). Respecte a la relació amb el filtrat glomerular renal, s'ha descrit a altres estudis com la cTnT i la hs-cTnT són potents predictors pronòstics de mortalitat i esdeveniments cardiovascular en pacients amb insuficiència renal. No està ben definit encara com es metabolitza la troponina T però s'assumeix que es fa principalment per via renal i en part podria explicar l'augment dels nivells (141). No obstant, un estudi en pacients amb insuficiència renal sense cardiopatia coneguda va evidenciar que no tots els pacients amb molt baix filtrat glomerular tenen nivells detectables de hs-cTnT (142). Els possibles mecanismes són també diversos i inclouen la disfunció endotelial i l'estrés oxidatiu.

A l'anàlisi de regressió de Cox ambdós biomarcadors (hs-cTnT i NTproBNP) es van associar amb la mortalitat i dita associació es va mantenir de forma independent a l'anàlisi multivariat. Per *bootstraping* es van identificar els punts de tall òptims i es va dividir la població en quatre grups segons els nivells dels biomarcadors per sobre o per sota del punt de tall. La combinació d'ambdós biomarcadors per sobre dels punts de tall va augmentar significativament el risc de mort (HR 7,42), identificant així un subgrup de molt al risc (supervivència del 28% als 5 anys pels dos biomarcadors elevats vs 86% pels pacients amb biomarcadors per sota del punt de tall segons les corbes de Kaplan-Meier). Avaluant individualment els dos marcadors, tenir un nivells de hs-cTnT per sobre del punt de tall va condicionar un pitjor pronòstic que no pas l'elevació aïllada d'NT-proBNP (HR 3.88 vs HR 1.73).

En incloure els 2 biomarcadores individualment en un model amb factors de risc de mortalitat establerts la hs-cTnT va millorar l'estratificació pronòstica en major grau que l'NT-proBNP, segons les mesures de discriminació, calibratge i reclassificació (NRI de 7,7% i 1,5%, respectivament). En afegir la hs-cTnT al model clínic que incorporava l'NT-proBNP, també va millorar l'AUC, el calibratge i la reclassificació.

En resum, la hs-cTnT millora l'estratificació del risc de mort en una població ambulatòria, de manera aïllada o en combinació amb l'NT-proBNP sobre un model de factors de risc establerts. A més a més la hs-cTnT s'erigeix com a marcador molt potent.

7.1.3 Hs-cTnT, ST2 i NT-proBNP

Lupón J, de Antonio M, Galán A, Vila J, Zamora E, Urrutia A, Bayes-Genis A. Combined Use of the Novel Biomarkers High-Sensitivity Troponin T and ST2 for Heart Failure Risk Stratification vs Conventional Assessment. Mayo Clin Proc 2013;88(3):234-43. Erratum in: Mayo Clin Proc. 2013 May;88 (5):532.

L'objectiu de l'article va ser valorar si una estratègia multimarcador de tres biomarcadors de mecanismes fisiopatològics de la IC (hs-cTnT per lesió miocàrdica, ST2 per fibrosi i NT-proBNP estrès miocardíac) podria millorar la capacitat pronòstica sobre un model clínic en una població amb IC crònica.

L'estudi es derivà de 864 pacients (72% homes, edat mitja 68,2 anys, 91,7% en classe II-III de la NYHA, FE 36%, etiologia isquèmica 52,2%), seguits durant una mediana de 3,4 anys de mediana (305 defuncions). Pel que fa a les causes de mort, en 168 pacients l'etiologia fou cardiovascular, i d'entre aquestes la IC fou la més freqüent en el 54% dels casos, seguida per la mort sobtada en el 17.8% i infart agut de miocardi en 8.9% dels casos.

A l'anàlisi de Cox, els 3 biomarcadors van ser predictius de mort per qualsevol causa, aquesta associació es va mantenir a l'anàlisi multivariant. En avaluar les morts de causa cardiovascular la combinació de ST2 i hs-cTnT romangueren associats amb la mortalitat però no l'NT-proBNP.

Després de determinar els millors punts de tall pronòstics per *bootstrapping*, d'entre les possibles combinacions en parelles dels biomarcadors, la combinació de nivells d'ST2 i hs-cTnT per sobre dels punts de tall va ser la que es va associar a un major risc de mortalitat (HR 11.69) i per tant va identificar un subgrup de molt alt risc. L'NT-proBNP només va aportar valor afegit en pacients amb valors d'ST2 i hs-cTnT per sota del punt de tall en algun dels altres dos biomarcadors estudiats.

Sobre el model de variables clíniques es van crear 4 models més de combinacions de biomarcadors. L'AUC del model de variables clíniques va ser de 0.76, comparable a altres models que s'utilitzen a al pràctica clínica (121), i va millorar amb l'addició de qualsevol combinació de biomarcadors. Tots els models van estar ben calibrats, però la combinació de hs-cTnT i ST2 va presentar els valors més baixos dels diferents *scores*. Afegir NT-proBNP al model dels altres dos biomarcadors no va millorar els paràmetres de reclassificació.

La combinació de hs-cTnT i ST2 per sobre del punt de tall va identificar més defuncions per qualsevol causa i més morts de causa cardiovascular que no pas la presencia simultània dels tres biomarcadors elevats.

Resumint, segons els nostres resultats, els mecanismes de lesió, estirament miocardíac i fibrosi miocardíaca representats pels nivells de hs-cTnT i ST2 condicionen un major mortalitat en la IC crònica, i l'NT-proBNP no aporta informació addicional quant aquest dos biomarcadors estan elevats. Tanmateix, en el subgrup de pacients amb nivells de hs-cTnT i/o ST2 per sota del punt de tall l'NT-proBNP si va aportar valor pronòstic. Aquestes troballes són importants per diversos motius: 1) indiquen que no necessàriament l'estratificació pronòstica és millor amb la suma més i més marcadors; 2) desafien el paper del NT-proBNP com l'únic biomarcador establert: i 3) obren noves vies d'investigació per valorar si disminuir els nivells d'aquests biomarcadors milloraria el pronòstic.

Com a breu síntesi d'aquest bloc, podem dir que els novells biomarcadors ST2 i hs-cTnT no només han demostrat tenir un potent valor predictiu de mort en pacients amb IC, superior al del biomarcador profusament establert NT-proBNP, sinó que combinats milloren les mesures de rendiment predictiu, pel que es podria replantejar el paper de l'NTproBNP com a únic marcador pronòstic.

7.2. Comparació de biomarcadors d'un mateix procés fisiopatològic

7.2.1 Hs-cTnT i sc-cTnI

de Antonio M, Lupón J, Galán A, Vila J, Zamora E, Urrutia A, Díez C, Coll R, Altimir S, Bayes-Genis. Head-to-head comparison of high-sensitivity troponin T and sensitive-contemporary troponin I regarding heart failure risk stratification. Clin Chim Acta 2013;426:18-24.

L'objectiu va ser comparar dos biomarcadors de lesió miocardíaca, hs-cTnT i troponina I amb sensibilitat contemporània (sc-cTnI) disponibles a la pràctica clínica de manera comercial, en termes de la seva capacitat pronòstica per sobre d'un model de variables clíniques.

Es van incloure 876 pacients (FE mediana 34%, etiologia isquèmica 52%) amb IC crònica. Durant una mediana de seguiment de 3.45 anys van morir 311 pacients.

Ambdós assaigs analítics van ser predictius de mortalitat i afegeixen informació pronòstica a un model amb factors de risc ben establerts.

Tots els pacients van presentar nivells detectables de hs-cTnT mentre que només el 31,5% dels pacients tenien nivells detectables de sc-cTnI. Si tenim en compte els valors per sobre del percentil 99, el 59,5% dels pacients tenien nivells de hs-cTnT per sobre del percentil 99, mentre que només el 15,6% d'ells tenia nivells sc-cTnI per sota del percentil 99. Els pacients amb alts nivells de hs-cTnT sovint tenien baixos nivells de sc-cTnI, però inesperadament, alguns pacients amb alts nivells de sc-cTnI presentaven nivells relativament baixos de hs-cTnT, tot i ser un assaig analític molt més sensible. Aquesta diferència en la incidència de detecció de les dues troponines té diverses explicacions possibles. La diferència en la sensibilitat dels assaigs, sent el hs-cTnT més sensible és probablement un dels motius principals. Una altra hipòtesi especulativa és que tinguin un patró d'alliberament diferent o bé presentin canvis estructurals en context de la IC. Aquestes darreres hipòtesis també podria ajudar a explicar la discrepància observada en alguns casos.

Hi ha pocs estudis que comparin les dues troponines en IC. Ilva et al (143), utilitzant assaigs convencionals mostrà que els nivells de cTnI eren més alts que els de cTnT (51% enfront de 30%) en la IC aguda. Amb l'ús d'assaigs d'alta sensibilitat, en un petit estudi de pacients amb IC crònica amb indicació de cateterisme cardíac dret (144) es van comparar ambdues troponines amb tècniques d'alta sensibilitat, i contràriament a les nostres troballes, van trobar valors de la hs-cTnI ser per sobre del percentil 99 en una major proporció de pacients que en el cas de la hs-cTnT (47.6% vs 35,9%). La comparació de diferents assaigs de troponina es complexa per la manca d'estandardització i consens internacional dels assaigs de la cTnI que presenten diferències en la imprecisió analítica. Hi ha nombrosos assaigs analítics per la cTnI, sc-cTnI i la hs-cTnI però només hi ha un únic assaig per cTnT i hs-cTnT. A més a més, hi ha múltiples factors que poden afectar els mesuraments de cTnI com la modificació postranscripcional (per degradació proteolítica i fosforilació) i la formació de complexes amb altres (molècules (heparina, anticossos heteròfils i autoanticossos (145). No obstant, l'assaig sc-cTnI utilitzat en aquest estudi reconeix epítops de la part central de la molècula de troponina, que són més estables, no s'afecten per modificacions postranscripcionals, i la interferència amb anticossos heteròfils s'ha minimitzat.

Malgrat les diferències en la prevalença, ambdues troponines van ser predictives de mortalitat per qualsevol causa de manera independent en l'anàlisi multivariable. Els nostres resultats estan d'acord amb estudis previs que mostren que tant sc-cTnI i hs-cTnT s'associen amb una major morbiditat i mortalitat en IC (59, 68, 70-72, 132). L'addició de sc-cTnI i hs-cTnT a un model amb factors de risc de mortalitat establerts (incloent NT-proBNP) va millorar les mesures de rendiment, sobretot quan s'utilitzen els punts de tall estimats per *bootstraping*. Tanmateix, la hs-cTnT prediu millor la mortalitat que la sc-cTnI i va identificar una proporció més gran de morts durant el seguiment.

El millor punt de tall per a hs-cTnT va ser de 16 ng/L, lleugerament superior a la sensibilitat funcional de referència (13 ng/L), i 1 ng/L per a sc-cTnI. La utilització d'aquests punts de tall va mostrar millors resultats globals amb finalitats pronòstiques que no pas l'ús del percentil 99. En el cas de la sc-cTnI el punt de tall estimat per *bootstraping* està molt per sota del coeficient variació al percentil 99 <10% (50 ng/L), i malgrat tenir valor pronòstic, des del punt de vista clínic probablement fora més recomanable utilitzar els valors del percentil 99.

En l'anàlisi del subgrup segons els valors de FE, en els pacients amb FEVE ≥45% ambdues troponines també foren predictives de mortalitat en l'anàlisi univariant. En canvi, no van millorar les mesures de discriminació i reclassificació, suggerint que la seva aplicació en aquest subgrup de pacients pot ser limitada. Cal destacar que en els pacients amb FEVE ≥45% l'elevació de sc-cTnI , tot i ser menys freqüent, comporta un pitjor pronòstic.

7.2.2 Galectina-3 i ST-2

Bayes-Genis A, de Antonio M, Vila J, Peñafiel J, Galán A, Barallat J, Zamora E, Urrutia A, Lupón J. Head-to-head comparison of 2 myocardial fibrosis biomarkers for long-term heart failure risk stratification: ST2 versus galectin-3. J Am Coll Cardiol 2014;63(2):158-66.

En aquest treball es compararen dos marcadors de fibrosi miocardíaca i remodelatge, la galectina-3 i l'ST2 en quant a la seva capacitat pronòstica addicional a un model de variables clíniques establertes que conté NT-proBNP.

La fibrosi és un component fonamental del remodelatge estructural advers en la IC i en la progressió de la disfunció cardíaca (32) i hi ha múltiple substàncies implicades. L'ST2 i la

galectina-3 s'han associat a remodelatge ventricular i pronòstic en la IC crònica (60, 82, 84).

La població d'estudi va ser de 876 pacients (edat mediana 70 anys, FE mediana 34%) amb IC crònica, havent-se enregistrat 392 defuncions durant un seguiment de 4.2 anys.

Tant l'ST2 com la galectina-3 es van associar amb un major risc de mortalitat global i cardiovascular en l'anàlisi bivariant. No obstant això, en incorporar altres variables en l'anàlisi multivariant la galectina-3 únicament es va correlacionar amb la mortalitat global mentre que l'ST2 va mantenir l'associació amb la mortalitat global i cardiovascular. Pel que fa a les mesures de rendiment del model, l'ST2 va millorar la discriminació, calibratge i reclassificació del model, però l'addició de galectina-3 al model no va comportar cap milloria. Concretament, no va augmentar significativament la discriminació segons l'avaluació de l'estadístic C del model de referència. En canvi, la incorporació d'ST2 al model va millorar l'estadístic C, que va augmentar de 0.757a 0.77 (p=0,004). En totes les anàlisis de calibratge l'ST2 va ser més precís. El model amb ST2 va augmentar significativament l'IDI i l'NRI per totes les causes i la mortalitat cardiovascular, indicant una bona capacitat de reclassificació. Per contra, la galectina-3 no va tenir efecte o fins i tot va empitjorar la reclassificació. En l'anàlisi de la mortalitat cardiovascular la galectinal-3 va empitjorar la reclassificació amb un NRI -4,2.

Els estudis previs que havien valorat el valor predictiu de la galectina-3 en conjunció amb els pèptids natriurètics han estat contradictoris. En una sèrie de 232 pacients (85) la galectina-3 va ser predictiva de la mortalitat de manera significativa, fins i tot després d'ajustar per NT-proBNP. Per contra, Felker et al. i Gullestad et al. (86,87), en sèries més grans de pacients amb IC crònica i amb seguiment a llarg termini, trobaren que la galectina-3 va ser predictiva d'esdeveniments en l'anàlisi univariant però aquesta associació no va persistir després de l'ajust per a altres factors predictius. Contràriament, l'ST2 s'ha demostrat ser un biomarcador robust en totes les cohorts estudiades amb o sense biomarcadors addicionals, incloent-hi els pèptids natriurètics (60, 62, 146, 147).

Una possible hipòtesi per explicar l'escàs valor complementari de la galectina-3 en la nostra població d'estudi, en la qual el remodelatge pot estar en una fase avançada, és que la galectina-3 estès implicada en les fase inicials del procés. De fet, estudis recents han mostrat que nivells més alts de galectina-3 s'associen amb major risc d'IC de nova aparició en població aparentment sana (89). D'altra banda, pacients amb un infart agut

de miocardi i disfunció ventricular mostren nivells més alts de Gal-3 (88). No obstant això, Nativí et al. (148) van reportar recentment que en pacients trasplantats els nivells de galectina-3 es mantenien elevats mesos després del trasplantament, el que pot suggerir que la galectina-3 és un marcador sistèmic d'inflamació i menys específic de la IC.

La comparació de dos biomarcadors de fibrosi de nova generació mostra com l' ST2 aporta valor addicional als factors de risc establerts i NT-proBNP, mentre que el valor additiu de galectina-3 és trivial. Nous estudis haurien de confirmar si aquesta superioritat de ST2 és present en totes les etapes del continu de la IC.

7.2.3 Cistatina-C i filtrat glomerular renal

Zamora E, Lupón J, de Antonio M, Vila J, Galán A, Gastelurrutia P, Urrutia A, Bayes-Genis A. Limited value of cystatin-C over estimated glomerular filtration rate for heart failure risk stratification. PLoS One 2012;7(12):e51234

Comparem en aquest estudi el valor pronòstic del filtrat glomerular renal estimat amb la fórmula de Cockroft-Gault i els nivells de la cistatina-C en pacients amb IC crònica. És una proteïna de mida petita que es filtra lliurament pels glomèruls i és bon estimador del filtrat glomerular (95, 149, 150). Les seves concentracions s'associen a la mortalitat en la cardiopatia isquèmica (151) i en la IC aguda (96). En la IC crònica també s'ha relacionat amb el pronòstic però l'evidència prové d'estudis petits (97,152). Hi ha estudis que suggereixen que la seva capacitat com biomarcador s'associa no només al fet d'estimar la funció renal sinó que està en relació amb el remodelatge i la rigidesa de la paret vascular (153).

Varen participar 879 pacients (72% homes, edat mediana 70.4 anys, 52% etiologia isquèmica, FE mediana 34%, 65% en classe funcional II NYHA), dels quals 312 van morir al llarg d'un seguiment de 3.46 anys.

Tant la cistatina-C com el filtrat glomerular estimat per la fórmula de Cockroft-Gault van ser predictius de mortalitat en l'anàlisi bivariant. Les corbes de supervivència segons els valors dels biomarcadors dividits en quartils van mostrar una clara influència en el pronòstic. En les anàlisis multivariants de regressió ambdós es van mantenir com factors predictius independents. No obstant això, en el model multivariant que incorporava ambdues variables i la interacció entre elles, només el filtrat glomerular renal va romandre independentment associat a mort per qualsevol causa.

El valor predictiu a llarg termini d'acord amb l'AUC (discriminació) i altres mesures de calibratge va ser similar pels dos biomarcadors. D'altra banda, prenent el model amb filtrat glomerular estimat com model de referència, les mesures de reclassificació (IDI i NRI) van empitjorar significativament amb la cistatina-C.

L'addició conjunta dels dos al model de variables de risc clíniques no va millorar significativament les mesures de discriminació, calibratge i reclassificació, excepte quan es va incloure en el model la variable d'interacció entre elles; llavors, el calibratge i algun paràmetre de reclassificació com l'IDI si van millorar i el test de la raó de versemblança si va esdevenir significatiu.

En les corbes de supervivència, a l'analitzar el valors dels dos biomarcadors conjuntament, afegir la cistatina-C al filtrat glomerular renal únicament va millorar l'estratificació pronòstica en els pacients amb insuficiència renal moderada (30-60 ml/min/1,73m2).

En conclusió, en el nostre estudi, la cistatina-C i el filtrat glomerular renal estimat amb la fórmula de Cockroft-Gault van presentar un valor predictiu pronòstic a llarg termini similar sobre un model de factors de risc clínic en pacients amb IC crònica. La cistatina-C va millorar la capacitat pronòstica únicament en el subgrup de pacients amb disfunció renal moderada.

Com a breu síntesi final d'aquest bloc, podem dir que des del punt de vista pronòstic, d'entre els marcadors de fibrosi-remodelatge cardíac estudiats ST2 és superior a galectina-3, d'entre els marcadors de dany miocardíac hs-cTnT és superior a sc-cTnI i pel que fa relació a marcadors de la funció renal cistatina-C no és superior a filtrat glomerular renal estimat amb la fórmula de Cockroft-Gault. No obstant, la cistatina-C millora la discriminació pronòstica en pacients amb insuficiència renal moderada.

7.3. Valor pronòstic de biomarcadors segons l'estat de la funció renal

ST2 i NTproBNP

Bayes-Genis A, Zamora E, de Antonio M, Galán A, Vila J, Urrutia A, Díez C, Coll R, Altimir S, Lupón J. Soluble ST2 serum concentration and renal function in heart failure. J Card Fail 2013;19(11):768-75.

La insuficiència renal és molt prevalent en la insuficiència cardíaca i, a part de condicionar el pronòstic, pot afectar els nivells d'alguns biomarcadors com l'NT-proBNP. L'objectiu principal del treball fou valorar l'associació entre l'ST2 i la funció renal i analitzar el seu valor pronòstic en pacients amb IC crònica i insuficiència renal. En l'estudi es van incloure altres biomarcadors, com l'NTproBNP, la hs-cTnT i la cistatina-C, per tal de comprovar fins quin punt l'ST2 millorava la capacitat predictiva per sobre dels altres biomarcadors. A la literatura hi ha poques referències de la influència de la funció renal sobre l'ST2. Dieplinger et al (154) van conduir l'avaluació analítica i clínica del nou assaig ST2 Pressage en plasma humà. En aquest estudi pilot, les concentracions plasmàtiques d'ST2 van ser similars en pacients amb insuficiència renal i els individus sans. Cal remarcar que era un estudi petit amb 15 pacients amb IC i sense disfunció renal i 15 pacients amb disfunció renal sense IC. Més recentment, un altre estudi de 69 pacients (155) va observar que la concentració sèrica d'ST2 es correlaciona amb la severitat de la malaltia renal crònica, però no es van aportar dades sobre la funció cardíaca. No hi ha dades a la literatura sobre l'ST2 en coexistència d'IC i disfunció renal.

Durant 3.46 anys es van seguir 879 pacients (edat mediana 70 anys, 71.8% homes) amb IC crònica, amb un total del 312 defuncions.

Els nivells d'ST2, NT-proBNP i hs-cTnT es van correlacionar inversament amb el filtrat glomerular renal, però la correlació va ser molt més intensa per l'NT-proBNP. Els pacients es van dividir d'acord amb els valors dels filtrat glomerular renal en tres categories. En els pacients més simptomàtics (classe funcional NYHA III-IV) els nivells d'ST2 van ser semblants en els tres subgrups de pacients, mentre que els nivells d'NT proBNP va augmentar d'acord amb l'empitjorament de la funció renal. De tal manera, en formes més avançades de la malaltia, les concentracions d'ST2 es veuen menys afectades que les d'NT-proBNP per la funció renal.

En les corbes de supervivència, la combinació dels dos marcadors va millorar la capacitat pronòstica en la població amb insuficiència renal. El subgrup de població amb dos

biomarcadors per sobre dels punts de tall determinats per *bootstrapping* tenia una supervivència significativament menor. La combinació d'ST2 i hs-cTnT en pacients amb insuficiència renal va obtenir resultats similars. En incorporar l'ST2 i l'NT-proBNP en un model pronòstic de variables clíniques, cistatina C i hs-cTnT ambdós van mostrar la seva capacitat per estratificar els pacients amb insuficiència renal segons el pronòstic, però l'addició d'ST2 per sobre de tots els altres biomarcadors va millorar significativament el rendiment. Fins i tot, la reclassificació obtinguda va ser més gran en el subgrup de pacients amb insuficiència renal que en el total de la cohort estudiada. En l'anàlisi multivariant de regressió de Cox l'ST2 es va mantenir com un factor pronòstic independent, mentre que l'NTproBNP va perdre la significació estadística. Atès que la insuficiència renal condiciona un estat inflamatori afegit a la de la IC, i l'ST2 és també un marcador d'inflamació, podria justificar la bona capacitat predictiva de l'ST2 en la insuficiència renal.

En resum, segons els nostres resultats, la capacitat pronòstica de l'ST2 es manté (i fins i tot és millor) en pacients amb insuficiència renal i d'altra banda, la combinació amb NT-proBNP i altres marcadors millora la capacitat pronòstica d'un model de variables clíniques en pacients amb insuficiència renal.

7.4. Elaboració d'una calculadora de risc per pacients amb IC, amb la incorporació de biomarcadors i variables clíniques: Barcelona Bio-HF- risk calculator (BCN Bio-HF calculator)

Lupón J, de Antonio M, Vila J, Peñafiel J, Galán A, Zamora E, Urrutia A, Bayes-Genis. Development of a novel heart failure risk tool: the barcelona bio-heart failure risk calculator (BCN bio-HF calculator). PLoS One 2014;9(1):e85466.

La predicció del risc en IC és complexa però és important perquè condiciona les decisions que prenem en quant al tractament farmacològic i no farmacològic. Els models pronòstics poden ser útils per millorar la nostra precisió. N'existeixen diversos, més o menys sofisticats i més o menys fàcils d'aplicar o utilitzar en la pràctica diària. Cap dels models desenvolupats fins ara incorpora biomarcadors.

Per passar de la teoria dels estudis previs a la pràctica, es va elaborar una calculadora accessible online (http://www.BCNBioHFcalculator.cat) que permet el càlcul de l'estimació pronòstica individual de forma ràpida i senzilla en forma de risc de mort a 1,2 i 3 anys i d'esperança de vida.

En la població de derivació es van incloure 864 pacients ambulatoris amb IC, amb predomini d'homes (72%), edat mitjana de 68.2, etiologia isquèmica i amb un seguiment de 3.4 anys.

Després d'avaluar 23 variables, s'inclogueren finalment a la calculadora 8 models (1 sense biomarcadors i 7 amb diferents combinacions) amb 11 variables clíniques significatives: edat, sexe, classe funcional NYHA, FE, filtrat glomerular renal, sodi, hemoglobina, dosi diària diürètic de nansa i tractament amb beta-blocadors, IECAs/ARA-II i estatines.

La calculadora ha demostrat un bon calibratge en els seus 8 models (mesurat pel test de Hosmer-Lemeshow) i una bona discriminació. En l'anàlisi de validació interna *10-fold-cross validation* amb 1000 replicacions l'estadístic C mitjà fou 0,79. Pel que fa als paràmetres de reclassificació s'obtingueren millors resultats en els models amb més d'un biomarcador, sent el més òptim el de la combinació de hs-cTnT i ST2.

La incorporació dels biomarcadors modifica substancialment el pronòstic. A títol d'exemple, un home de 68 anys d'edat, en classe III NYHA, FEVE 30%, sodi 130

mmol/L, filtrat glomerular renal 45 ml/min/m 2, hemoglobina 12 g/dl, amb dosi de 60 mg de furosemida i tractament amb estatines, IECA i beta-blocadors presenta un risc de mort del 22%, 42% i 60% als 1,2 i 3 anys, respectivament. En afegir els següents nivells de biomarcadors: hs-cTnT 14 ng/L, ST2 40 ng/ml i NTproBNP 900 ng/L, el risc es redueix a 10%, 21% i 32%. No obstant, això, si introduïm nivells més alts de biomarcadors, hs-cTnT 70 ng/L, ST2 140 ng/ml, i NTproBNP 2500 ng/L el risc augmenta 35%, 62% i 80%, respectivament.

Els models de predicció de risc de mortalitat en IC presenten una àmplia variació en el grau de validació i en la forma d'expressar el pronòstic, que va des de la classificació en categories de risc (baix-alt risc, risc baix-mitjà-alt, decils de risc) (120,156-158), passant per l'esperança de vida (124) o la mortalitat individual (121, 124, 156,158). En la majoria d'aquests models el gruix de la població de derivació no prenia cap dels tractaments actuals basats en l'evidència, que modifiquen el curs de la malaltia com els IECA/ARA II, beta-blocadors i espironolactona/eplerenona, o estaven inclosos en assaigs clínics, o bé es van dissenyar per pacients hospitalitzats (117,159). En el *Seattle Heart Failure Model* (121*)*, l'efecte relatiu dels fàrmacs no es va poder obtenir de la cohort de derivació i es van estimar a partir dels assaigs publicats o metanàlisis. En la nostra cohort contemporània de pacients ambulatoris, el 87% rebien tractament amb beta-blocadors , el 90% amb d'IECA/ARA II, i el 40% amb espironolactona/eplerenona. Considerant l'efecte de reducció sobre la mortalitat de dits fàrmacs és recomanable que estiguin inclosos en una calculadora de risc. De fet, el risc estimat varia significativament amb tractament, tant en el model sense biomarcadors com en el que sí conté biomarcadors.

Alguns models (157, 158, 121) s'han derivat i validat en poblacions nombroses provinents d'assaigs clínics, amb la limitació conseqüent que poden ser poc representatives de la població de la pràctica clínica habitual. A més, molts d'aquests estudis es van fer fa més d'una dècada, i cap inclou mesures de biomarcadors. Un dels models pronòstics més recents, el 3C-HF (124), realitzat en una població mixta de pacients ambulatoris inclosos tant prospectiva com retrospectivament sí va incloure el tractament contemporani de la IC però cap biomarcador. A l'estudi Val-HeFT, la inclusió del BNP al *Seattle Heart Failure Model Seattle* va augmentar el AUC en 0,03 (121). A la calculadora BCN Bio-HF, es van incloure tres biomarcadors complementaris disponibles al mercat que proporcionen informació sobre la necrosi dels miòcits (hs-cTnT), fibrosi, remodelatge i inflamació (ST2), i l'estrès miocardíac (NT-proBNP). Altres biomarcadors que s'estan analitzant en la IC no estan disponibles al mercat i altres reflecteixen les vies que es superposen els utilitzats

aquí. Tal i com ha quedat exposat als apartats previs, el nostre grup de treball (160-162) i altres investigadors ha valorat prèviament la utilitat pronòstica d'aquests tres biomarcadors. La calculadora pot funcionar amb la disponibilitat de cap, un, dos (qualsevol combinació) o els tres biomarcadors (d'aquí la creació de 8 models independents). Aquesta característica és única d'aquesta nova eina, i en combinació amb l'ús de metodologia estadística d'última generació amb paràmetres de discriminació, calibratge i la reclassificació, aporten solidesa al model.

El *Seattle Heart Failure Model* (121) és probablement el model més àmpliament utilitzat. Es va validar prospectivament en diversos assaigs. L'AUC de validació varià entre 0,68-0,81, amb una AUC global de 0,73 i una AUC de menys de 0,70 en les tres principals cohorts. Pel 3C-HF (124) l'AUC va oscil·lar entre 0.68 i 0.83. L'ús del coeficient de correlació "Dxy" de Somers en l'anàlisi de l'estadístic C incorpora informació de les dades censurades i és més correcte des del punt de vista de la supervivència que els models de regressió logística.

Vam aplicar el *Seattle Heart Failure Model* i l'*score* 3C-HF a la nostra població (encara que amb algunes limitacions al no disposar de les dades de totes les variables). L'estadístic C utilitzant el coeficient de correlació Somers "Dxy" va ser de 0.71 i 0.73 respectivament.

Convé ressaltar que tant el *Seattle Heart Failure Model* com la calculadora Bio-HF BCN proporcionen el risc individual de mort en diversos moments i donen una estimació de l'esperança de vida. Es més, la calculadora BCN Bio-HF permet avaluar els pacients de forma dinàmica, ja que és sensible als canvis en les concentracions plasmàtiques dels biomarcadors i permet observar com varia el risc de cada pacient de forma individual amb les modificacions dels nivells aconseguides amb els tractaments adients. La darrera determinació és la que marca el pronòstic en cada moment.

Moltes de les variables del nostre model s'inclouen també en *Seattle Heart Failure Model*, encara que aquest en té més. Algunes variables que poden ser considerades clínicament importants, com els dispositius, antagonistes de l'aldosterona i la pressió arterial sistòlica, van ser excloses del nostre model per manca de significació estadística en el model multivariable. En el cas de dispositius podria haver estat influenciat pel nombre reduït de pacients amb DAI o TRC.

Recentment, Ky et al. (102) va mostrar que l'addició d'un panell de biomarcadors més complex que consta de proteïna C reactiva d'alta sensibilitat, mieloperoxidasa, BNP, forma soluble del receptor tirosina-quinasa 1, troponina I, ST2, creatinina i àcid úric afegit al *Seattle Heart Failure Model* millorava la precisió de la predicció a 1 any per mort de totes les causes, amb un estadístic C de fins a 0,8. Els nostres models clínic i amb biomarcadors van mostrar un rendiment semblant tot i ser molt menys complexes i incloure menys biomarcadors. Determinar quins biomarcadors es poden implementar realment a la pràctica clínica dependrà de factors com ara el cost i la facilitat de l'assaig, entre d'altres.

7.5. Limitacions

Els nostres estudis presenten com a possibles limitacions:

1) La població d'estudi, que tot i tenir l'avantatge de ser un població del "món real", té les seves peculiaritats que podrien fer que els resultats no necessàriament siguin extrapolables a altres poblacions. Es tracta d'una cohort relativament jove, de predomini masculí, amb fracció d'ejecció deprimida, ben tractats farmacològicament però amb baixa utilització de DAI i TRC i seguits en un hospital terciari. Malgrat això, el seguiment estricte dels pacients i l'adequació dels tractament dona solidesa als resultats. La mida de la cohort no és menyspreable i el seguiment és suficientment llarg per treure conclusions. A més a més, la calculadora s'ha validat posteriorment en una cohort externa de Boston amb IC amb FE deprimida (Rev Esp Cardiología, en premsa).

2) No s'han practicat determinacions seriades dels biomarcadors que podrien haver millorat la capacitat pronòstica dels models i aportar informació sobre el com es modifiquen amb el tractament de la IC.

3) Els biomarcadors s'han mesurat de mostres congelades de sèrum dels pacients i desconeixem si en el cas d'algun dels biomarcadors estudiats això pot afectar els seus nivells. Tanmateix, en cap dels assaigs utilitzats s'ha descrit que s'afectin en les temperatures utilitzades i molts dels estudis publicats a la literatura utilitzen mostres congelades.

8. CONCLUSIONS

En la valoració de diversos biomarcadors de diferents procesos fisiopatològics en pacients amb insuficiència cardíaca crònica seguits en una Unitat d'Insuficiència Cardíaca Multidisciplinària hem observat que:

1. La combinació de ST2 i NT-proBNP sobre un model de factors de risc establerts millora l'estratificació pronòstica de mortalitat global.

2. La hs-cTnT té una bona capacitat pronòstica i es correlaciona amb diverses variables clíniques. L'ús complementari a l' NT-proBNP sobre un model de factors de risc clàssics confereix una major capacitat predictiva de mortalitat.

3. En l'estratègia multimarcador amb hs-cTnT, ST2 i NT-proBNP, el model amb ST2 i hs-cTnT va mostrar un millor rendiment, pel que fa a la reclassificació, que el model amb els tres biomarcadors.

4. La comparació de dos biomarcadors de fibrosi, ST2 i galectina-3 va mostrar que només l'ST2 afegia valor complementari sobre el model de factors de risc clàssics, tot i que els dos van ser marcadors independents de mortalitat. L'ST2 va ser també predictiu de mortalitat cardiovascular.

5. En l'anàlisi comparatiu dels biomarcadors de lesió miocardíaca, hs-cTnT i sc-TnI ambdós van ser predictius de mortalitat, però la hs-cTnT va mostrar globalment millors mesures de rendiment.

6. La cistatina-C i el filtrat glomerular estimat per la fórmula de Cockroft-Gault tenen un rendiment pronòstic similar. Avaluats conjuntament, la cistatina-C va afegir capacitat predictiva al filtrat glomerular en el subgrup de pacients amb insuficiència renal moderada.

7. L'ST2 manté la seva capacitat pronòstica en els pacients amb insuficiència renal i fins i tot estratifica millor el risc en el subgrup de pacients amb disminució del filtrat glomerular renal.

8. La calculadora de risc mortalitat en IC *BCN Bio-HF risk calculator* és una eina pronòstica que per primera vegada incorpora biomarcadors disponibles en la pràctica clínica i que milloren la capacitat predictiva del model. Expressa el risc individual de

mort a 1, 2 i 3 anys i l'esperança de vida. És accessible *online* (wwwBCNbiohfcalculator.cat) i de fàcil utilització.

D'aquestes conclusions se'n poden derivar aplicacions a la pràctica clínica habitual de pacients amb IC crònica:

A. La determinació de biomarcadors de forma individual amb un procediment senzill com una analítica aporta informació pronòstica addicional als pèptids natriurètics. Aquests, però, presenten limitacions en algunes situacions clíniques i els seus nivells es veuen afectats per altres condicions no cardíaques, de tal manera que en certs escenaris com la insuficiència renal altres biomarcadors com l'ST2 poden ser més útils si només es pot disposar d'un únic marcador.

B. Una millora en l'estratificació de risc pot ser de gran utilitat en la consulta clínica per millorar el maneig de la IC per diversos motius: 1) per identificar pacients d'alt risc tributaris d'un seguiment més estret i intensificació de tractament farmacològic 2) per racionalitzar la indicació de teràpies costoses i complexes, sobretot en fases més avançades la malaltia (dispositius o trasplantament/assistències ventriculars).

C. La calculadora *BCN Bio-HF calculator* permet estimar, de manera fàcil i accessible *online,* el risc individual de mort en pacients amb IC amb paràmetres clínics i es poden incorporar al càlcul biomarcadors que milloren significativament la capacitat pronòstica del model. A més, permet revalorar el pronòstic en el temps segons l'evolució dels nivells de biomarcadors.

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