A New Biomarker Tool for Risk Stratification in “De Novo” Acute Heart Failure (OROME)

Rosa Agra Bermejo (rosinagra@msn.com)
Hospital Clinico Universitario de Santiago de Compostela

Carla Cacho-Antonio
Complexo Hospitalario Universitario de Santiago de Compostela: Complejo Hospitalario Universitario de Santiago de Compostela

Eva Gonzalez-Babarro
Complexo Hospitalario de Pontevedra: Complejo Hospitalario Universitario de Pontevedra

Adriana Rozados-Luis
Complexo Hospitalario Universitario de Santiago de Compostela: Complejo Hospitalario Universitario de Santiago de Compostela

Marinela Couselo-Seijas
Complexo Hospitalario Universitario de Santiago de Compostela: Complejo Hospitalario Universitario de Santiago de Compostela

Inés Gómez-Otero
Complexo Hospitalario Universitario de Santiago de Compostela: Complejo Hospitalario Universitario de Santiago de Compostela

Alfonso Varela-Román
Complexo Hospitalario Universitario de Santiago de Compostela: Complejo Hospitalario Universitario de Santiago de Compostela

José N López-Canoa
Complexo Hospitalario de Pontevedra: Complejo Hospitalario Universitario de Pontevedra

Isabel Gómez-Rodríguez
Complexo Hospitalario de Pontevedra: Complejo Hospitalario Universitario de Pontevedra

María Pata
BIOSTATECH

Sonia Eiras
Complexo Hospitalario Universitario de Santiago de Compostela: Complejo Hospitalario Universitario de Santiago de Compostela

Jose R González-Juanatey
Complexo Hospitalario Universitario de Santiago de Compostela: Complejo Hospitalario Universitario de Santiago de Compostela

Original investigation

Keywords: OROSOMUCOID, OMENTIN, NT-PROBNP, HEART FAILURE

Posted Date: January 29th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-154510/v1

Abstract

Background: Inflammation is one of the mechanisms involved on heart failure (HF) pathophysiology. Thus, the acute phase reactant protein, orosomucoid, was associated with a worse post-discharge prognosis in *de novo* acute HF (AHF). However, the presence of anti-inflammatory adipokine, omentin, might protect and reduce the severity of the disease. We wanted to evaluate the value of omentin and orosomucoid combination for stratifying risk of these patients.

Methods and Results: Two independent cohorts of patients admitted for de novo AHF in two centers were included in the study (n=218). Orosomucoid and omentin circulating levels were determined by ELISA at discharge. Patients were follow-up for 317 (3-575) days. A predictive model was determined for primary endpoint, death and/or HF readmission. Differences in survival were evaluated using a Log-rank test. According cut-off values of orosomucoid and omentin, patients were classified on UpDown (high orosomucoid and low omentin levels), equal (both proteins high or low) and DownUp (low orosomucoid and high omentin levels). The Kaplan Meier determined worse prognosis for the UpDown group (Long-rank test p=0.02). The predictive model that includes the combination of orosomucoid and omentin groups (OROME) + NT-proBNP values achieved a higher C-index=0.84 than the predictive model with NT-proBNP (C-index=0.80) or OROME (C-index=0.79) or orosomucoid alone (C-index=0.80).

Conclusions: The orosomucoid and omentin determination stratifies *de novo* AHF patients in high, mild and low risk of rehospitalization and/or death for HF. Its combination with NT-proBNP improves its predictive value in this group of patients.

Background

Heart failure (HF) is the leading cause of hospitalization for patients > 65 years. In acute HF (AHF), exists a 20–30% of re-hospitalization within the first 3–6 months after discharge. It is coupled with an increase of acute phase response reactants, measured by C-reactive protein (CRP) or interleukin-6 (IL-6) levels [1] and a raise of other several serum inflammatory cytokines levels such as tumor necrosis factor alfa (TNF-α) and interleukin-1b (IL-1β) which are associated with the degree of disease severity, poor outcomes and mortality [1]. A number of diagnostic and/or prognostic plasma biomarkers of worsening HF have been proposed during hospitalization and in chronic stable patients. Therefore, some examples of these biomarkers are: growth differentiation factor 15 (GDF15) that belongs to transforming growth factor beta superfamily and regulates inflammatory and apoptotic pathways; troponin T (TnT) that is a structural and contractile protein; ST2, member of interleukin 1 receptor family and galectin 3, lectin family protein, that are involved in adverse cardiac remodeling and fibrosis; and TNF-a [2,3,4]. However, the European Society of Cardiology HF guidelines only recommend in clinical practice the determination of B-type natriuretic peptide (BNP) and N-terminal pro-brain natriuretic peptide (NT-proBNP)[5]. These proteins were considered biomarkers for evaluating dysnoeic patients [6] and, in consequence, AHF. Moreover, they were included in the HF diagnostic algorithm and proposed for a more accurate follow-up of the patients and risk stratification. Several authors have suggested the combination of biomarkers associated with

fibrosis (ST2 and Galectin 3) [7], inflammation [8] or myocardial load/damage (BNP and TnI) [9] as a better diagnostic and prognostic tool. Physiological orosomucoid levels improve the endothelial healing process [10]. However, their high levels were associated with the pro-inflammatory profile of monocytes [11]. Thus, recent data from our group showed orosomucoid as a new prognosis biomarker in *de novo* AHF patients [12]. In consideration that omentin a) is an anti-atherogenic and anti-inflammatory protein and b) improves the protector effect of orosomucoid against lipotoxicity [13] c) is associated with better prognosis in patients with chronic stable HF [14], our aim was to analyse the predictive prognosis value of the combined orosomucoid and omentin determination in acute *de novo* HF patients.

Material And Methods

Study population

This is a prospective observational study with two independent cohorts. One of the cohort (DEXA) has included 96 consecutive patients admitted at the Cardiology Department of Clinical Hospital of Santiago de Compostela between 2014 and 2015. The other cohort (SEC-ROVI) has included 122 patients admitted at the Cardiology Department of Clinical Hospital of Santiago de Compostela and Pontevedra between 2018 and 2019. Heart failure diagnostic was made according to the recommendations of the European Society of Cardiology [5].

The only exclusion criterion was the previous HF history. The database collected demographic, clinical (electrocardiogram and echocardiogram parameters within 24 hours after admission) and laboratory analysis (haemogram, basic biochemistry and coagulation rate, lipid and glucose profile, as well as specialized parameters such as levels of electrolytes and pro brain natriuretic peptide (NT-proBNP), registered after admission.

The study complies with the Declaration of Helsinki and was approved by the Clinical Research Ethics Committee of Galicia. All patients provided an informed consent.

Orosomucoid and omentin plasma levels analysis

Blood samples were obtained at discharge and were centrifuged at 1800 xg for 15 minutes. Isolated plasma was stored at -80ºC until be used. Orosomucoid levels from DEXA cohort were analysed as it was previously described[12] and from SEC-ROVI cohort by ELISA with a detection limit of 59 ng/mL (SEA816Hu, Cloud Clone). Omentin levels were measured by ELISA with a detection limit of 6.5 pg/mL (SEA933Hu, Cloud Clone Corp, Houston, USA) following manufacture´s protocol.

Endpoint definition and follow-up

The primary endpoint was death from any cause and/or HF rehospitalization. Follow-up information was recorded from medical history. The mean of follow-up was 317 (3-575) days.

Statistical analysis

Continuous variables were represented by the mean and standard deviation (SD) or median and interquartile range if the distribution of the variables were not normal. Differences between the patients of each project were performed by ANOVA test, or a Kruskall-Wallis test in the absence of normality. The categorical variables were represented with the absolute and relative frequencies and the differences between projects were evaluated using the χ^2 test, or with the χ^2 test with permutation if the expected frequency in any of the cells is less than 5. Variables differences between patients who suffered or not event (rehospitalization for HF and/or death) were determined by ANOVA test, or Kruskall-Wallis test, according the normality of continuous variables. The differences on categorical variables were evaluated using the χ2 test, or with the χ2 test with permutation if the expected frequency in any of the cells was less than 5. For both continuous and categorical variables, the odds ratio (OR) was obtained by maximum likelihood estimation (MLE), and its 95% confidence interval (normal approximation) were represented. All survival analyzes were carried out using the Kaplan-Meier method. In addition, the differences in survival were evaluated based on each of the three categorized markers (according to the optimal cut-off point), using a Log-rank test. The optimal cut-off point for the markers was estimated with a methodology based on correlation with survival time: Maximally selected rank statistics. The cut-off point was the one that provides the most significant split based on the standardized log-rank test.

For the predictive models several steps were performed:

a) analysis of missing values followed by multiple imputation of lost values with MICE algorithm (Multiple Imputation by Chained Equations)[15]. Fifteen imputations were made. Performance of imputation was evaluated comparing the distributions of original and imputed values. Overimputation was used for evaluating the predictive distribution fitting. The pooling of estimated parameters was made following Rubin [16] and Enders [17] rules.

b) analysis of the predictors distribution to assess the presence of outliers c) possible transformations for each of the quantitative predictors, a univariate Cox model was estimated, and it was compared with predictor models with the logarithmic transformation, square root transformation, and restricted cubic splines. The presence of outliers followed comparison with a model in which a truncation (winsoring) of the predictor had been carried out: a reasonable option was choose the percentiles 1 and 99. Although only splines were applied to the markers d) categorical variables were included in the predictive model as a pathology score, and a drug score. To create these scales, a univariate Cox regression was carried out with each drugs and pathologies, assigning a weight to each of the variables based on the Hazard ratio (HR) obtained, and proceeding to add them to obtain the corresponding scores e) interactions with the markers and among them were tested by bivariate Cox models. Risk of proportionality was tested over time f) the selection of the best predictive model was carried out with LASSO method, and stepwise backward selection with validation through different resampling methods (bootstrap and randomization) g) an internal validation of the model was carried out using bootstrap h) The discrimination capacity of the model (ability to distinguish event-free patients from those who present it) was estimated using the concordance index C, which is a generalization of the area under the curve (AUC). Time-dependent ROC curves were estimated i) The model optimum was evaluated and corrected in the final parameters.

Optimum is defined as the difference between the real model and the estimated one j) The calibration of the model was carried out through bootstrap resampling, comparing the mean predicted by the model with the observed one k) The prognostic models were presented in the form of a nomogram (which predicted survival at 3, 6 and 12 months), and using Kaplan-Meier curves with patients grouped according to the predicted risk profiles. The statistical analysis was performed by R software (R Core Team, 2020). The packages mice [15,18], miceadds [19] and Hmisc [20] for imputation and pooling, timeROC[21] for obtaining time-dependent ROC curves, glmpath [22] for model selection, and rms[20] for model selection, validation, calibration and prognostic models presentation.

Results

Baseline characteristics of patients

After missing values of 17 patients, we included the main clinical characteristics of patients from the two cohorts (n = 201) (Supplementary Table 1). The mean age was 71.0 [61.0;79.0] years old with a BMI of 29.0 [26.8;33.6] kg/m^2. From all patients 65.7% were men, 39.6% in-took alcohol, 22.2% were smokers, 35.3% were diabetic, 68.2% were hypertensive, 47% had edemas at admission and 12.9%, chronic obstructive pulmonary disease (COPD). The main differences between two cohorts (DEXA and SEC-ROVI) were alcohol intake, BMI, percentage of patients with edemas at admission, hepatomegaly and previous stroke.

Quantile levels of orosomucoid and omentin

Orosomucoid levels were stratified in 3 quantiles (Q1, the lowest levels, Q2 and Q3, the highest levels). Those patients with highest orosomucoid levels (Q3) were characterized by higher glucose 130 [98.2;177] *vs.* (122 [106;183] in Q1 or 163 [121;243] mg/dL in Q2, p = 0.008) and creatinine levels 1.21 [0.86;1.47] *vs.* 0.95 [0.77;1.10] in Q1 or 0.87 [0.75;1.02] mg/dL in Q2, p = 0.001. The percentage of patients with an event was higher in the Q3 group 16 (31.4%) *vs.* 6 (10.3%) in Q1 or 9 (15.0%) in Q2, p = 0.013 (Table 1). Regarding omentin, a higher percentage of smokers and alcohol intake was represented on the Q1 group (lowest omentin levels) (Table 2).

Table 1
Clinical characteristics differences among orosomucoid quantiles groups

Orosomucoid	Q1	Q2	Q3	p.overall
	N = 52	N = 57	N = 51	
Sex (Women)	19 (36.5%)	20 (35.1%)	17 (33.3%)	0.943
Age	70.5 [61.0;79.2]	70.0 [62.0;78.0]	73.0 [60.0;78.0]	0.988
Alcohol:	18 (36.0%)	26 (46.4%)	13 (25.5%)	0.080
Tobacco	10 (20.0%)	19 (33.3%)	7 (13.7%)	0.045
BMI	30.4 [27.6;35.7]	28.9 [26.6;33.6]	28.5 [26.6;32.5]	0.292
Previous peripheral artery	5 (9.62%)	3 (5.26%)	5 (9.80%)	0.642
Ascites	0 (0.00%)	0 (0.00%)	1 (2.00%)	1.000
DM	19 (36.5%)	21 (36.8%)	17 (33.3%)	0.917
Edemas_admission	27 (51.9%)	25 (43.9%)	29 (56.9%)	0.392
COPD	7 (13.5%)	7 (12.3%)	7 (13.7%)	0.972
Hepatomegalia	2 (4.35%)	5 (10.4%)	9 (18.0%)	0.102
HLP:	29 (55.8%)	29 (50.9%)	29 (56.9%)	0.799
HTA	37 (71.2%)	36 (63.2%)	38 (74.5%)	0.417
Previous myocardial infarction	4 (7.69%)	7 (12.3%)	8 (15.7%)	0.452
Previous Stroke	3 (5.77%)	3 (5.26%)	3 (5.88%)	1.000
Heart rate	69.2 (14.2)	72.2 (13.1)	69.6 (12.8)	0.465
Systolic blood preasure_admission	141 (27.3)	139 (27.1)	143 (26.7)	0.745
Salicylic Acid	13 (25.0%)	21 (38.2%)	17 (33.3%)	0.339
Amiodarone	5 (9.62%)	5 (9.09%)	10 (19.6%)	0.192
ARB	12 (23.1%)	6 (10.9%)	14 (27.5%)	0.088
Betablockers	39 (75.0%)	47 (85.5%)	39 (76.5%)	0.352
Digoxin	15 (28.8%)	11 (20.0%)	11 (22.0%)	0.532
Diuretics	48 (92.3%)	51 (92.7%)	49 (96.1%)	0.780
ACEI	33 (63.5%)	40 (72.7%)	28 (54.9%)	0.161

Orosomucoid	Q1	Q2	Q3	p.overall
Ivabradine:	2 (3.85%)	4 (7.27%)	3 (5.88%)	0.836
Metformin:	15 (28.8%)	22 (40.0%)	9 (17.6%)	0.041
Nitrates:	3 (5.77%)	3 (5.45%)	3 (5.88%)	1.000
Creatinine_admission (mg/dL)	0.95 [0.77;1.10]	0.87 [0.75;1.02]	1.21 [0.86;1.47]	0.001
LVEF_admission				0.756
< 40%	18 (40.0%)	29 (51.8%)	20 (48.8%)	
40–49%	11 (24.4%)	9 (16.1%)	7 (17.1%)	
> 50%	16 (35.6%)	18 (32.1%)	14 (34.1%)	
Glucose_admission (mg/dL)	122 [106;183]	163 [121;243]	130 [98.2;177]	0.008
HB_admission (g/dL)	13.5 (2.16)	13.7 (1.93)	13.4 (1.85)	0.673
K_admission (mmol/L)	4.41 (0.55)	4.38 (0.52)	4.60 (0.68)	0.108
Na_admission (mmol/L)	140 [139;143]	140 [138;142]	141 [138;144]	0.232
NT-ProBNP_admission (pg/mL)	2297 [1180;3518]	2064 [916;4380]	3645 [1372;7324]	0.111

Table 2
Clinical characteristics differences among omentin quantiles groups

Omentin	Q1	Q2	Q3	p.overall
	N = 51	N = 55	N = 49	
Sex (Women)	16 (31.4%)	16 (29.1%)	22 (44.9%)	0.196
Age	72.0 [62.5;79.5]	68.0 [60.0;78.0]	68.0 [61.0;79.0]	0.269
Alcohol:	26 (54.2%)	20 (36.4%)	10 (20.4%)	**0.003**
Tobacco	16 (32.7%)	14 (25.5%)	5 (10.2%)	**0.026**
BMI	30.4 [27.6;33.9]	29.2 [26.5;33.9]	28.8 [27.6;33.6]	0.621
Previous peripheral artery	5 (9.80%)	5 (9.09%)	3 (6.12%)	0.828
Ascites	0 (0.00%)	1 (1.92%)	0 (0.00%)	1.000
DM	18 (35.3%)	19 (34.5%)	20 (40.8%)	0.775
Edemas_admission	25 (49.0%)	25 (45.5%)	29 (59.2%)	0.355
COPD	9 (17.6%)	6 (10.9%)	5 (10.2%)	0.465
Hepatomegalia	4 (10.0%)	6 (11.5%)	5 (10.6%)	1.000
HLP:	24 (47.1%)	31 (56.4%)	29 (59.2%)	0.440
HTA	30 (58.8%)	41 (74.5%)	38 (77.6%)	0.085
Previous myocardial infarction	4 (7.84%)	10 (18.2%)	5 (10.2%)	0.233
Previous Stroke	3 (5.88%)	2 (3.64%)	3 (6.12%)	0.827
Heart rate	72.6 (12.2)	67.4 (14.5)	71.7 (12.4)	0.108
Systolic blood preasure	139 (24.7)	137 (26.7)	146 (27.3)	0.169
Salicylic Acid	16 (32.0%)	21 (38.2%)	12 (25.0%)	0.360
Amiodarone	4 (8.00%)	10 (18.2%)	3 (6.25%)	0.110
ARB	8 (16.0%)	11 (20.0%)	12 (25.0%)	0.540
Betablockers	36 (72.0%)	47 (85.5%)	39 (81.2%)	0.219
Digoxin	8 (16.0%)	11 (20.0%)	17 (36.2%)	**0.047**
Diuretics	45 (90.0%)	53 (96.4%)	45 (93.8%)	0.471
ACEI	32 (64.0%)	36 (65.5%)	31 (64.6%)	0.988
Ivabradine:	3 (6.00%)	3 (5.45%)	3 (6.25%)	1.000

Omentin	Q1	Q2	Q3	p.overall
Metformin:	18 (36.0%)	14 (25.5%)	12 (25.0%)	0.386
Nitrates:	2 (4.00%)	6 (10.9%)	1 (2.08%)	0.155
Creatinine_admission (mg/dL)	1.03 [0.85;1.27]	0.97 [0.80;1.27]	0.88 [0.72;1.08]	0.069
LVEF_admission				0.602
< 40%	22 (46.8%)	24 (50.0%)	19 (45.2%)	
40–49%	6 (12.8%)	11 (22.9%)	8 (19.0%)	
> 50%	19 (40.4%)	13 (27.1%)	15 (35.7%)	
Glucose_admission (mg/dL)	136 [113;214]	130 [100;189]	152 [107;190]	0.325
HB_admission (g/dL)	13.3 (2.28)	13.6 (1.88)	13.6 (1.79)	0.767
K_admission (mmol/L)	4.52 (0.55)	4.45 (0.54)	4.41 (0.70)	0.621
Na_admission (mmol/L)	140 [138;142]	140 [138;143]	141 [139;143]	0.179
NT-ProBNP_admission (pg/mL)	2242 [1186;4669]	2440 [908;4408]	2202 [1128;4784]	0.925

Markers correlation

The correlation among markers showed that neither orosomucoid nor omentin showed statistically significant correlations with any clinical variable (Supplementary Fig. 1).

Cut-off values of orosomucoid and omentin

We performed the imputation of the lost values using the MICE algorithm (Multiple imputation by chained equations). Confidence intervals obtained by overimputation showed very coverage for all the variables (for all the imputed variables, the overimputed confidence interval included the observed values in more than 90% of the cases). Distribution of imputed and original values was very close. Afterwards, the median was defined as the optimal cut-off point, threshold to consider high or low values of the markers, for orosomucoid and omentin. The omentin and orosomucoid cut-off values was 6.637 and 1.409, respectively. Then, following categories were generated: a) DownUp: Orosomucoid values below optimal and omentin values above optimal b) Equal: both proteins were simultaneously above the optimum, or below it c) UpDown: orosomucoid values above optimal and omentin values below optimal. The main differences among groups were the higher percentage of Chronic obstructive pulmonary disease (COPD), creatinine and potassium levels at admission in the UpDown group (Table 3).

Table 3
Clinical characteristics differences among categorized (OROME) groups

Orosomucoid/Omentin (OROME)	DownUp	Equal	UpDown	p.overall
	N = 43	N = 89	N = 22	
Sex (Women)	21 (48.8%)	27 (30.3%)	6 (27.3%)	0.080
Age	67.0 [59.5;79.0]	71.0 [61.0;78.0]	72.5 [62.8;78.0]	0.652
Alcohol:	13 (30.2%)	35 (40.7%)	8 (36.4%)	0.509
Tobacco	6 (14.0%)	25 (28.7%)	4 (18.2%)	0.143
BMI	28.9 [27.4;35.1]	29.9 [27.1;32.8]	28.6 [26.4;34.1]	0.987
Previous peripheral artery	2 (4.65%)	8 (8.99%)	2 (9.09%)	0.696
DM	16 (37.2%)	35 (39.3%)	5 (22.7%)	0.347
Edemas_admission	25 (58.1%)	43 (48.3%)	11 (50.0%)	0.566
COPD	1 (2.33%)	15 (16.9%)	4 (18.2%)	0.041
Hepatomegalia	2 (4.76%)	9 (12.2%)	4 (18.2%)	0.236
HLP:	24 (55.8%)	50 (56.2%)	9 (40.9%)	0.418
HTA	34 (79.1%)	57 (64.0%)	17 (77.3%)	0.153
Previous myocardial infarction	5 (11.6%)	11 (12.4%)	3 (13.6%)	1.000
Previous Stroke	3 (6.98%)	4 (4.49%)	1 (4.55%)	0.873
Heart rate	69.7 (14.1)	71.3 (13.2)	68.4 (12.4)	0.628
Systolic blood preasure_admission	144 (28.3)	139 (26.4)	140 (23.6)	0.555
Salicylic Acid	13 (30.2%)	28 (32.2%)	7 (31.8%)	0.975
Amiodarone	4 (9.30%)	9 (10.3%)	4 (18.2%)	0.601
ARB	8 (18.6%)	20 (23.0%)	3 (13.6%)	0.595
Betablockers	38 (88.4%)	67 (77.0%)	16 (72.7%)	0.210
Digoxin	14 (32.6%)	18 (20.9%)	4 (18.2%)	0.274
Diuretics	41 (95.3%)	79 (90.8%)	22 (100%)	0.253
ACEI	31 (72.1%)	54 (62.1%)	13 (59.1%)	0.452
Ivabradine:	1 (2.33%)	7 (8.05%)	1 (4.55%)	0.458
Metformin:	12 (27.9%)	28 (32.2%)	4 (18.2%)	0.426

Orosomucoid/Omentin (OROME)	DownUp	Equal	UpDown	p.overall
Nitrates:	3 (6.98%)	4 (4.60%)	2 (9.09%)	0.715
Creatinine_admission (mg/dL)	0.84 [0.70;1.00]	1.00 [0.82;1.28]	1.21 [0.94;1.30]	0.001
LVEF_admission				0.918
< 40%	17 (43.6%)	41 (51.2%)	7 (41.2%)	
40–49%	8 (20.5%)	14 (17.5%)	3 (17.6%)	
> 50%	14 (35.9%)	25 (31.2%)	7 (41.2%)	
Glucose_admission (mg/dL)	127 [107;192]	137 [108;193]	132 [111;189]	0.919
HB_admission (g/dL)	13.6 (1.91)	13.5 (2.08)	13.2 (1.80)	0.785
K_admission (mmol/L)	4.25 (0.54)	4.52 (0.62)	4.63 (0.46)	0.017
Na_admission (mmol/L)	140 [139;143]	140 [138;143]	142 [139;144]	0.192
NT-ProBNP_admission	2360 [1110;3815]	2086 [1126;5261]	3431 [1052;5368]	0.677

Follow up and events

During the follow-up, 16% of patients were rehospitalized for HF and/or death. These patients were older, mostly hypertensive, with high proBNP and high creatinine levels at admission with respect to those without event (Supplementary table 2). Patients in the UpDown group had the worse prognosis at 90, 180 and 365 days. The Kaplan-Meier curves showed that these differences were statistically significant ($p < 0.05$) (Fig. 1).

After testing the number of events in the NT-proBNP, orosomucoid and omentin categorized groups by quantiles and the combined orosomucoid and omentin group regarding cut-off values (Up or down), we observed that all the events in the DownUp group, low orosomucoid and high omentin levels, were recorded six months after discharge (Supplementary Fig. 2).

Predictive models for rehospitalization for HF and/or death

Pathologies were grouped in a Score, assigning 3 points if the patient had hypertension, 2 points if they have COPD, 1.2 points if they have hyperlipidemia, and 1 point to the remaining pathologies. For the treatments, the variable Pharmacological-score was generated, assigning 4 points if the patient takes nitrates, 3 points if the patient takes amiodarone, 2 points if the patient takes acetylsalicylic acid, and 1 point to the other drugs. Three different methodologies, Lasso method and Backwards Step-down deletion with and without bootstrapped validation (200 bootstrap samples) with AIC as stopping rule, were performed for selecting the variables for the best predictive model. Among the most frequently selected covariates were LVEF, K^+, Creatinine, BMI, and pharmacological and pathology scores. NT-

ProBNP was selected in every model. With regard to Omentin and Orosomucoid, their selection was increased when their interaction with any of the scores were also included (Supplementary table 3). After testing the predictive models that includes same clinical variables BMI, pathology and pharmacological scores, LVEF and K^+ and the biomarkers, we observe that the inclusion of NT-proBNP determined a c-index = 0.8016 (optimism corrected C-index = 0.798) (Central Illustration), the inclusion of orosomucoid depicted a c-index = 0.8036 (optimism corrected C-index = 0.788) (Central Illustration), the inclusion of ometin a c-index = 0.7953 (optimism corrected C-index = 0.772) and the combination of categorized orosomucoid and omentin (OROME) had similar c-index = 0.7889 (optimism corrected C-index = 0.778) (Central Illustration). But the inclusion of NT-proBNP and the combined orosomucoid and omentin (OROME) showed the best c-index = 0.8488 (Central Illustration). These results indicated the improvement of the predictive model with all three biomarkers. (Central Illustration).

The AUC were sustained in the time (Supplementary Fig. 3a) with model OROME + NT-proPNB outperformed model NT proBNP (Supplementary Fig. 3b). Models including only combination of orosumucoid and omentin (OROME) or orosomucoid performed as well as NT proBNP model (Supplementary Fig. 3c and d).

Calibration plots showed very good concordance between predicted and observed survival probabilities for the selected model (Supplementary Fig. 4).

Nomogram of finally selected model (including BMI, pathology and pharmacological scores, LVEF, K^+, NT-proBNP and OROME) is presented in Fig. 2. It shows predicted survival probabilities at several follow-up times. The total points are obtained by adding the individual points of each predictor. These points are obtained by drawing a vertical straight line from the predictor value to Points, (i.e., 42.5 points are assigned to a concentration of NT-ProBNP of 15000 ng/ml).

Discussion

Our results show that the combination of plasma orosomucoid and omentin values improved the prognostic risk stratification of *de novo* AHF patients. Therefore, low orosomucoid and high omentin levels were associated with the lowest risk of suffering adverse outcomes during the follow-up compared with UpDown or equal group (high orosomucoid and low omentin levels or both proteins high or low). Adding these two new biomarkers to NT-ProBNP significally improves the risk stratification of acute de novo HF patients.

To the best of our Knowledge we describe for the first time the prognostic value of these biomarkers in AHF patients. Our findings may have implications not only for the risk stratification at hospital discharge because these new biomarkers can be considered as a potential therapeutic target and useful for the development of new treatment strategies and help to improve the patient care pathway.

Nowadays *de novo* HF represents one third of hospitalized patients for AHF [23]. Although their mortality in hospital is similar to worsening HF, they have better post-discharge follow-up, less associated

comorbidities and less advance HF syndrome [24,25]. However, their long-term prognosis after hospitalization for HF remains poor. Our data showed that 16 % patients had an event during the 1-year mean follow-up. Thus, many efforts are focussed on these patients with the aim of achieving hemodynamic stability, preventing recurrent HF, and reducing mortality[12].

The present study shows that admitted patients for *de novo* AHF with high orosomucoid and low omentin levels presented the highest risk of death or requiring re-hospitalization for HF during an early or mid-term follow-up. However, any patients with low plasma orosomucoid and high omentin levels had an event within 100 days after discharge. These patients represent the 40% of the AHF patients. Therefore, there is an improvement on risk stratification of patients with *de novo* AHF using the combination of two proteins (high concentration of orosomucoid and low concentration of omentin).

One of the main issues, in the AHF management, is based on the identification of patients with worse prognosis for improving their management and trying to find an optimal medical treatment and an efficient follow-up plan. With this objective has been used clinical parameters (heart rate at the admission [26], blood pressure, renal failure [23], protein biomarkers (NT-proBNP [27], ST2 [28], galectin-3 [29]) and several scores. However, all of them have some limitations. Recent data showed that a quite cardiac specific marker, DKK3, had also a limited additional prognostic value regarding NT-proBNP[30]. Even, a multimarker panel was suggested in patients with AHF and renal mild or moderate impairment or the combination of two biomarkers in dyspnoeic patients. However, this is the first time that a protective and a deleterious marker combination were tested in AHF the new-onset for stratifying patients according mortality risk or readmission for HF. The combination of NT-proBNP, orosomucoid and omentin took out the best predictive model for rehospitalization for HF and/or death. A nomogram strategy might change the clinical follow-up of the patients (Fig. 3).

The pathogenesis of *de novo* HF is not completely known and an inflammatory mechanism has been suggested [1]. The association between inflammation and HF has been recognized after many studies demonstrated that pro-inflammatory biomarkers are elevated in patients with a variety of cardiomyopathies and the clinical presentation of the HF syndrome. The plasma levels of inflammatory biomarkers have correlated with the prognosis and severity of the disease in both reduced and preserved LVEF-HF patients [29 ,24, 25].

In this sense, low levels of omentin, an anti-inflammatory cytokine, have been described in chronic HF patients [14] and higher levels of orosomucoid have been related to worse outcome in AHF patients [12]. These proteins have a protective effect on cardiomyoblasts, in part, mediated by an anti-lipotoxicity effect [13].

Omentin might play a cardioprotective role against an inflammatory process and high orosomucoid levels since its high levels are able to reduce the probability of death or readmission for HF in those patients with high orosomucoid levels.

The role of our discovery in the pathogenesis and prognosis of HF has potential therapeutic and diagnostic implications. We could not prove if these proteins are a direct cause of HF, but if it is confirmed in further studies, the treatment targeting this inflammatory pathway should be beneficial; however, if it is only a marker of the disease it could help us to identify patients who are in a more advanced state in the moment of the diagnosis.

Limitations

The number of patients included is small but is comparable to other studies that assessed similar objectives and outcome and the results are statistically significant and we think that this issue will have future clinical relevance. The adjustment for other clinical parameters and biomarkers may influence our results.

Conclusion

The combination of orosomucoid, omentin and NT-proBNP could be a new biomarker strategy for risk stratification in patients with *de novo* HF. Our findings should be considered as hypothesis-generating and needs to be confirmed in future large-scale cohorts of patients with the spectrum of HF clinical presentation.

CLINICAL PERSPECTIVES

Acute HF (AHF) is associated with poor outcomes and mortality. The determination of orosomucoid and omentin could helps to stratify the risk of this population.

Acute *de novo* HF patients with low orosomucoid and high omentin levels are associated with better outcomes.

Adding these two new biomarkers to NT-ProBNP significally improves the risk stratification of acute de novo HF patients.

TRANSLATIONAL OUTLOOK:

The determination of OROME may have implications for the risk stratification at in " de novo" AHF patients. Indeed, these new biomarkers could be considered as a potential therapeutic target and useful for the development of new treatment strategies in order to improve the patient care pathway.

Abbreviations

AHF: acute heart failure

HF: heart failure

LVEF: left ventricular ejection fraction

NT-proBNP : N-terminal pro-brain natriuretic peptide

Declarations

Acknowledges

We would like to thank patient's participation.

Ethics approval an consent to participate : The study complies with the Declaration of Helsinki and was approved by the Clinical Research Ethics Committee of Galicia. All patients provided an informed consent.

Consent for publication : not applicable

Availability of data and materials: not applicable

Competing interests : not apllicable

Funding : The present study was supported by Complejo Hospitalario Universitario de Santiago de Compostela y Pontevedra (SERGAS), Health Research Institute of Santiago de Compostela (IDIS), Sociedad Española de Cardiología (SEC-ROVI, 2017), Fondo de Investigaciones Sanitarias Instituto de Salud Carlos III (ISCIII) Madrid, Spain (PI18/00821), (PI16/01282), European Regional Development Fund (FEDER) and INTERREG-POCTEP-0227-CODIGOMAIS-1-E.

Authors' contributions : all authors contributed to this publication

Acknowledgements : We would like to thank patient's participation.

References

1. Goonewardena SN, Stein AB, Tsuchida RE, Rattan R, Shah D, Hummel SL. Monocyte Subsets and Inflammatory Cytokines in Acute Decompensated Heart Failure. *J Card Fail* 2016;22.
2. Jankovic-Tomasevic R, Pavlovic SU, et al. Prognostic utility of biomarker growth differentiation factor-15 in patients with acute decompensated heart failure. *Acta Cardiol* 2016;
3. Metra M, Nodari S, Parrinello G, et al. The role of plasma biomarkers in acute heart failure. Serial changes and independent prognostic value of NT-proBNP and cardiac troponin-T. *Eur J Heart Fail* 2007; .
4. Boulogne M, Sadoune M, Launay JM, Baudet M, Cohen-Solal A, Logeart D. Inflammation versus mechanical stretch biomarkers over time in acutely decompensated heart failure with reduced ejection fraction. *Int J Cardiol* 2017;
5. Ponikowski P, Voors AA, Anker SD, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. Eur. Heart J. 2016.

6. Januzzi JL, Camargo CA, Anwaruddin S, et al. The N-terminal Pro-BNP investigation of dyspnea in the emergency department (PRIDE) study. *Am J Cardiol* 2005; 95(8):948-954.
7. Wang CH, Yang NI, Liu MH, Hsu KH, Kuo LT. Estimating systemic fibrosis by combining galectin-3 and ST2 provides powerful risk stratification value for patients after acute decompensated heart failure. *Cardiol J* 2016;23(5):563-572.
8. Ueland T, Gullestad L, Nymo SH, Yndestad A, Aukrust P, Askevold ET.Inflammatory cytokines as biomarkers in heart failure. *Clin Chim Acta* 2015**;** .
9. Behnes M, Espeter F, Hoffmann U, et al. Diagnostic and long-term prognostic value of sensitive troponin i in symptomatic patients suspected of acute heart failure. *Clin Lab* 2015;
10. Fandiño-Vaquero R, Fernández-Trasancos A, Álvarez E, et al. Orosomucoid secretion levels by epicardial adipose tissue as possible indicator of endothelial dysfunction in diabetes mellitus or inflammation in coronary artery disease. *Atherosclerosis* 2014 ;235(2):281-8
11. Eiras S, Varela-Román A, Andrade MC, et al. Non classical Monocytes Levels, Increased by Subcutaneous Fat-Secretome, Are Associated with Less Rehospitalization after Heart Failure Admission. *J Cardiovasc Transl Res* 2017;10(1):16-26.
12. Agra RM, Varela-Román A, González-Ferreiro R, et al. Orosomucoid as prognosis factor associated with inflammation in acute or nutritional status in chronic heart failure. *Int J Cardiol* 2017; 228:488-494.
13. Lage R, Moscoso I, Fernández-Trasancos Á, et al. Differential behaviour of epicardial adipose tissue-secretomes with high and low orosomucoid levels from patients with cardiovascular disease in H9C2 cells. *Mol Cell Endocrinol* 2015; 416:77-87**.**
14. Narumi T, Watanabe T, Kadowaki S, et al. Impact of serum omentin-1 levels on cardiac prognosis in patients with heart failure. *Cardiovasc Diabetol* 2014;13:84.
15. Buuren S van. Multiple imputation of discrete and continuous data by fully conditional specification. *Stat Methods Med Res* 2007**;** 16(3):219-42.
16. Rubin DB. Multiple imputation in sample surveys - A phenomenlogical Bayesian approach to nonresponse. *Proceedings of the survey research methods section of the American Statistical Association.* 1978.
17. Galbraith S. Applied Missing Data Analysis by Craig K Enders. *Aust N Z J Stat* 2012;54.
18. Groothuis-oudshoorn K. Journal of Statistical Software MICE : Multivariate Imputation by Chained. *J Stat Softw* 2011..
19. Robitzsch A, Grund S. miceadds: Some Additional Multiple Imputation Functions, Especially for 'mice'. *R Packag version* 2020;
20. Harrell FE. Hmisc: Harrell miscellaneous. *R Packag version 44-0* 2020.

21. Blanche P, Dartigues JF, Jacqmin-Gadda H. Estimating and comparing time-dependent areas under receiver operating characteristic curves for censored event times with competing risks. *Stat Med* 2013; 32(30):5381-97.

22. Park, M. Y. and Hastie T. No Title. *J R Stat Soc Ser B* 2008;69:659–677.

23. Hummel A, Empen K, Dörr M, Felix SB. De Novo Acute Heart Failure and Acutely Decompensated Chronic Heart Failure. *Dtsch Aerzteblatt Online* 2015.

24. Lassus JPE, Harjola VP, Peuhkurinen K, et al. Cystatin C, NT-proBNP, and inflammatory markers in acute heart failure: Insights into the cardiorenal syndrome. *Biomarkers* 2011;16(4):302-10.

25. Banno A, Kohsaka S, Yoshikawa T. Elevated inflammatory markers in heart failure patients with lower BMI. J. Card. Fail. 2011; 17(3):264.

26. Lourenço P, Ribeiro A, Cunha FM, et al. Is there a heart rate paradox in acute heart failure? *Int J Cardiol* 2016; 203:409-14.

27. Fonarow GC, Peacock WF, Phillips CO, Givertz MM, Lopatin M. Admission B-Type Natriuretic Peptide Levels and In-Hospital Mortality in Acute Decompensated Heart Failure. *J Am Coll Cardiol* 2007; 49(19):1943-50.

28. Shah R V., Januzzi JL. ST2: A novel remodeling biomarker in acute and chronic heart failure. Curr. Heart Fail. Rep. 2010; 7(1):9-14.

29. Lainscak M, Coletta AP, Sherwi N, Cleland JGF. Clinical trials update from the Heart Failure Society of America Meeting 2009: FAST, IMPROVE-HF, COACH galectin-3 substudy, HF-ACTION nuclear substudy, DAD-HF, and MARVEL-1. *European Journal of Heart Failure* 2010; 12(2):193-6.

30. Piek A, Suthahar N, Voors AA, Boer RA de, Silljé HHW. A combined bioinformatics, experimental and clinical approach to identify novel cardiac-specific heart failure biomarkers: is Dickkopf-3 (DKK3) a possible candidate? *Eur J Heart Fail* 2020; https://doi.org/10.1002/ejhf.1988.

Figures

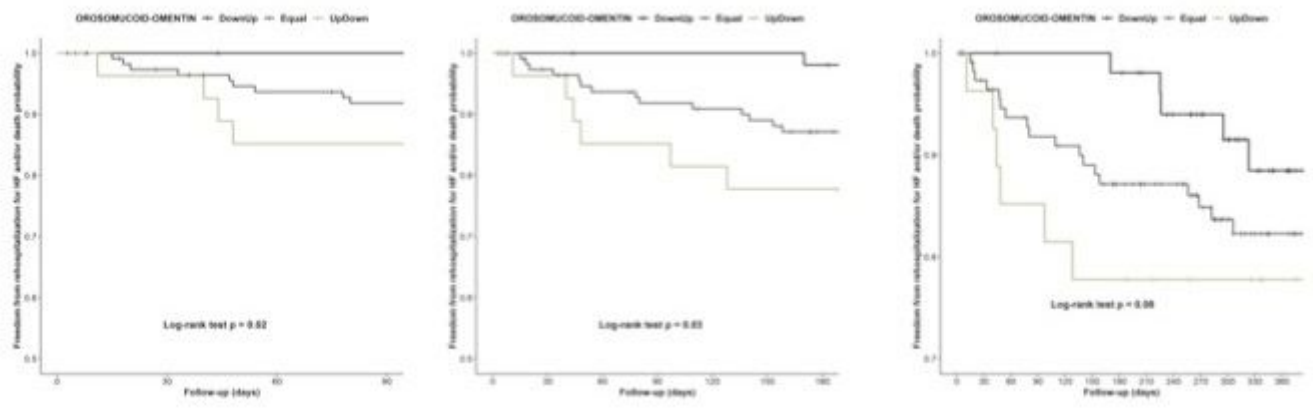

Figure 1

Kaplan Meier curves display the rehospitalization for HF and/or death probability for the three categorized groups (DownUp-low orosomucoid and high omentin, Equal-both protein low or high, UpDown-high oromucoid and low omentin levels) at 3, 6 and 12 months after discharge.

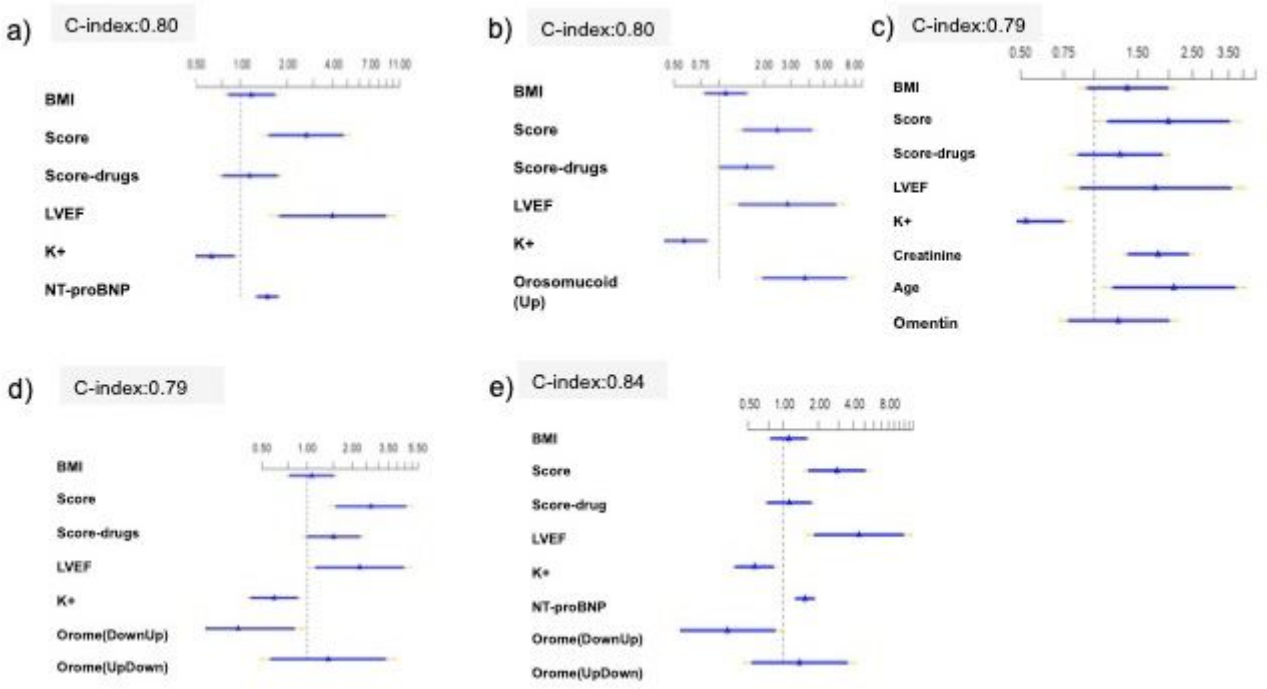

Figure 2

Predictive models with a) NT-proBNP, b) Orosomucoid, c) OROME, d) NT-proBNP and OROME and hazard ratio and 95% confidence interval for events of each variable

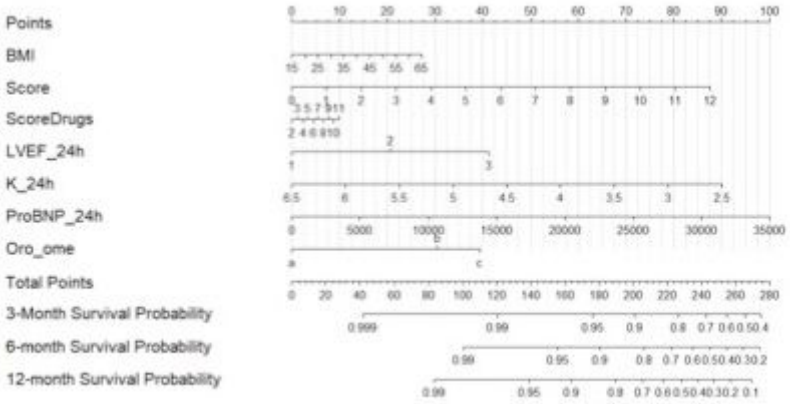

Figure 3

Nomogram of finally selected model (including BMI, pathology and pharmacological scores, LVEF, K+, NT-proBNP and combination of orosomucoid and omentin)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SuplemmentaryCHf.pdf