JACC: HEART FAILURE VOL. 8, NO. 5, 2020

PUBLISHED BY ELSEVIER

Clinical Role of CA125 in Worsening Heart Failure

A BIOSTAT-CHF Study Subanalysis

Julio Núñez, MD, PhD,[a,b] Antoni Bayés-Genís, MD, PhD,[b,c] Elena Revuelta-López, PhD,[b,d] Jozine M. ter Maaten, MD, PhD,[e] Gema Miñana, MD, PhD,[a,b] Jaume Barallat, MD, PhD,[f] Adriana Cserkóová, PhD,[d] Vicent Bodi, MD, PhD,[a,b] Agustín Fernández-Cisnal, MD,[a] Eduardo Núñez, MD, MPH,[a] Juan Sanchis, MD, PhD,[a,b] Chim Lang, MD, PhD,[g] Leong L. Ng, MD, PhD,[h] Marco Metra, MD, PhD,[i] Adriaan A. Voors, MD, PhD[e]

ABSTRACT

OBJECTIVES The aim of this study was to evaluate the association between antigen carbohydrate 125 (CA125) and the risk of 1-year clinical outcomes in patients with worsening heart failure (HF).

BACKGROUND CA125 is a widely available biomarker that is up-regulated in patients with acute HF and has been postulated as a useful marker of congestion and risk stratification.

METHODS In a large multicenter cohort of patients with worsening HF, either in-hospital or in the outpatient setting, the independent associations between CA125 and 1-year death and the composite of death/HF readmission (adjusted for outcome-specific prognostic risk score [BIOSTAT risk score]) were determined by using the Royston-Parmar method (N = 2,356). In a sensitivity analysis, the prognostic implications of CA125 were also adjusted for a composite congestion score (CCS). Data were validated in the BIOSTAT-CHF (Biology Study to Tailored Treatment in Chronic Heart Failure validation) cohort (N = 1,630).

RESULTS Surrogates of congestion, such as N-terminal pro-B-type natriuretic peptide and CCS, emerged as independent predictors of CA125. In multivariable survival analyses, higher CA125 was associated with an increased risk of mortality and the composite of death/HF readmission ($p < 0.001$ for both comparisons), even after adjustment for the CCS ($p < 0.010$ for both comparisons). The addition of CA125 to the BIOSTAT score led to a significant risk reclassification for both outcomes (category-free net reclassification improvement = 0.137 [$p < 0.001$] and 0.104 [$p = 0.003$] respectively). All outcomes were confirmed in an independent validation cohort.

CONCLUSIONS In patients with worsening HF, higher levels of CA125 were positively associated with parameters of congestion. Furthermore, CA125 remained independently associated with a higher risk of clinical outcomes, even beyond a predefined risk model and clinical surrogates of congestion. (J Am Coll Cardiol HF 2020;8:386-97)

From the [a]Cardiology Department, Hospital Clínico Universitario de Valencia, Universitat de Valencia, INCLIVA, Valencia, Spain; [b]CIBER Cardiovascular, Madrid-Spain; [c]Cardiology Department and Heart Failure Unit, Hospital Universitari Germans Trias i Pujol, Badalona, Department of Medicine, Autonomous University of Barcelona, Barcelona, Spain; [d]ICREC Research Program, Germans Trias i Pujol Health Science Research Institute, Can Ruti Campus, Badalona, Spain; [e]Cardiology Department, University of Groningen, University Medical Centre Groningen, Groningen, the Netherlands; [f]Biochemistry Department, Hospital Universitari Germans Trias I Pujol, Badalona, Spain; [g]Division of Molecular and Clinical Medicine, School of Medicine, University of Dundee, Dundee, United Kingdom; [h]Department of Cardiovascular Sciences University of Leicester, Clinical Sciences Wing Glenfield General Hospital Leicester, Leicester, United Kingdom; and the [i]Cardiology Department of Medical and Surgical Specialties, Radiologic Sciences, and Public Health, University of Brescia, Brescia, Italy. The BIOSTAT-CHF was funded by the European Commission (FP7-242209-BIOSTAT-CHF; EudraCT 2010-020808-29) and CIBER Cardiovascular (16/11/00420 and 16/11/00403). Dr. Metra has received consulting honoraria as a committee member for clinical trials from Amgen, Fresenius, and Vifor Pharma; has received consulting honoraria as advisory board member from Bayer; and has received honoraria for speeches from Abbott and Edwards. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.
The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the *JACC: Heart Failure* author instructions page.

Manuscript received September 27, 2019; revised manuscript received December 2, 2019, accepted December 3, 2019.

ISSN 2213-1779/$36.00 https://doi.org/10.1016/j.jchf.2019.12.005

Congestion plays a major role in the pathogenesis of acute heart failure (AHF) syndromes; however, its severity and organ distribution are largely heterogeneous (1,2). Due to the limited accuracy of routine clinical assessment for identifying and monitoring systemic congestion, there is a growing interest in searching for a reliable marker for congestion. For instance, recent evidence shows that the biologically active form of adrenomedullin (bio-ADM) is positively associated with symptoms and signs of congestion and a higher risk of adverse outcomes in patients with worsening heart failure (HF) (3). Along this same line, plasma carbohydrate antigen 125 (CA125) has also emerged as a potential biomarker of congestion in this same setting (4,5). In fact, plasma levels of this glycoprotein are elevated in up to 60% to 70% of patients with AHF, and higher levels were correlated to congestion severity and adverse prognosis. Interestingly, this biomarker has also shown promising properties for monitoring the clinical course and guiding therapy following an episode of AHF (4–6). For instance, in a clinical trial of 380 patients with a recent episode of AHF, CA125-guided therapy was associated with a reduction of 1-year death/AHF-related risk (7). This effect was attributed to a better individualization of patients' decongestive therapy. Despite this evidence, the pathophysiology and clinical role of this glycoprotein in AHF management are not well established. Moreover, its prognostic ability for predicting clinical outcomes must be confirmed, particularly in settings in which traditional prognosticators and clinical surrogates of congestion are included.

The goals of the current study were to: 1) evaluate the independent association between CA125 and the risk of 1-year clinical outcomes (death/AHF readmission); 2) determine whether the prognostic value of this biomarker remains significant after adjusting for clinical proxies of congestion; and 3) relate its values to other HF biomarkers and clinical surrogates of HF severity/congestion.

SEE PAGE 398

METHODS

STUDY SAMPLES. BIOSTAT-CHF cohort (Derivation cohort). The BIOSTAT-CHF (Biology Study to Tailored Treatment in Chronic Heart Failure) was a multicenter, multinational, prospective, observational study that included 2,516 patients with worsening signs and/or symptoms of HF from 69 centers in 11 European countries (8). The recruitment period was 24 months, from December 2010 to December 2012; median follow-up was 21 months (interquartile range: 15 to 27 months). Patients were included after presentation with either new-onset or worsening HF, which was defined as left ventricular ejection fraction (LVEF) ≤40% and/or brain natriuretic peptide >400 pg/ml or N-terminal pro-B-type natriuretic peptide (NT-proBNP) >2,000 pg/ml. Patients were expected and encouraged to be up-titrated to recommended treatment doses.

All patients enrolled in BIOSTAT-CHF provided written informed consent to participate in the study. BIOSTAT-CHF was conducted in concordance with the Declaration of Helsinki, national ethics, and legal requirements, as well as relevant European Union legislation; the study was also approved by national and local ethics committees. The characteristics of the BIOSTAT-CHF cohort have been described elsewhere (8). In brief, most patients were hospitalized for AHF, and the remainder presented with worsening signs and/or symptoms of HF at outpatient clinics.

All deaths and hospitalizations were recorded. The primary outcome of interest was time to a composite of death or unscheduled hospitalization for HF. The co-primary endpoint was time to all-cause mortality. The secondary endpoint was to identify the clinical determinants of CA125.

Validation cohort. Data were validated in the BIOSTAT-CHF validation cohort consisting of 1,738 patients with HF from 6 centers in Scotland, United Kingdom (described in detail elsewhere [9]). In summary, patients ≥18 years of age diagnosed with HF with a previous hospital admission for HF requiring diuretic treatment and current treatment with furosemide ≥20 mg/day or equivalent were included. They had previously not been treated or had received ≤50% of target doses of angiotensin-converting enzyme inhibitors/angiotensin receptor blockers and/or beta-blockers.

CONGESTION ASSESSMENT. A composite congestion score (CCS) was calculated for individual patients at baseline by using a modified and validated algorithm as described by Ambrosy et al. (10). The CCS was calculated by summing the individual scores for orthopnea, peripheral edema, and jugular venous distension. The presence of orthopnea and jugular venous distension contributed to the CCS with 1 point for each. However, peripheral edema used different weights to match its severity: the absence of edema, 0; edema limited to the ankles, 0.33; edema below the

ABBREVIATIONS AND ACRONYMS

AHF = acute heart failure
bio-ADM = biologically active form of adrenomedullin
CA125 = carbohydrate antigen 125
CCS = composite congestion score
cfNRI = category-free net reclassification improvement
CI = confidence interval
eGFR = estimated glomerular filtration rate
GDF = growth differentiation factor
HF = heart failure
HR = hazard ratio
IDI = integrated discrimination improvement
LVEF = left ventricular ejection fraction
NT-proBNP = N-terminal pro-B-type natriuretic peptide
NYHA = New York Heart Association

TABLE 1 Baseline Characteristics Among Quartiles of CA125

	Q1	Q2	Q3	Q4	p Value
Epidemiology					
Age, yrs	67 ± 12	69 ± 12	71 ± 11	68 ± 13	<0.001
Male	441 (74.7)	434 (73.8)	425 (72.2)	442 (75.0)	0.671
Race					0.418
White	581 (98.5)	581 (98.8)	585 (99.3)	584 (99.2)	
Asian	3 (0.5)	3 (0.5)	2 (0.3)	3 (0.5)	
Black	4 (0.7)	1 (0.2)	0 (0.0)	0 (0.0)	
Other	2 (0.3)	3 (0.5)	2 (0.3)	2 (0.3)	
Medical history					
HF hospitalization in the last yr	192 (32.5)	190 (32.3)	178 (30.2)	178 (30.2)	0.719
Hypertension	399 (67.6)	377 (64.1)	362 (61.5)	335 (56.9)	0.001
Diabetes	176 (29.8)	187 (31.8)	197 (33.4)	205 (34.8)	0.294
COPD	97 (16.4)	108 (18.4)	98 (16.6)	103 (17.5)	0.810
Valve surgery	31 (5.3)	40 (6.8)	46 (7.8)	54 (9.2)	0.067
IHD	336 (57.8)	336 (57.9)	316 (54.9)	299 (51.8)	0.116
Coronary revascularization (CABG or PCI)	216 (36.6)	185 (31.5)	209 (35.5)	187 (31.7)	0.147
Stroke	44 (7.5)	53 (9.0)	69 (11.7)	52 (8.8)	0.083
PAD	54 (9.2)	58 (9.9)	73 (12.4)	71 (12.1)	0.197
Symptoms and signs					
Extent of peripheral edema*					
Not present	263 (56.3)	233 (48.6)	161 (33.1)	131 (24.9)	<0.001
Ankle	137 (29.3)	147 (30.7)	156 (32.0)	140 (26.6)	
Below knee	57 (12.2)	83 (17.3)	129 (26.5)	175 (33.3)	
Above knee	10 (2.1)	16 (3.3)	41 (8.4)	80 (15.2)	
Elevated JVP†	68 (17.7)	101 (26.3)	154 (39.9)	197 (50.0)	<0.001
Hepatomegaly	52 (8.8)	62 (10.6)	102 (17.4)	120 (20.5)	<0.001
Orthopnea	129 (21.9)	178 (30.3)	245 (41.6)	267 (45.6)	<0.001
Pulmonary rales >1/3 up lung fields‡	35 (15.6)	48 (19.0)	76 (21.8)	75 (19.5)	0.330
Composite congestion score§	0.51 ± 0.75	0.81 ± 0.86	1.16 ± 0.93	1.42 ± 0.97	<0.001
Composite congestion score§					<0.001
0	251 (72.5)	188 (53.9)	139 (39.3)	118 (31.3)	
1	67 (19.4)	114 (32.7)	129 (36.4)	124 (32.9)	
2	25 (7.2)	43 (12.3)	74 (20.9)	109 (28.9)	
3	3 (0.9)	4 (1.1)	12 (3.4)	26 (6.9)	
NYHA functional class					<0.001
I	17 (2.9)	22 (3.8)	8 (1.4)	7 (1.2)	
II	278 (47.8)	230 (40.1)	182 (32.0)	132 (23.2)	
III	246 (42.3)	272 (47.5)	288 (50.7)	335 (59.0)	
IV	40 (6.9)	49 (8.6)	90 (15.8)	94 (16.5)	
Vital signs					
Heart rate, beats/min	74 ± 17	78 ± 18	82 ± 21	85 ± 20	<0.001
SBP, mm Hg	127 ± 21	126 ± 20	125 ± 23	121 ± 23	<0.001
DBP, mm Hg	76 ± 12	75 ± 12	74 ± 14	74 ± 14	0.036
Laboratory					
Hemoglobin, g/dl‖	13.4 ± 1.8	13.3 ± 1.9	13.0 ± 1.9	13.1 ± 2.0	0.003
Serum creatinine, mg/dl¶	1.24 ± 0.62	1.27 ± 0.62	1.34 ± 0.62	1.35 ± 0.61	0.005
eGFR (MDRD formula), mg/dl/1.73 m^2¶	67 ± 25	65 ± 26	62 ± 25	62 ± 24	0.001
BUN, mmol/l#	14.6 ± 11.6	14.3 ± 11.0	14.6 ± 11.2	15.9 ± 13.0	0.132
Sodium, mmol/l**	140 ± 3	139 ± 4	139 ± 4	138 ± 4	<0.001
Potassium, mmol/l**	4.3 ± 0.6	4.3 ± 0.6	4.2 ± 0.6	4.2 (0.6)	0.001
NT-proBNP, pg/dl††	1,293 (482–2,740)	2,014 (901–4,132)	3,902 (1,873–7,095)	4,629 (2,455–8,768)	<0.001
CA125, U/ml††	10.7 (8.0–13.3)	23.6 (19.8–30.7)	73.6 (56.1–97.8)	263.4 (182.0–415.1)	<0.001
Bio-ADM, pg/ml††	27.4 (19.4–39.0)	29.5 (22.0–45.4)	37.5 (24.3–60.1)	46.0 (26.7–88.8)	<0.001
Electrocardiogram					
Atrial fibrillation	217 (36.8)	278 (47.3)	293 (49.7)	278 (47.2)	<0.001
LBBB	129 (22.4)	119 (20.3)	133 (22.7)	136 (23.3)	0.639

Continued on the next page

TABLE 1 Continued

	Q1	Q2	Q3	Q4	p Value
Echocardiography					
Mitral valve regurgitation††	224 (40.4)	254 (45.5)	289 (51.1)	294 (52.9)	<0.001
LVEDD, mm‡‡	62 ± 9	61 ± 10	61 ± 10	61 ± 10	0.104
LVESD, mm§§	50 ± 11	49 ± 11	50 ± 12	51 ± 12	0.417
Interventricular wall thickness, mmǁǁ	10.6 ± 2.3	10.8 ± 2.8	10.7 ± 2.6	10.5 ± 2.5	0.654
Posterior wall thickness, mm¶¶	10.1± ± 2.2	10.4 ± 2.2	10.5 ± 2.4	10.3 ± 2.3	0.115
LVEF, %##	32 ± 9	31 ± 10	32 ± 11	30 ± 12	0.015
Left atrial diameter, mm***	46.3 ± 7.5	47.0 ± 8.2	48.0 ± 8.0	48.6 ± 8.0	<0.001
Medical treatment					
Diuretics	589 (99.8)	587 (99.8)	589 (100.0)	589 (100.0)	0.572
Loop diuretics	586 (99.3)	586 (99.7)	588 (99.8)	586 (99.5)	0.572
Aldosterone antagonists	325 (55.1)	317 (53.9)	292 (49.6)	322 (54.7)	0.206
Beta-blockers	518 (87.8)	497 (84.5)	474 (80.5)	471 (80.0)	0.001
ACE inhibitors/ARBs	451 (76.4)	432 (73.5)	416 (70.6)	410 (69.6)	0.039

Values are mean ± SD, n (%), or median (interquartile range). *Data available in 1,959 patients. †Data available in 1,548 patients. ‡Data available in 1,211 patients. §Data available in 1,426 patients. ǁData available in 2,152 patients. ¶Data available in 2,212 patients. #Data available in 1,961 patients. **Data available in 2,177 patients. ††Data available in 2,235 patients. ‡‡Data available in 1,990 patients. §§Data available in 1,366 patients. ǁǁData available in 1,711 patients. ¶¶Data available in 1,618 patients. ##Data available in 2,190 patients. ***Data available in 1,736 patients.

ACE = angiotensin-converting enzyme; ARB = angiotensin receptor blockers; bio-ADM = bio-adrenomodullin; BUN = blood urea nitrogen; CA125 = carbohydrate antigen 125; CABG = coronary artery bypass graft surgery; COPD = chronic obstructive pulmonary disease; DBP = diastolic blood pressure; eGFR = estimated glomerular filtration rate; HF = heart failure; IHD = ischemic heart disease; JVP = jugular venous pressure; LBBB = left bundle branch block; LVEDD = left ventricle end-diastolic diameter; LVEF = left ventricle ejection fraction; LVESD = left ventricle end-systolic diameter; MDRD = Modification of Diet in Renal Disease; NT-proBNP = N-terminal pro–B-type natriuretic peptide; NYHA = New York Heart Association; PAD = peripheral artery disease; PCI = percutaneous coronary intervention; SBP = systolic blood pressure.

knees, 0.66; and edema above the knees, 1. By summing integer and noninteger scores, the final CCS was treated in the analysis as a continuous variable. Just for a sensitivity analysis, the CCS was categorized as follows: no congestion, CCS = 0; mild congestion, CCS = 1 or 2; and severe congestion, CCS ≥3. This classification was achieved by reclassifying edema into 2 categories: 0 = no edema, edema limited to ankles, and edema below the knees; and 1 = edema above the knees (11). In addition, the presence of hepatomegaly and pulmonary rales in the upper one-third of the pulmonary fields were assessed.

CA125 MEASUREMENT. CA125 was measured by using the ARCHITECT CA 125 II assay (lot. 81007M800), a chemiluminescent microparticle immunoassay, on the ARCHITECT *i* System (Abbott Laboratories, Abbott Park, Illinois). The specificity and the precision of the ARCHITECT CA 125 II assay were ≤12% and 10% total coefficient of variation, respectively. The sensitivity of the assay was ≤1.0 U/ml. Analytical sensitivity corresponds to the upper limit of the 95% confidence interval (CI) and represents the lowest concentration that can be distinguished from zero. The normal range of CA125 established for this assay is 35 U/ml.

CA125 was measured from frozen samples. Clinical endpoint adjudications were blinded to CA125 status. NT-proBNP was measured by using electrochemiluminescence on a Cobas e411 analyzer, using standard methods (Roche Diagnostics GmbH, Mannheim, Germany).

A great number of other biomarkers from multiple pathophysiological domains, including markers of inflammation, apoptosis, remodeling, myocyte stress, angiogenesis, endothelial function, and renal function, were also measured (Supplemental Table 1).

STATISTICAL ANALYSIS. Baseline characteristics among CA125 quartiles (Q1-Q4) were compared by using analysis of variance, Kruskal-Wallis, or chi-squared tests, as appropriate.

Correlation heatmap and dendrogram. Spearman correlation was determined between CA125 and age, systolic blood pressure, urea, estimated glomerular filtration rate (eGFR), serum sodium, serum potassium, NT-proBNP, New York Heart Association (NYHA) functional class, CCS, bio-ADM, growth differentiation factor (GDF)-15, interleukin-6, endothelin-1, proenkephalin, renin, and aldosterone. Because of listwise deletion, the final sample in which the correlation analysis was based included 978 patients. The same sets of variables were included in hierarchical cluster analysis (agglomerative type) with the ward.D2 linkage method and Spearman correlation matrix as a dissimilarity measure. No significant differences were found in those included versus those excluded (Supplemental Table 2).

Association between congestion and CA125. With log-transformed CA125 as the dependent variable, the following covariates were included in the final linear regression model: CCS, age, systolic blood pressure, serum sodium, NT-proBNP, heart rate, pulmonary

rales/crackles, and hepatomegaly. The added value of each covariate on the model's R^2 was used as an indicator of the predictor's importance.

CA125 as a clinical outcome predictor. By means of a flexible parametric regression modeling (Royston-Parmar model), we determined the independent prognostic effect of CA125 on both clinical outcomes. Estimates of risk were adjusted for the outcome-specific prognostic risk score (BIOSTAT-risk score) (9). The BIOSTAT risk score for mortality included age, blood urea nitrogen, NT-proBNP, serum hemoglobin, and the use of a beta-blocker. The BIOSTAT risk score for the composite endpoint included age, previous HF hospitalization, peripheral edema, systolic blood pressure, NT-proBNP, hemoglobin, high-density lipoprotein, sodium, and use of beta-blockers.

The linearity assumption for CA125 and the prognostic risk score was tested with multivariable fractional polynomials (12). Risk estimates from the Royston-Parmar model are presented as hazard ratios (HRs) with 95% CIs. Harrell C-statistics was used as the metric for the model's performance. In a sensitivity analysis, the CCS, together with the BIOSTAT-risk score, was also included in the prognostic models (n = 1,426) to evaluate how much of its effect is mediated by clinical surrogates of congestion. The added value of CA125 on the model's discriminative ability was estimated with changes in C-statistics, the integrated discrimination improvement (IDI), and category-free net reclassification improvement (cfNRI).

A 2-sided p value of <0.05 was set as the threshold for statistical significance. Stata 15.1 (Stata Statistical Software, Release 15 [2017]; StataCorp LP, College Station, Texas) was used for the main analysis. Risk reclassification analyses (survIDINRI and SurvC1 modules) and dendrograms were implemented in R version 3.5.2 (R Foundation for Statistical Computing, Vienna, Austria). A more detailed description of statistical analyses is presented in Supplemental Table 1.

RESULTS

A total of 2,356 from 2,516 patients included in the BIOSTAT-CHF cohort were included in the current analysis. The mean age of the sample was 69 ± 12 years; 614 (26.1%) were female, 1,287 (55.6%) had a history of ischemic heart disease, and 1,884 (89.3%) had an LVEF ≤40%. Median (interquartile range) of CA125 and NT-proBNP were 38.6 U/ml (16 to 125 U/ml), and 2,699 pg/ml (1,179 to 5,764 pg/ml), respectively. Baseline characteristics across quartiles of CA125 are shown in **Table 1**. Overall, higher values of CA125 were found in older patients, those with higher rates of atrial fibrillation, worse prior NYHA functional class, and more severe AHF severity proxies (lower systolic and diastolic blood pressures, hemoglobin, and eGFR). Likewise, patients in the upper quartiles exhibited a worse echocardiographic profile (lower LVEF, higher left atrial diameter, and a higher proportion of mitral valve regurgitation) and greater evidence of clinical congestion. Regarding the use of medications at baseline, lower rates of beta-blockers and angiotensin-converting enzyme inhibitors/angiotensin receptor blockers were found in the upper quartiles.

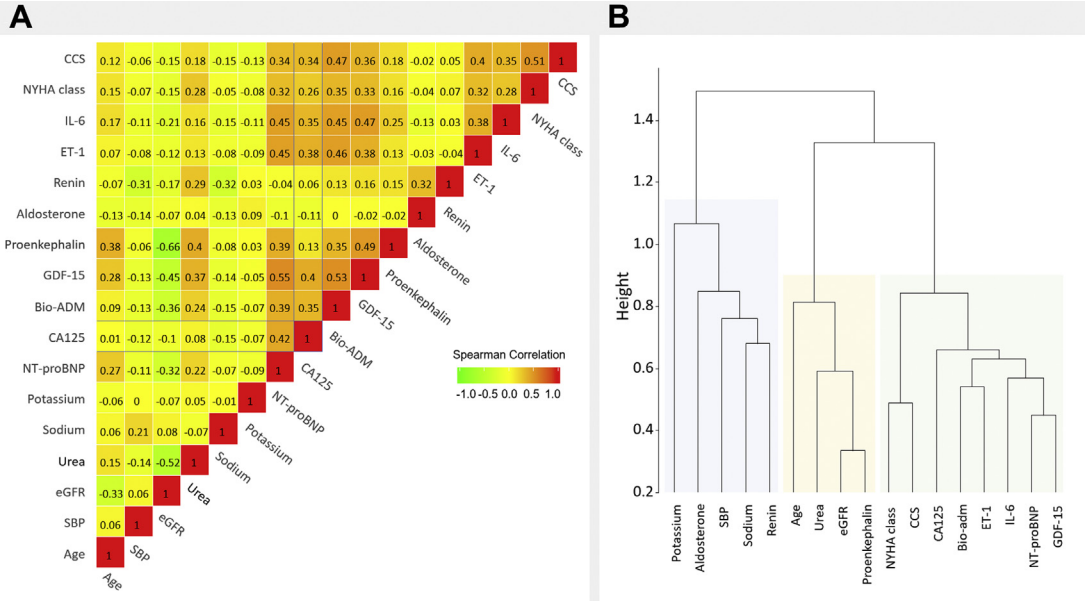
In the validation cohort (n = 1,630 from 1,738 patients of the validation cohort), the distribution of baseline characteristics among quartiles of CA125 showed an overall higher risk profile as CA125 values increased. Higher levels of CA125 were associated with a history of HF hospitalizations, higher NYHA functional class and heart rate, poor renal dysfunction, and higher NT-proBNP. An inverse association was found between CA125 and age, systolic blood pressure, hemoglobin, sodium, and potassium (Supplemental Table 3).

HEATMAP AND DENDROGRAM. **Figure 1A** displays the Spearman correlation heatmap coefficients. CA125 had the strongest correlation with NT-proBNP (rho = 0.42; p < 0.001), followed by GDF-15 (rho = 0.40; p < 0.001), endothelin-1 (rho = 0.38; p < 0.001), interleukin-6 (rho = 0.35; p < 0.001), bio-ADM (rho = 0.35; p < 0.001), the CCS (rho = 0.34; p < 0.001), NYHA functional class (rho = 0.26; p < 0.001), heart rate (rho = 0.23; p < 0.001), and serum sodium (rho = -0.15; p < 0.001).

In a hierarchical cluster analysis (**Figure 1B**), CA125 clustered with the combined effect of bio-ADM, endothelin-1, interleukin-6, NT-proBNP, and GDF-15, which, in turn, clustered together with the combined effect of NYHA functional class and the CCS.

Similar findings were obtained in the validation cohort (Supplemental Figure 1). CA125 correlated with NT-proBNP (rho = 0.52; p < 0.001), GDF-15 (rho = 0.37; p < 0.001), bio-ADM (rho = 0.30; p < 0.001), NYHA functional class (rho = 0.24; p < 0.001), proenkephalin (rho = 0.20; p < 0.001), heart rate (rho = 0.20; p < 0.001), serum potassium (rho = -0.16; p < 0.001), systolic blood pressure (rho = -0.15; p < 0.001), urea (rho = 0.15; p < 0.001), eGFR (rho = -0.13; p < 0.001), and serum sodium (rho = -0.13; p < 0.001). The dendrogram showed that CA125 clustered with NT-proBNP. Both clustered together with the combined effect of NYHA class, bio-ADM, and GDF-15 (Supplemental Figure 2).

FIGURE 1 Heatmap and Dendrogram



(A) Biomarker position of antigen carbohydrate 125 (CA125) in a correlation heatmap. Correlations are based on Spearman's rho as a correlation coefficient. **(B)** Biomarker position of CA125 in hierarchical cluster analysis. N = 978. bio-ADM = bio-adrenomedullin; CCS = composite congestion score; eGFR = estimated glomerular filtration rate; ET = endothelin, GDF = growth differentiation factor; IL = interleukin; NT-proBNP = N-terminal pro-B-type natriuretic peptide; NYHA = New York Heart Association; SBP = systolic blood pressure.

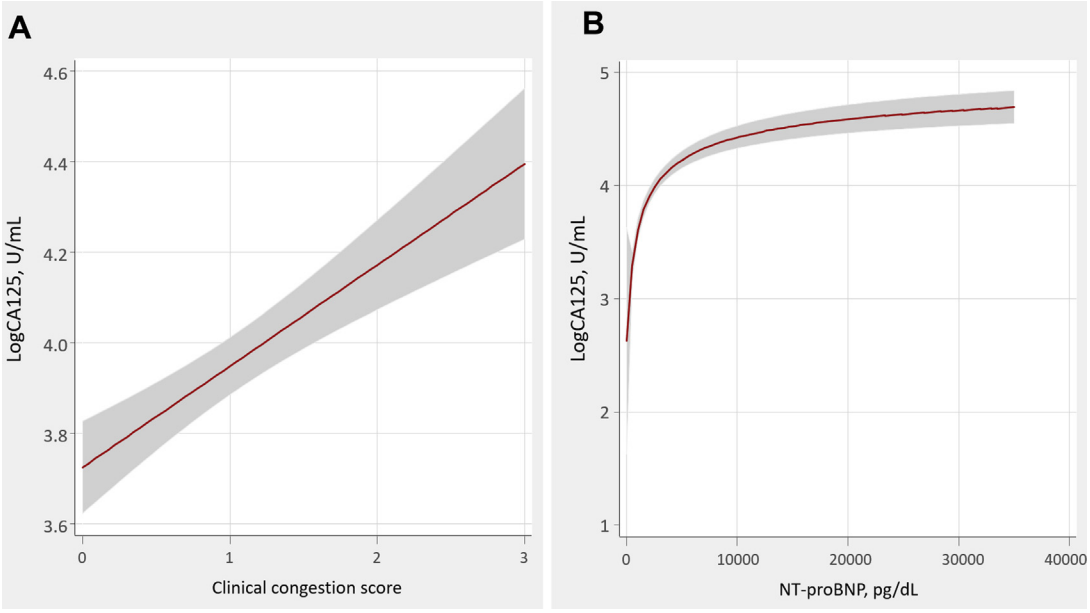
CA125 AS A MARKER OF CONGESTION. The analysis showed that the most important predictors of log-transformed CA125 (line-up based on the magnitude of its contribution to the total R^2 of the model) were: NT-proBNP ($\Delta R^2 = 0.184$; $p < 0.001$), CCS ($\Delta R^2 = 0.040$; $p < 0.001$), age ($\Delta R^2 = 0.020$; $p < 0.001$), serum sodium ($\Delta R^2 = 0.015$; $p < 0.001$), heart rate ($\Delta R^2 = 0.006$; $p = 0.001$), pulmonary rales/crackles ($\Delta R^2 = 0.006$; $p = 0.004$), systolic blood pressure ($\Delta R^2 = 0.003$; $p = 0.027$), and hepatomegaly ($\Delta R^2 = 0.0008$; $p = 0.226$). The full model R^2 was 0.277. The detailed model is shown in Table 2. The contribution of adding the block of all congestion-related variables (NT-proBNP, CCS, serum sodium,

TABLE 2 CA125 Predictors

	Coefficient	SE	t Value	p Value	95% CI	Omnibus p Value	R^2	Change in R^2
NT-proBNP, pg/ml (FP2[-0.5])	18.622	6.593	2.82	0.005	5.687 to 31.557	<0.001	0.1844	
NT-proBNP, pg/ml (FP2[-0.5])	-9.760	1.583	-6.17	0.000	-12.865 to -6.654			
Age, yrs (FP[1])	-0.015	0.003	-5.12	<0.001	-0.021 to -0.009		0.2039	0.0195
Composite congestion score (FP1[1])	0.223	0.040	5.62	0.000	0.145 to 0.301		0.2455	0.0416
Serum sodium, mEq/l (FP2[1])	0.000	0.000	-3.45	0.001	0.000 to 0.000	<0.001	0.2604	0.0149
Serum sodium, mEq/l (FP2[2])	0.000	0.000	3.43	0.001	0.000 to 0.000			
Heart rate, beats/min (FP[1])	0.006	0.002	3.26	0.001	0.002 to 0.000		0.2666	0.0061
Pulmonary rales/crackles	Reference					0.004	0.2729	0.0064
Single base	0.331	0.106	3.13	0.002	0.124 to 0.539			
Bi-basilar	0.160	0.076	2.11	0.035	0.011 to 0.310			
SBP, mm Hg (FP[1])	-0.003	0.002	-2.16	0.031	-0.006 to 0.000		0.2757	0.0028
Hepatomegaly	0.109	0.090	1.21	0.226	-0.068 to 0.285		0.2766	0.0008

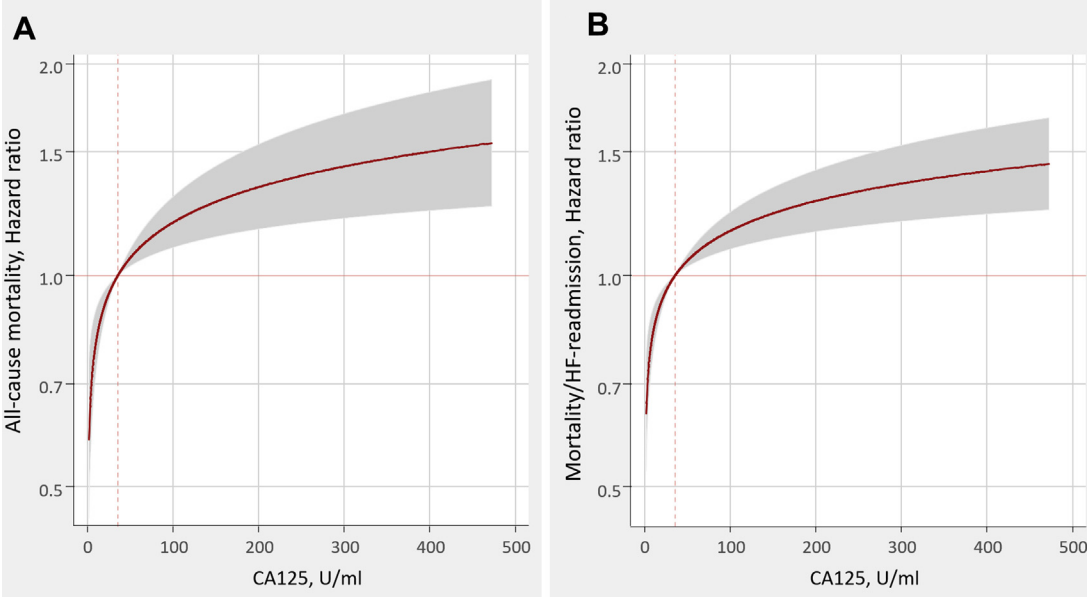
FP = fractional polynomials; other abbreviations as in Table 1.

FIGURE 2 CCS and NT-proBNP as Predictors of Log-Transformed CA125



(A) CCS and log-transformed CA125. **(B)** NT-proBNP and log-transformed CA125. Covariates used for adjustment were: age (years), systolic blood pressure (mm Hg), heart rate (beats/min), serum sodium (mmol/l), NT-proBNP (pg/ml), hepatomegaly, pulmonary rales, and the CCS. N = 1,268; model R^2 = 0.277. Abbreviations as in Figure 1.

FIGURE 3 1-Year Multivariable Analysis



(A) All-cause mortality. **(B)** Mortality/heart failure (HF) readmission. The analysis adjusted by the BIOSTAT risk score for mortality (N = 2,356). Hazard ratio depiction along the continuum of CA125 (35 U/ml as reference). CA125 = carbohydrate antigen 125.

pulmonary rales/crackles, and hepatomegaly) to age, heart rate, and systolic blood pressure represents a $\Delta R^2 = 0.22$ ($p < 0.001$), which is equivalent to 79.4% of the total model R^2. Figure 2 shows the independent association between CCS and NT-proBNP with log-transformed CA125.

In the validation cohort, the independent variables associated with log-transformed CA125 were, in order of importance: NT-proBNP ($\Delta R^2 = 0.256$; $p < 0.001$), serum potassium ($\Delta R^2 = 0.016$; $p < 0.001$), serum sodium ($\Delta R^2 = 0.015$; $p < 0.001$), heart rate ($\Delta R^2 = 0.014$; $p < 0.001$), systolic blood pressure ($\Delta R^2 = 0.005$; $p = 0.004$), age ($\Delta R^2 = 0.003$; $p = 0.033$), and prior admission for AHF ($\Delta R^2 = 0.002$; $p = 0.015$). The full model R^2 was 0.313.

CLINICAL ENDPOINTS. Derivation cohort. *Mortality.* During a 1-year follow-up, 369 deaths were registered. Unadjusted mortality rates (per 100 person-years) significantly differed across quartiles of CA125 (8.53, 12.17, 21.21, and 29.33 for Q1, Q2, Q3, and Q4, respectively; $p < 0.001$). Kaplan-Meier curves are shown in Supplemental Figure 3.

In a multivariable survival analysis that included the BIOSTAT risk score for mortality as a covariate, the continuum of CA125 revealed a positive, sigmoid-shaped association with the risk of mortality (C-statistics = 0.757; overall; $p < 0.001$) (Figure 3A). The adjusted association between CA125 (log CA125 and quartiles) and mortality is presented in Table 3.

Composite of death and/or rehospitalization for AHF. At a median follow-up of 1 year, 678 combined endpoints were ascertained. The rates (per 100 person-years) of this composite endpoint significantly differed among CA125 quartiles (18.6, 30.3, 41.7, and 56.6 for Q1, Q2, Q3, and Q4, respectively; $p < 0.001$). Kaplan-Meier curves are shown in Supplemental Figure 4.

Multivariable analysis revealed that CA125 was significantly associated with this endpoint, through a positive and also sigmoid-shaped curve (C-statistics = 0.719; overall; $p < 0.001$) (Figure 3B). Risk estimates (log CA125 and quartiles) for the composite endpoint are presented in Table 3. In the derivation cohort, CA125 (log CA125 and quartiles) remained significantly associated with the risk of the combined endpoint.

Sensitivity analysis. In a sensitivity analysis, to which the CCS was added as an additional covariate (n = 1,426), the results were in line with the main findings (Figures 4A and 4B). Furthermore, we also found that the predictive ability for all-cause mortality of CA125 for both endpoints (here dichotomized at 35 U/ml) was consistent across all levels of the categorical CCS (Supplemental Figures 5 and 6).

TABLE 3 CA125 and Clinical Outcomes in the BIOSTAT-Derivation and Validation Cohorts

	HR (95% CI)	p Value	Omnibus p Value
Derivation cohort (n = 2,356)			
All-cause mortality*†			
Log CA125	1.18 (1.09–1.28)	<0.001	
CA125 quartiles			
Q1	Ref.	Ref.	0.0015
Q2	1.22 (0.84–1.77)	0.299	
Q3	1.50 (1.07–2.12)	0.020	
Q4	1.84 (1.31–2.57)	<0.001	
All-cause mortality or HF hospitalization*‡			
Log CA125	1.15 (1.09–1.22)	<0.001	
CA125 quartiles			
Q1	Ref.	Ref.	<0.001
Q2	1.34 (0.04–1.73)	0.024	
Q3	1.46 (1.14–1.87)	0.003	
Q4	1.72 (1.36–2.20)	<0.001	
Validation cohort (n = 1,623)			
All-cause mortality*§			
Log CA125	1.49 (1.39–1.59)	<0.001	
CA125 quartiles			
Q1	Ref.	Ref.	<0.001
Q2	1.17 (0.86–1.61)	0.311	
Q3	1.78 (1.33–2.38)	<0.001	
Q4	3.25 (2.49–4.24)	<0.001	
All-cause mortality or HF hospitalization*‖			
Log CA125	1.39 (1.30–1.47)	<0.001	
CA125 quartiles			
Q1	Ref.	Ref.	<0.001
Q2	1.08 (0.84–1.38)	0.549	
Q3	1.58 (1.25–1.99)	<0.001	
Q4	2.59 (2.09–3.22)	<0.001	

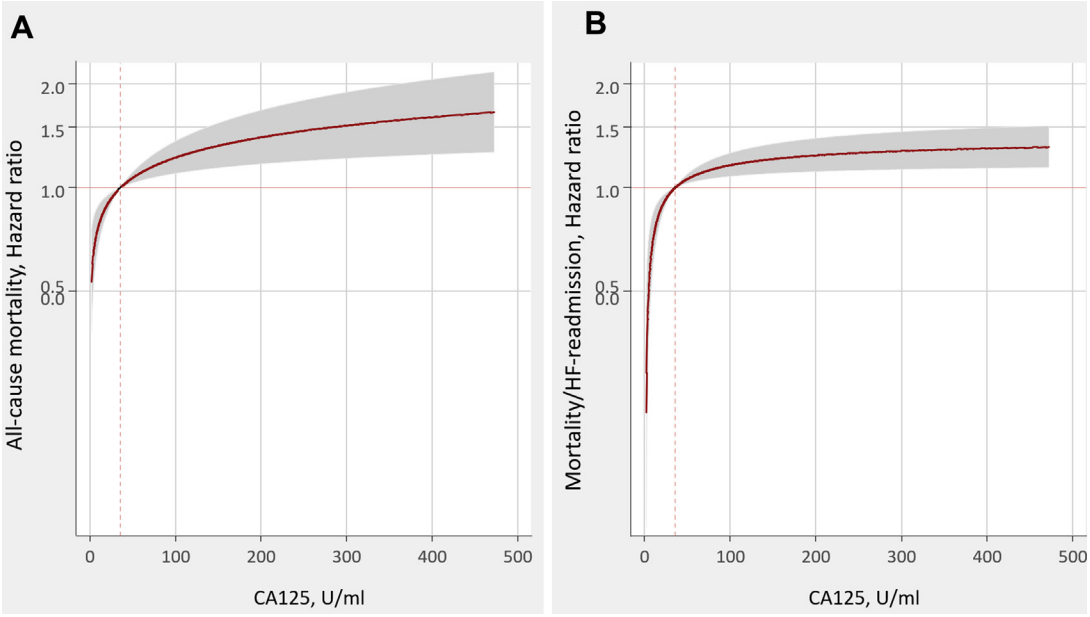
*Multivariate estimates of risk adjusted for BIOSTAT-risk score. The BIOSTAT risk score for mortality included age, BUN, amino-terminal pro-brain natriuretic peptide, serum hemoglobin, and the use of a beta-blocker. The BIOSTAT risk score for the composite endpoint included age, previous HF hospitalization, peripheral edema, SBP, amino-terminal pro-brain natriuretic peptide, hemoglobin, high-density lipoprotein, sodium, and use of beta-blocker at baseline. †C-statistic of the model = 0.757. ‡C-statistic of the model: 0.719. §C-statistic of the model: 0.719. ‖C-statistic of the model: 0.674.

Log CA125 = log-transformed CA125; other abbreviations as in Table 1.

Survival model's performance. *All-cause mortality.* Supplemental Table 4 summarizes the survival model's performance. The models that included CA125 showed a significant risk reclassification compared with the BIOSTAT risk score (IDI = 0.008 [95% CI: 0.001 to 0.020; p = 0.010] and cfNRI = 0.137 [95% CI: 0.073 to 0.184; $p < 0.001$]) or BIOSTAT risk score + NT-proBNP (IDI = 0.005 [95% CI: 0.001 to 0.013; p = 0.043] and cfNRI = 0.106 [95% CI: 0.021 to 0.161; p = 0.027]). However, the ΔC statistic did not significantly differ when adding CA125 to the BIOSTAT risk score (Δ = 0.004; CI: -0.001 to 0.010) or BIOSTAT + NT-proBNP (Δ = 0.001; CI: -0.002 to 0.004).

Composite of death and/or rehospitalization for AHF. The addition of CA125 led to a significant risk reclassification compared with the BIOSTAT risk score

FIGURE 4 1-Year Multivariable Analyses Including the CCS



(A) All-cause mortality. **(B)** Mortality/HF readmission. The analysis adjusted by the 1-year BIOSTAT risk score for the composite endpoint and the CCS (N = 1,426). Hazard ratio depiction along the continuum of CA125 (35 U/ml as reference). Abbreviations as in Figures 1 and 3.

(IDI = 0.009 [95% CI: 0.003 to 0.017; p < 0.001] and cfNRI = 0.104 [95% CI: 0.061 to 0.150; p = 0.003]) and borderline compared with the BIOSTAT risk score + NT-proBNP (IDI = 0.004 [95% CI: 0.001 to 0.010; p = 0.040] and cfNRI = 0.050 [95% CI: -0.013 to 0.086; p = 0.106]). The ΔC statistic was significantly higher compared with the BIOSTAT risk score (Δ = 0.006; 95% CI: 0.001 to 0.010) but not compared with the BIOSTAT risk score + NT-proBNP (Δ = 0.002; 95% CI: -0.001 to 0.005).

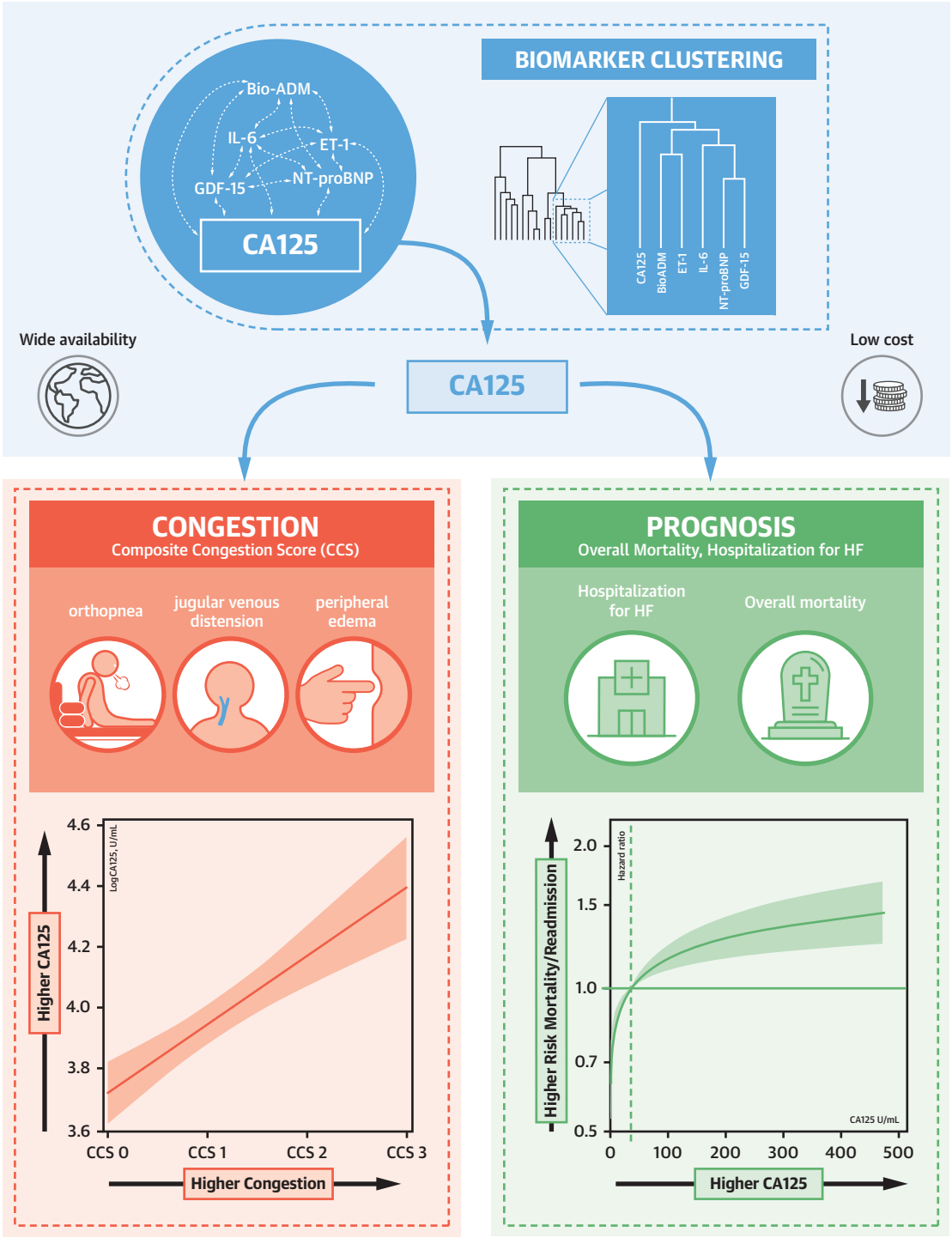
Validation cohort. In a univariate as well as in multivariable context, CA125 was also shown to be a significant and independent predictor for 1-year all-cause mortality and for the composite of mortality and/or AHF readmission (Table 3).

DISCUSSION

The current study confirms the role of CA125 as a surrogate of congestion and its utility as a prognostic biomarker in worsening HF, confirming findings from previous smaller studies. In fact, the current data from a subanalysis of the BIOSTAT-CHF and the validation cohorts indicate that CA125 was strongly associated with higher risk of 1-year all-cause mortality and the combined all-cause death and hospitalization for HF (Central Illustration). The merit of this study stems from rigorous model adjustment, including clinical surrogates of systemic congestion. Moreover, through a cluster analysis, CA125 grouped with recognized biomarkers of congestion and inflammation. Congruent results were also found in the validation cohort.

CA125 AS A MARKER OF CONGESTION. Symptoms and signs of congestion are found in most patients with AHF; however, its presence and severity are largely heterogeneous (1,2). Unfortunately, traditional clinical assessment of congestion through symptoms and signs has shown limited accuracy. Thus, current experts recommend an integrative multiparameter-based evaluation of congestion using clinical assessment and biomarkers, and supplemented with technical assessments (1-3). Despite these recommendations, a well-validated tool for the clinical assessment of congestion is still an unmet need. In the last years, CA125 has emerged as a novel useful biomarker of congestion (4,5). The pathophysiology of CA125 in AHF remains largely unknown. What is known is that this large glycoprotein is synthesized by mesothelial cells in response to an increase in hydrostatic pressures and/or inflammatory mediators (4,5). In patients with AHF, CA125 has been shown to be related to symptoms or signs of fluid overload, such as peripheral edema, serosal effusion, pulmonary wedge pressure, and increasing

CENTRAL ILLUSTRATION CA125 as a Biomarker in Patients With Worsening Heart Failure



Núñez, J. et al. J Am Coll Cardiol HF. 2020;8(5):386–97.

Carbohydrate antigen 125 (CA125) correlates with parameters of congestion. CA125 is associated with higher risk of adverse events and is widely available in daily clinical setting. ADM = adrenomedullin; ET = endothelin; GDF = growth differentiation factor; IL = interleukin; HF = heart failure; NT-proBNP = N-terminal pro-brain natriuretic peptide.

cardiac pressures (4,5,13,14). Consistent with this notion, a recent meta-analysis reported that patients with significant serosal effusions had higher CA125 levels (15). However, in patients with refractory congestive HF treated with continuous peritoneal dialysis, CA125 decreased in parallel with decongestion and despite the peritoneal irritation induced by the presence of an osmotic solution into the peritoneum (16). These findings suggest that increases in CA125 associated with serosal effusion are parallel processes caused by common pathophysiological mechanisms, and not necessarily a cause-effect phenomenon. Interestingly, in the current study, we found that CA125 was strongly associated with well-validated proxies of clinical congestion. In addition, their values were also positively related to biochemical surrogates of increased filling pressures/congestion such as NT-proBNP and bio-ADM (3). In a recent analysis of 2,179 patients with worsening HF, ter Maaten et al. (3) reported that bio-ADM was related to symptoms and signs of congestion. Interestingly, in the cluster analysis, CA125 and bio-ADM grouped together. These findings suggest that both biomarkers may play a crucial role in identifying the degree of congestion in patients with worsening HF.

Future studies should investigate the exact role of CA125 in the identification of intravascular versus extravascular congestion. In addition, a formal comparison with other novel biomarkers of congestion is still required.

CA125 AND RISK STRATIFICATION. Previous studies have reported a significant association between CA125 and the risk of death and HF readmission in various AHF scenarios (4–6,13–15,17). To the best of our knowledge, this study is the largest in AHF to confirm the prognostic value of CA125 independent of standard prognosticators. In addition, the current findings also support that the value of these biomarkers for risk stratification is independent of traditional symptoms/signs of congestion. Interestingly, the association between CA125 and adverse clinical events remained significant in those with mild, moderate, and severe congestion. Such behavior opens an avenue for its incorporation in multiparametric congestion scores.

CA125 ASSESSMENT IN AHF: READY FOR CLINICAL IMPLEMENTATION? In our opinion, the following aspects may contribute to endorsing use of CA125 in routine clinical practice: 1) the lack of standardized biomarkers for assessing congestion in daily clinical practice; 2) wide availability and low cost (≈1 €); 3) independent predictive ability beyond clinical and biochemical markers of congestion (including natriuretic peptides) (4–6,17); 4) longitudinal trajectories highly associated with risk of adverse outcomes (4–6); and 5) potential utility for guiding depletive therapy (7,18). Regarding this last point, the CHANCE-HF (Carbohydrate Antigen-125-Guided Therapy in Acute Heart Failure) trial, performed in 380 patients discharged for AHF and high CA125, found that CA125-guided therapy (uptitrating or downtitrating depletive treatment when CA125 increased or decreased during follow-up) was superior to the standard of care in terms of reducing the risk of the composite of 1-year death or AHF readmissions (7). More recently, a CA125-guided diuretic strategy (intensive diuretic therapy in patients with CA125 >35 U/ml and more conservative approach when CA125 is ≤35 U/ml) was evaluated in an open-label randomized study that enrolled 160 patients with AHF and renal dysfunction at presentation (mean eGFR 33.7 ± 11.3 ml/min/1.73 m^2) (18). In this trial, the CA125-guided strategy significantly improved eGFR and other renal function parameters at 72 h.

In summary, and beyond logistic issues, there are promising data supporting the clinical utility of CA125 as an HF biomarker, not only as a spot prognosticator but also for monitoring the course of the disease/congestion status and guiding depletive therapy. Lastly, and along this line, given the long half-life of CA125 (7 to 12 days) (4,5), it can be measured during early decompensation without noticing significant changes (17). Conversely, we have found meaningful and significant changes at 30 days and further follow-up, with these changes strongly associated with prognosis (4–6).

STUDY LIMITATIONS. First, these results do not apply to patients with stable chronic HF. Second, the lack of assessment of echocardiographic parameters of right ventricular failure and a more detailed evaluation of symptoms and signs of congestion preclude evaluating the contribution of these parameters to CA125. Third, we could not validate in the external cohort (BIOSTAT-validation cohort) the regression formula obtained in the main cohort given the lack of availability of several biomarkers and congestion parameters. Instead, a similar statistical approach followed on the main cohort was applied to the validation cohort, using the set of covariates available. Fourth, the clinical utility of this biomarker in underrepresented AHF phenotypes, such as HF with preserved ejection fraction, and patients with greater renal dysfunction, remains to be determined. Fifth, we cannot evaluate the influence of new treatments, such as sacubitril/valsartan, on the magnitude or direction of these findings. Sixth, because this is a cohort predominantly of white patients, these findings cannot be extrapolated to other races. Finally, we have not compared fresh samples versus frozen

samples and therefore cannot exclude storage/freeze/thaw artifacts.

CONCLUSIONS

In patients with signs and/or symptoms of worsening HF, circulating levels of CA125 were independently and positively associated with clinical surrogates of congestion. Their levels were also highly predictive of 1-year all-cause mortality and of the composite of mortality and HF hospitalization. In addition, such prognostic effect was shown to be independent of the severity of systemic congestion. Further studies are warranted to confirm the role of CA125 as a marker of congestion and unravel the role of this glycoprotein in the pathophysiology of AHF syndromes.

ADDRESS FOR CORRESPONDENCE: Dr. Adriaan A. Voors, Department of Cardiology, University Medical Center Groningen, Hanzeplein 1, 9713 GZ, Groningen, the Netherlands. E-mail: a.a.voors@umcg.nl.

PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: The current study confirms the role of CA125 as a surrogate of congestion and its utility as a prognostic biomarker in patients with worsening HF. The current data from a subanalysis of the BIOSTAT-CHF and the validation cohorts indicate that CA125 was strongly associated with a higher risk of 1-year all-cause mortality and the combined event of all-cause death and hospitalization for HF. Moreover, through a cluster analysis, CA125 grouped with recognized biomarkers of congestion and inflammation.

TRANSLATIONAL OUTLOOK: Measurement of CA125 constitutes a valuable tool, both for estimating the degree of congestion in worsening HF, which can be very useful for clinical management, as well as for risk stratification. The fact that this biomarker is widely available, inexpensive, and with standardized measurements favors its routine use in clinical practice.

REFERENCES

1. Girerd N, Seronde MF, Coiro S, et al., INI-CRCT, Great Network, and the EF-HF Group. Integrative assessment of congestion in heart failure throughout the patient journey. J Am Coll Cardiol HF 2018;6:273–85.

2. Mullens W, Damman K, Harjola VP, et al. The use of diuretics in heart failure with congestion—a position statement from the Heart Failure Association of the European Society of Cardiology. Eur J Heart Fail 2019;21:137–55.

3. Ter Maaten JM, Kremer D, Demissei BG, et al. Bio-adrenomedullin as a marker of congestion in patients with new-onset and worsening heart failure. Eur J Heart Fail 2019;21:732–43.

4. Núñez J, Miñana G, Núñez E, Chorro FJ, Bodí V, Sanchis J. Clinical utility of antigen carbohydrate 125 in heart failure. Heart Fail Rev 2014;19:575–84.

5. Llàcer P, Bayés-Genís A, Núñez J. Carbohydrate antigen 125 in heart failure. New era in the monitoring and control of treatment. Med Clin (Barc) 2019;152:266–73.

6. Núñez J, Núñez E, Bayés-Genís A, et al. Long-term serial kinetics of N-terminal pro B-type natriuretic peptide and carbohydrate antigen 125 for mortality risk prediction following acute heart failure. Eur Heart J Acute Cardiovasc Care 2017;6:685–96.

7. Núñez J, Llàcer P, Bertomeu-González V, et al., CHANCE-HF Investigators. Carbohydrate Antigen-125-Guided Therapy in Acute Heart Failure: CHANCE-HF: a randomized study. J Am Coll Cardiol HF 2016;4:833–43.

8. Voors AA, Anker SD, Cleland JG, et al. A systems BIOlogy Study to TAilored Treatment in Chronic Heart Failure: rationale, design, and baseline characteristics of BIOSTAT-CHF. Eur J Heart Fail 2016;18:716–26.

9. Voors AA, Ouwerkerk W, Zannad F, et al. Development and validation of multivariable models to predict mortality and hospitalization in patients with heart failure. Eur J Heart Fail 2017;19:627–34.

10. Ambrosy AP, Pang PS, Khan S, et al., EVEREST Trial Investigators. Clinical course and predictive value of congestion during hospitalization in patients admitted for worsening signs and symptoms of heart failure with reduced ejection fraction: findings from the EVEREST trial. Eur Heart J 2013;34:835–43.

11. Rubio-Gracia J, Demissei BG, Ter Maaten JM, et al. Prevalence, predictors and clinical outcome of residual congestion in acute decompensated heart failure. Int J Cardiol 2018;258:185–91.

12. Royston P, Sauerbrei W. Multivariable Model-building: A Pragmatic Approach to Regression Analysis Based on Fractional Polynomials for Modelling Continuous Variables. Chichester, UK: Wiley; 2008.

13. D'Aloia A, Faggiano P, Aurigemma G, et al. Serum levels of carbohydrate antigen 125 in patients with chronic heart failure: relation to clinical severity, hemodynamic and Doppler echocardiographic abnormalities, and short-term prognosis. J Am Coll Cardiol 2003;41:1805–11.

14. Nagele H, Bahlo M, Klapdor R, Schaeperkoetter D, Rodiger W. Ca 125 and its relation to cardiac function. Am Heart J 1999;137:1044–9.

15. Li KHC, Gong M, Li G, Baranchuk A, et al. International Health Informatics Study (IHIS) Network. Cancer antigen-125 and outcomes in acute heart failure: a systematic review and meta-analysis. Heart Asia 2018;10:e011044.

16. Núñez J, Miñana G, González M, et al. Antigen carbohydrate 125 in heart failure: not just a surrogate for serosal effusions? Int J Cardiol 2011;146:473–4.

17. Núñez J, Sanchis J, Bodí V, et al. Improvement in risk stratification with the combination of the tumour marker antigen carbohydrate 125 and brain natriuretic peptide in patients with acute heart failure. Eur Heart J 2010;31:1752–63.

18. Núñez J, Llàcer P, García-Blas S, et al., IMPROVE-HF Investigators. CA125-Guided Diuretic Treatment Versus Usual Care in Patients With Acute Heart Failure and Renal Dysfunction. Am J Med 2020;133:370–80.e4.

KEY WORDS CA125, carbohydrate antigen 125, congestion, outcome, worsening heart failure

APPENDIX For supplemental information including tables and figures, please see the online version of this paper.