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Anne Marie Dupuy, Nils Kuster, Anne Sophie Bargnoux, Sylvain Aguilhon, Fabien Huet, Florence Leclercq, Jean-Luc Pasquié, François Roubille and Jean Paul Cristol*

Long term pronostic value of suPAR in chronic heart failure: reclassification of patients with low MAGGIC score

Abstract

Objectives: Inflammation is a hallmark of heart failure (HF) and among inflammatory biomarkers, the most studied remains the C-reactive protein (CRP). In recent years several biomarkers have emerged, such as sST2 and soluble urokinase–type plasminogen activator receptor (suPAR). This study set out to examine the relative importance of long-time prognostic strength of suPAR and the potential additive information on patient risk with chronic HF in comparison with pronostic value of CRP and sST2.

Methods: Demographics, clinical and biological variables were assessed in a total of 182 patients with chronic HF over median follow-up period of 80 months. Inflammatory biomarkers (i.e., CRP, sST2, and suPAR) were performed.

Results: In univariate Cox regression analysis age, NYHA class, MAGGIC score and the five biomarkers (N-terminal pro brain natriuretic peptide [NT-proBNP], high-sensitive cardiac troponin T [hs-cTnT], CRP, sST2, and suPAR) were associated with both all-cause and cardiovascular mortality.

In the multivariate model, only NT-proBNP, suPAR, and MAGGIC score remained independent predictors of all-cause mortality as well as of cardiovascular mortality. Risk classification analysis was significantly improved with the addition of suPAR particularly for all-cause short- and long-term mortality. Using a classification tree approach, the same three variables could be considered as significant classifier variables to predict all-cause or cardiovascular mortality and an algorithm were reported. We demonstrated the favorable outcome associated with patients with a low MAGGIC score and a low suPAR level by comparison to patients with low MAGGIC score but high suPAR values.

Conclusions: The main findings of our study are (1) that among the three inflammatory biomarkers, only suPAR levels were independently associated with 96-month mortality for patients with chronic HF and (2) that an algorithm based on clinical score, a cardiomyocyte stress biomarker and an inflammatory biomarker could help to a more reliable long term risk stratification in heart failure.

Keywords: biomarkers; heart failure; low grade inflammation; prognosis; soluble urokinase–type plasminogen activator receptor (suPAR).

Introduction

The link between inflammation and heart failure (HF) has been established for decades [1]. This pathophysiological pathway suggests an important role of inflammatory biomarkers. C-reactive protein (CRP) remains the most studied inflammatory biomarker, and is considered through many publications as a risk of similar importance to traditional cardiovascular risk factors such as age, sex, hypercholesterolemia, hypertension or smoking [2]. However, inconsistent relationship was reported between high level of CRP and mortality risk [3]. Among the emerging inflammatory biomarkers, sST2, which has been widely studied [4], has been shown to be an independent predictor of mortality and as a promising guide to decision-making. However, the relationship between poor outcome and sST-2 level seems to be limited in the time [5]. More recently, the soluble

*Corresponding author: Jean Paul Cristol, Department of Biochemistry, Centre Ressources Biologiques de Montpellier, University Hospital of Montpellier, Montpellier cédex 5 34295, France; and PhyMedExp, University of Montpellier, INSERM U1046, CNRS UMR 9214, Montpellier, France, Fax: +33 4 67 33 83 93, E-mail: jp-cristol@chu-montpellier.fr

Anne Marie Dupuy, Department of Biochemistry, Centre Ressources Biologiques de Montpellier, University Hospital of Montpellier, Montpellier, France

Nils Kuster and Anne Sophie Bargnoux, Department of Biochemistry, Centre Ressources Biologiques de Montpellier, University Hospital of Montpellier, Montpellier, France; and PhyMedExp, University of Montpellier, INSERM U1046, CNRS UMR 9214, Montpellier, France

Sylvain Aguilhon, Fabien Huet, Florence Leclercq and Jean-Luc Pasquié, Cardiology Department, University Hospital of Montpellier, Montpellier, France

François Roubille, and PhyMedExp, University of Montpellier, INSERM U1046, CNRS UMR 9214, Montpellier, France; Cardiology Department, University Hospital of Montpellier, Montpellier, France

urokinase-type plasminogen activator receptor (suPAR) was identified as a risk biomarker for common diseases (cancer, diabetes) including cardiovascular disease and death in the general population [6]. suPAR is the soluble form of the cell membrane-bound protein uPAR, which is expressed mainly in immune cells, endothelial cells and smooth muscle cells. uPAR is released during inflammation (even in low grade inflammation) or immune activation, and therefore the suPAR level reflects the extent of immune activation in the individual [7]. suPAR is of particular interest compared to other inflammatory biomarkers because it has a distinct pathophysiological pathway than CRP and studies show that it appears to outperform CRP in risk prediction for cardiovascular diseases [8]. We aimed to evaluate the long-time prognostic strength of suPAR and the potential additive information on patient risk with chronic HF to well-established prognostic biomarkers as CRP, natriuretic peptides and sST2 and clinical score such as MAGGIC score. The final goal of this study is to propose a decisional tree in order to stratify the long-term risk.

Materials and methods

This retrospective study was based in a previous biologic bank build in 2011 for which we have already studied specific biomarkers such as sST2, GDF-15, collagen metabolism and now inflammatory biomarkers [5, 9, 10]. This registry of patients with diagnostic of stable HF based on criteria of the European Society of Cardiology has been described in details elsewhere [9, 10]. For each patient, we calculated the MAGGIC project heart failure risk score which included 13 routinely available clinical variables [11]. At inclusion of patients, routine parameters such as urea, electrolytes, creatinine, N-terminal pro brain natriuretic peptide (NT-proBNP), (NT-proBNP), high-sensitive cardiac troponin T (hs-cTnT), and CRP, were performed on Cobas 8000 (Roche Diagnostic, Meylan, France) using e701 and e602 modules. In addition, venous blood was collected in dry and EDTA tubes, immediately centrifuged and frozen (-80°C) on several aliquots until tested. sST2 plasma concentrations were measured with a high sensitivity sandwich monoclonal immunoassay (Presage[®] ST2 assay, Critical Diagnostics, San Diego, CA, distributed in France by Eurobio society) using an EDTA plasma aliquot never thawed. In June 2019, the determination of suPAR levels (from plasma aliquot never thawed) was performed with suPARnostic[®]TurbiLatex reagents provided by ViroGates society (Birkerød, Danemark) and adapted on Cobas 8000 analyzer (module c502) using a turbidimetric method. Follow-up analysis was conducted over eight years from inclusion to endpoint defined as all-cause mortality. Cardiovascular death included death resulting from an acute myocardial infarction, sudden cardiac death, decompensated HF, stroke, cardiovascular procedures, and cardiovascular hemorrhage.

Statistical analysis

Categorical data are expressed as count (percentage). Continuous data are expressed as median [1st quartile; 3rd quartile]. In descriptive

statistics, comparisons between two groups were performed using the Mann–Whitney U test for continuous variables and the chi-square test for categorical variables.

Survival distributions were estimated using a Kaplan–Meier non-parametric estimator, and a log-rank test was used to compare multiple survival distributions.

Survival analysis was performed using the Cox proportional hazard model to evaluate associations between clinical and biological parameters and all-cause mortality. Because of skewed distributions, biomarkers concentrations were log-transformed before modeling. A univariate baseline model was fitted, including variables potentially associated with mortality in HF patients such as age, NYHA class, left ventricular ejection fraction (LVEF) and biomarkers level. Significant variables in univariate analysis were then considered in a multivariate model. Feature selection in multivariate analysis was performed by a stepwise selection procedure. Bayesian information criterion (BIC) was used as selection criterion.

Ability of markers to predict mortality at eight years of follow-up was assessed using time-dependent receiver operational characteristics (ROC) curves, as proposed by Heagerty et al. [12]. Ability of NT-proBNP, hs-cTnT, CRP, sST2, and suPAR for predicting 96-month mortality was estimated using area under the curve (AUC). Optimal cut-off was estimated by maximizing Youden index. The clinical benefit in risk prediction of adding a biomarker to the clinical model was further assessed by reclassification analysis, including the net reclassification improvement (NRI). Reclassification analysis was proposed to evaluate added usefulness of a new biomarker over pre-existing models [13].

In order to improve readability of results, a conditional inference tree has been grown from our data. This method uses a binary recursive partitioning framework developed by Strasser and Weber [14]. Roughly, the algorithm works in three steps: 1) Test the global hypothesis of independence between output variable (mortality in this study) and any of the input variable 2) If this hypothesis can be rejected, select the variable with the strongest association to the response 3) Implement a binary split of the data using the selected variable. Step 1 and 2 are repeated recursively. Test statistic considered in step 1 is corrected using Bonferroni method.

Analysis was performed using R 3.5.3 (R Core team, Vienna, Austria). Survival ROC version 1.03 and partykit 1.2-7 packages were used for time-dependent ROC curve analysis and classification tree, respectively. Significance level was set to $p < 0.05$.

Results

Out of 182 consecutive patients included from May 2010 to February 2011, biochemical measurements and vital status were available for 179, which were included in our analysis. Over a median follow-up period of 80 months (inter quartile range 12.3–90 months) there were 89 deaths. In the overall patient cohort, the 96-month mortality rate was 51%. Clinical and biochemical variables in survivors vs. deceased are reported in Table 1. Among all comorbidities, only diabetes and chronic kidney disease were associated with excess mortality. The NYHA class at baseline tended to be higher among decedents than survivors. Median

LVEF was not significantly different between the two groups of patients. All median biochemical parameters values including CRP, sST2 and suPAR biomarkers were statistically significantly higher in deceased patients vs.

alive. Concentrations of biomarkers were reported in Supplemental Table B. Mortality, assessed by Kaplan–Meier estimator, clearly increased across quartiles of CRP, sST2, and suPAR (Figure 1, $p < 0.001$ for all).

Table 1: Baseline characteristics of all patients with chronic HF according to all-cause mortality. Data presented as median [1st quartile; 3rd quartile] and number of patients with percentage of total.

Variable	Study population n=179	Alive n=90	Deceased n=89	p-Value
Demographic characteristics				
Age, years	75 [66; 82]	70 [62; 78]	79 [72; 84]	<0.001
Gender, n (%)				
Females	55 (30.7%)	33 (36.7%)	22 (24.7%)	–
Males	124 (69.3%)	57 (63.3%)	67 (75.3%)	–
Co-morbidities, n (%)				
Hypertension	114 (63.7%)	51 (56.7%)	63 (70.8%)	0.072
Diabetes	63 (35.2%)	23 (25.6%)	40 (44.9%)	0.007
COPD	40 (22.3%)	19 (21.1%)	21 (23.6%)	0.731
Chronic kidney disease	94 (52.6%)	35 (38.9%)	59 (66.3%)	<0.001
Pulmonary embolism	11 (6.1%)	5 (5.6%)	6 (6.7%)	0.774
Myocarditis	1 (0.6%)	1 (1.1%)	0 (0%)	1.000
Smoking habit	86 (48%)	47 (52.2%)	39 (43.8%)	0.293
Dyslipidemia	86 (48%)	45 (50%)	41 (46.1%)	0.659
Heart failure characteristics, n (%)				
NYHA class				
I	10 (5.6%)	7 (8%)	3 (3.4%)	
II	54 (30.5%)	34 (38.6%)	20 (22.5%)	
III	82 (46.3%)	38 (43.2%)	44 (49.4%)	
IV	31 (17.5%)	9 (10.2%)	22 (24.7%)	0.007
Ischemic cardiopathy	87 (53.7%)	43 (51.2%)	44 (56.4%)	0.538
Defibrillator	50 (27.9%)	25 (27.8%)	25 (28.1%)	1.000
Medication use, n (%)				
ACE inhibitors or ARBs	123 (68.7%)	70 (77.8%)	53 (59.6%)	0.009
Betablockers	120 (67%)	64 (71.1%)	56 (62.9%)	0.266
Ivabradine	7 (3.9%)	2 (2.2%)	5 (5.6%)	0.277
Aldosterone antagonists	53 (29.6%)	36 (40%)	17 (19.1%)	0.004
Diuretics	128 (71.5%)	63 (70%)	65 (70%)	0.736
Antiplatelet agent	14 (7.8%)	7 (7.8%)	7 (7.9%)	1.000
Anticoagulant therapy	18 (10.1%)	9 (10.1%)	9 (10%)	1.000
Statin	16 (8.9%)	7 (7.8%)	9 (10.1%)	0.621
Anti-arrhythmic	11 (6.1%)	6 (6.7%)	5 (5.6%)	1.000
Others	8 (4.5%)	2 (2.2%)	6 (6.7%)	0.164
Clinical measures				
Body mass index, kg/m ²	26.1 [23; 30]	27 [23; 31]	25 [23; 29]	0.268
LVEF, %	35 [25; 45]	35 [29; 47]	35 [25; 45]	0.216
Biomarkers				
Urea, mmol/L	9.5 [7; 14]	8 [6; 11]	12 [8; 18]	<0.001
Creatinine, μmol/L	102 [83; 138]	88 [76; 119]	117 [93; 156]	<0.001
eGFR CKD-EPI, mL/min/1.73 m ²	54 [38; 76]	65 [50; 86]	48 [33; 65]	<0.001
NT-proBNP, ng/L	2,503 [867; 5,645]	1,583 [5,554; 3,432]	3,312 [1,798; 9,655]	<0.001
Hs-cTnT, ng/L	43 [20; 133]	32 [15; 92]	56 [31; 155]	0.001
CRP, mg/L	6 [2; 26]	4 [2; 18]	13 [3; 30]	0.008
sST2, ng/mL	37 [20; 71]	26 [15; 48]	48 [28; 91]	< 0.001
suPAR, ng/mL	6 [3.7–8.5]	4.5 [3.0–6.2]	7 [4.5–10.6]	< 0.001
MAGGIC score	24 [19–28.2]	20 [16–24.5]	27 [24–30]	<0.001

COPD, chronic obstructive pulmonary disease; NYHA, New York Heart Association; ACE, angiotensin converting enzyme; ARB, angiotensin receptor blocker; LVEF, left ventricular ejection fraction; eGFR CKD-EPI, estimated glomerular filtration rate chronic kidney disease – epidemiology collaboration. hs-cTnT, high sensitivity cardiac troponin T; CRP, C reactive protein; sST2, soluble suppression of tumorigenicity 2; suPAR, soluble urokinase-plasminogen activator receptor. Bold p-values <0.05.

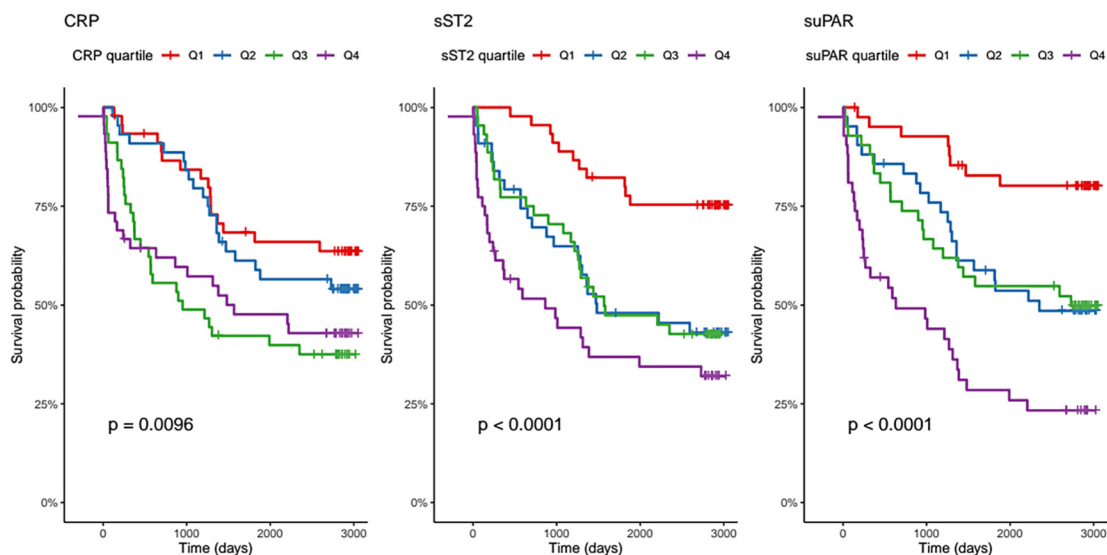


Figure 1: Kaplan Meier curves for all-cause mortality based on quartiles of CRP, sST2, and suPAR. CRP, C-reactive protein; sST2, soluble suppression of tumorigenicity 2; suPAR, soluble urokinase-type plasminogen activator receptor.

ROC curve analyses were used to predict 96-month all-cause mortality (Supplemental Data, Figure A). In this analysis, suPAR evidenced a slightly higher AUC (0.72) at 96-month compared to sST2 and CRP for which had a distinctly lower AUC. However, any statistically significant differences in AUCs were observed. Optimal cut-offs as defined by highest Youden index for sST2, suPAR, and CRP were 24.9, 6.91 ng/mL and 12.4 mg/L, respectively. The optimal cut-off value of suPAR was determined at 6.91 ng/mL higher than that of Koller et al. i.e., 4.4 ng/mL [15].

In univariate Cox regression analysis over the 96-month, age, NYHA class, MAGGIC score and the five biomarkers (NT-proBNP, hs-cTnT, CRP, sST2, and suPAR) were associated with both all-cause and cardiovascular mortality (Table 2). In the multivariate model, only NT-proBNP, suPAR, and MAGGIC score remained independent predictors of all-cause mortality as well as of cardiovascular mortality (Table 2). In our population, 38 patients had HF with preserved left ventricular ejection fraction (HFpEF), 138 patients with reduced LVEF (HFrEF) (for three patients, data were missing). Ability of HFpEF has been tested in our study population and showed that it was not a significant predictor in univariate analysis (HR=1.39, $p=0.29$). Levels of circulating biomarkers in HFpEF patients were thus not explored further.

Risk classification analysis was significantly improved with the addition of suPAR particularly for all-cause short- and long-term mortality. The improvement is less pronounced for cardiovascular mortality. At 42-months of follow-up, suPAR vs NT-proBNP had reclassified 31.1% ($p<0.002$) and vs. MAGGIC score 28.3% ($p<0.036$) of

patients. For cardiovascular mortality, suPAR added prognostic value over NT-proBNP for 35.5% ($p=0.004$) and over MAGGIC score for 31% ($p<0.06$) of patients (Supplemental Data, Table A).

Using a classification tree approach, the same three variables could be considered as significant classifier variables to predict all-cause or cardiovascular mortality. As shown in the classification tree model reported in Figure 2, MAGGIC score has the major impact on prediction of the dependent variable (all-cause mortality prediction). Looking at the most predictive leaves, we note that patients showing MAGGIC score >24 and a NT-proBNP $>1,150$ ng/L (log 3.067) have a low 96-months survival probability of 16% (95%CI, 9–28%) (see node7). On the contrary, in the left branch of the tree (MAGGIC score ≤ 24) patients with suPAR <6.91 (log 0.839) shown a very high 96-month survival probability of 87% (79–96%) (node 3), and patients with suPAR (log 0.839) >6.91 had a much lower survival probability of 31% (16–62) (node 4). Figure 2B revealed that the suPAR has the major impact on prediction of the dependent variable (cardiovascular mortality). Looking at the most predictive nodes, we note that patients with high suPAR concentrations >6.91 (log 0.839) have a much lower survival probability of 43% (28.4–65.1%) (node 5). On the contrary, in the left branch of the tree (suPAR <6.91) when the MAGGIC score is ≤ 24 , the probability of cardiovascular mortality is only 3.5% [0–8.1%] (see node 3). At the same level if MAGGIC score is >24 , the probability of cardiovascular mortality dramatically increases (see node 4).

Finally, in order to confirm the clinical relevance of these associations we performed Kaplan–Meier analysis

Table 2: (A) Univariate Cox regression analysis at global follow-up for prediction of all-cause and cardiovascular mortality, (B) Multivariate Cox regression analysis at global follow-up with BIC selection after adjusting of MAGGIC score and biological parameters for prediction of all-cause mortality and (C) cardiovascular mortality.

(A)	All-cause mortality		Cardiovascular mortality	
	Variable	HR [95% CI]	p-Value	HR [95% CI]
Age	1.045 [1.023 – 1.066]	<0.001	1.035 [1.004 – 1.066]	<0.025
Gender: Male	1.520 [0.939 – 2.461]	0.088	1.408 [0.686 – 2.891]	0.351
NYHA class	1.614 [1.234 – 2.109]	<0.001	1.710 [1.36 – 2.574]	0.010
LVEF	0.987 [0.972 – 1.003]	0.107	0.984 [0.996 – 1.008]	0.186
NT-proBNP (log 10)	2.844 [1.959 – 4.130]	<0.001	3.915 [2.201 – 6.963]	< 0.001
Hs-cTnT (log 10)	1.576 [1.199 – 2.051]	0.001	1.716 [1.159 – 2.542]	0.001
CRP (log 10)	1.736 [1.266 – 2.403]	0.001	2.329 [1.43 – 3.792]	0.001
sST2 (log 10)	3.463 [2.186 – 5.488]	<0.001	4.404 [2.231 – 8.692]	<0.001
suPAR (log 10)	12.056 [4.947 – 29.379]	<0.001	17.940 [4.755 – 67.681]	<0.001
MAGGIC score	1.157 [1.110 – 1.206]	<0.001	1.152 [1.083 – 1.226]	<0.001

(B)	At global follow-up	
	Variable	HR [95 % CI]
NT-proBNP (log 10)	1.647 [1,110 – 2,447]	0.013
suPAR (log 10)	4.485 [1,627 – 12,365]	0.003
MAGGIC score	1.118 [1,065 – 1,174]	<0.001

(C)	At global follow-up	
	Variable	HR [95 % CI]
NT-proBNP (log 10)	2.329 [1.23 – 4.41]	0.009
suPAR (log 10)	5.59 [1.25 – 24.97]	0.02
MAGGIC score	1.111 [1.03 – 1.20]	<0.004

NYHA, New York Heart Association; LVEF, left ventricular ejection fraction; hs-cTnT, high sensitivity cardiac troponin T; CRP, C-reactive protein; sST2, soluble suppression of tumorigenicity 2; suPAR, soluble urokinase-plasminogen activator receptor; BIC, Bayesian information criterion. Bold p-values <0.05.

using this combined approach and these cut-offs. As reported in Figure 3, we demonstrated the favorable outcome associated with patients with a low MAGGIC Score and a low suPAR level (Figure 3) by comparison to patients with low MAGGIC score but high suPAR values. Actually, suPAR is a relevant biomarker in patients with a low MAGGIC score, NT-proBNP in those with a high MAGGIC score (Figure 3).

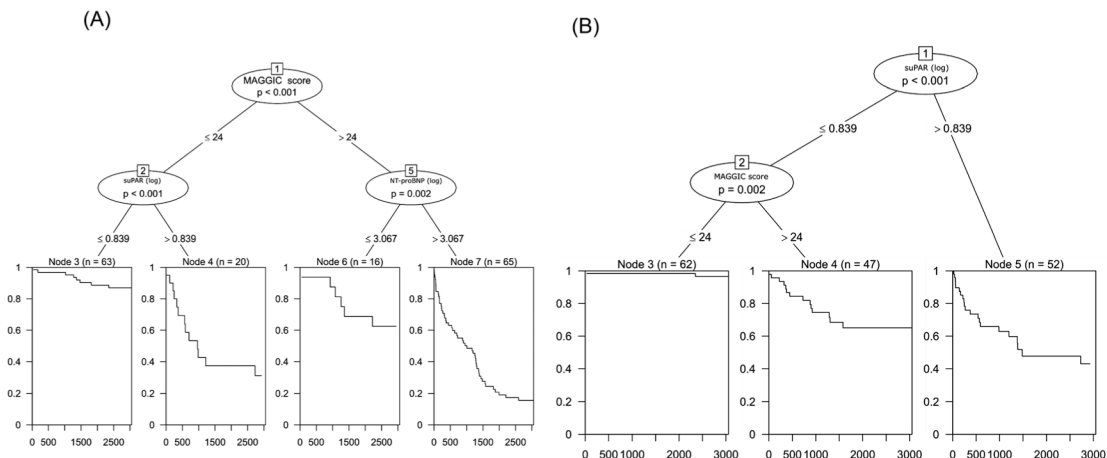


Figure 2: Representative regression tree obtained on 182 patients (A) for all-cause of mortality and (B) for cardiovascular mortality.

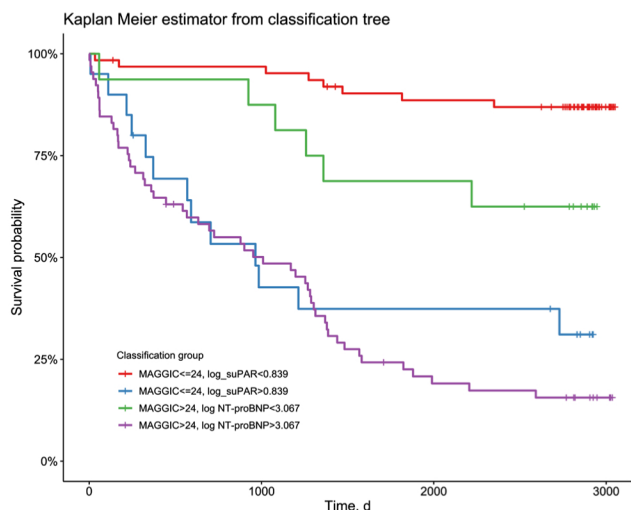


Figure 3: Kaplan Meier curves for all-cause mortality based on for all-cause mortality based on combined approach including MAGGIC score and suPAR. suPAR, soluble urokinase-type plasminogen activator receptor.

Discussion

The main findings of our study are that among the three inflammatory biomarkers, only suPAR levels were independently associated with 96-month mortality for patients with chronic HF, as sST2 and CRP were not. Multivariate Cox analysis and the classification tree approach highlighted the major importance of the clinical MAGGIC score, the classical NT-pro-BNP and the suPAR as an inflammatory biomarker. SuPAR was found to be a prognosis biomarker in patients with low MAGGIC Score whether for the prediction of all-cause and cardiovascular mortality. However, it should be noted that a limitation emerged from the reclassification analysis, while suPAR improved reclassification for prediction of all-cause death, its implication for CV death was less evident, which underlines the lack of specificity of this biomarker.

In univariate analysis the three biomarkers of inflammation, CRP, sST2, and suPAR were significantly associated with long term mortality.

CRP level is generally considered as the reference biomarker in cardiovascular disease, including chronic HF. Several meta-analyses [3, 16, 17] demonstrated that higher CRP levels can independently predict risk of all-cause mortality in general population. Although in many studies targeting HF, the relationship between high level of CRP and all-cause mortality risk was found [9, 16], others studies reported no association. These discordant results were attributed to the ethnic or age differences in the population studied [17, 18]. In the present study, CRP did not reach

significance in multivariate analysis including sST-2 and suPAR, suggesting interactions between these parameters (that are other “inflammatory” biomarkers).

sST2 has rapidly emerged as promising because of its pluripotent role in inflammation, mechanical strain, remodeling and fibrosis [19]. Despite its lack of specificity [20–23], sST2 appears consistently as promising in prognostic prediction of mortality in patients with chronic HF, particularly in combination with natriuretic peptides [19]. In previous study [9] we confirmed that sST2 alone is an important risk factor for 42-month all-cause or cardiovascular mortality in chronic HF patients. Even after adjustment for clinical variables including co-morbidities and several others biomarkers (including the gold-standard NT-proBNP), sST2 remains the strongest prognosis biomarker in after 3.5 years of follow-up. Here, the analysis of sST-2 long-term predictive value, was not in line with previous studies [4] performed mainly for short term heart failure risk stratification (<4-years follow up). These data suggest that sST2 which appears as a transition factor between inflammation and fibrosis could be a short or middle term prognosis factor.

Here, we found that in contrast to CRP and sST-2, suPAR was significantly associated to long term mortality risk on multivariate analysis. Several studies have compared the predictive power of CRP and suPAR. There is a known correlation between CRP and suPAR levels in the blood, and both reflect low grade inflammation and are positively related to smoking and physical inactivity. However, the bioclinical pathway is different between CRP and suPAR in respect to subclinical organ damage and metabolic dysfunctions (for example suPAR was not associated with obesity). Recently, suPAR was considered as superior for predicting mortality when compared to CRP [6, 8, 24] in general population. This could be explained by the fact that these two biomarkers have two distinct pathophysiological inflammatory pathways. Several authors speculate that suPAR may be a global biomarker of vascular inflammation and could reflect cellular inflammation while CRP mainly reflects inflammation associated with metabolic disturbances i.e., metabolic inflammation [24, 25]. CRP is synthesized in the liver in response to interleukine-6 originating from leucocytes in response to infection, from vascular smooth muscle cells in response to atherosclerosis [2] but originates also for one third from adipose tissue [26]. SuPAR is the soluble form of uPAR from the cell surface [27] mainly in inflammatory cells (neutrophils, monocytes, macrophages, and activated-T cells) and involved in adhesion, migration, angiogenesis, fibrinolysis and cell proliferation [7, 28, 29]. When inflammatory cells are activated by cytokines, the expression of uPAR is up-regulated, released into the extra cellular matrix in a

soluble form, thus suPAR level increases in the blood. SuPAR reflects an immune activation and is increased in various pathologies settings as cancer, diabetes, infection as well as cardiovascular diseases [25]. The association of CRP with severity of HF and outcomes was largely documented, while few data were available for suPAR in this domain [15]. However, for both biomarkers their role (marker or actor) in the development and progression of HF is not clear.

The concept of exploring different biomarkers involved in inflammation by different pathways could help in understanding the pathophysiology of the disease but also in the therapeutic decision-making. These three biomarkers explore distinct pathways involved in initiation, development and progression of HF which is multifactorial combining myocardial stress, local and systemic inflammation and ventricular remodeling. sT2 has a pluripotent role in inflammation, mechanical strain, remodeling and fibrosis. hs-CRP as well as suPAR are inflammatory biomarkers but represent two different inflammatory response system, and we confirm that suPAR in our population was more relevant than CRP. This biomarker is promising given that it can be assayed easily in routine lab like the CRP, and its variation is not linked to circadian rhythm [25]. In consequence, suPAR could reflect an alternative inflammatory pathway to CRP.

Beyond inflammatory biomarkers, multivariate Cox analysis selected 3 markers as potential mortality predictors, the clinical MAGGIC score, NT-pro-BNP, and suPAR levels. In order to provide a bioclinical algorithm to stratify the cardiac risk we use the classification and regression trees approach. This algorithm is presented in Figure 2. Interestingly classification tree approach converged with Cox analysis and selected the same predictors. The first stratification step will be the MAGGIC score for all-cause mortality. If the MAGGIC score is greater than 24, NT-proBNP should be considered. A high MAGGIC score and an elevated NT-pro-BNP level is predictor of poor outcome. By contrast if the MAGGIC score is low, the second stratification step could be the suPAR. Clearly, if the suPAR values are low, the prognosis seems really favorable. This result was observed also in the prediction of cardiovascular mortality. However, the first stratification step will be the suPAR. Looking at the nodes with the lowest and the highest probability of cardiovascular mortality, we note that the markers of the analysis remained the suPAR and the MAGGIC score while the NT-proBNP loses its impact.

Our study leads to the hypothesis that suPAR and MAGGIC score could be combined to predict mortality risk. This hypothesis further suggested that suPAR is mainly useful in patients with low MAGGIC score for prediction of

all-cause and cardiovascular mortality. By contrast if the MAGGIC score is high suPAR is less contributive for all-cause mortality. In prediction risk for cardiovascular mortality, high suPAR concentrations have the major impact. However, this hypothesis should be confirmed using an external cohort.

This risk stratification according to 3 parameters has been confirmed by Kaplan-Meier analysis according to combination of MAGGIC score with NT-ProBNP or suPAR. Clearly there are two opposite situations. In one hand, high MAGGIC score with high NT-proBNP is associated with poor outcome. On the other hand, low MAGGIC score and low suPAR level is associated with low mortality risk. However, low MAGGIC score with high suPAR has a greater risk than high MAGGIC score with low NT-proBNP. In other words, in patients with low MAGGIC score, suPAR could help to reclass the patients usually considered at low risk.

Our study has some obvious limitations. Indeed, our study was limited by the unicentric design and the relatively small sample size, especially for patients with HFpEF. Thus, the results appear limited to patients with HFrEF. However, these proofs of concept study, supporting an implication of suPAR in chronic HF as a prognostic marker on long-term follow-up (96 months) deserves further studies and a larger availability of suPAR for clinicians. In addition, suPAR does not appear to be predictive in our HFpEF subgroup. However, due to the small number of patients with HFpEF, a specific study is mandatory.

The pathophysiology of HF is multifactorial combining myocardial stress, local and systemic inflammation and ventricular remodeling. Therefore, the search for biomarkers specifically targeting one metabolic pathway could improve the understanding of this heterogeneous pathology and the combination of different biomarkers could be of great interest. This study reveals that an algorithm based on clinical score, a cardiomyocyte stress biomarker and an inflammatory biomarker could help to a more reliable long-term risk stratification in heart failure. Further studies are necessary to confirm this new direction and to define its therapeutic implications potential.

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