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Exploring collagen remodeling and regulation as prognosis biomarkers in stable heart failure

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ABSTRACT

We assessed the predictive ability of circulating biomarkers involved in collagen synthesis (procollagen type I N-terminal propeptide [PINP], and procollagen type III N-terminal propeptide [PIIINP], collagen degradation (c-terminal telopeptide of collagen type I [CTx] and mediators of cardiac fibrosis (Galectin-3 and soluble suppression of tumorigenicity 2 protein or sST2) as prognosis markers in 182 subjects with chronic heart failure (HF). In univariate analysis, all markers predicted mortality (except for PINP). A multivariate baseline model was fitted including variables potentially associated with mortality in HF patients. The baseline regression model included age, clinical data and biomarkers. We created four models from the baseline model augmented with the levels of hs-cTnT, CRP and NT-proBNP (model 1), CTx/PIIINP ratio, sST2 and Galectine-3 (model 2), NT-proBNP and sST2 (model 3) and NT-proBNP, CTx/PIIINP ratio and sST2 (model 4), to test whether these biomarkers have an incremental value for predicting mortality. After the addition of all biomarkers to the baseline model, age, CTx/PIIINP ratio and sST2 remained significant predictors. By contrast, Galectin-3 was not significantly associated with mortality. A multimarker strategy, demonstrated that the greatest prognostic improvement was attained with the combined addition of CTx/PIIINP ratio and sST2 highlighting the potential role of fibrosis pathways in risk stratification.

1. Introduction

Cardiac fibrosis which could be related to turn over of collagen, is a crucial component of cardiac remodeling and contributes to the progression of heart failure (HF). It is a major determinant of myocardial stiffness, left ventricular contractility and risk of cardiac arrhythmias. It is a complex phenomenon linked to collagen metabolism and highly regulated by paracrine mediators such as soluble suppression of tumorigenesis 2 protein (sST2) and galectin-3 (Gal-3). These two biomarkers were identified in macrophages and fibroblasts, and were considered key regulators of collagen synthesis [1], inflammation and immune response. Otherwise, although non-specific to the myocardium, serum markers of collagen turnover have been proposed for identification of myocardial fibrosis [2–4]. The aim of our study was to evaluate circulating biomarkers involved in collagen synthesis (such as PINP, PIIINP), collagen degradation (CTx), and mediators of cardiac fibrosis (Gal-3 and sST2) as prognosis markers in a population with chronic HF.

2. Methods

2.1. Study protocol and samples

The current work was a retrospective study which was based in a previous biologic bank build in 2011 for which we supplemented with specific biomarkers collagen metabolism. This registry of patients with diagnostic of stable HF based on criteria of the European Society of Cardiology, has been described in details in supplementary appendix (see Study population, follow up and outcomes).

At inclusion of patients, routine parameters such as urea, electrolytes, creatinine, NT-proBNP, hs-cTnT and CRP, were performed. In addition, venous blood was collected in dry and EDTA tubes, immediately centrifuged and frozen (−80 °C) on several aliquots until tested four years later before analysis of sST2, PINP, PIIINP, CTx and

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Gal-3. All assays were performed with the use of commercially available reagents (Principles of the methods are provided in Methods section in supplementary appendix).

2.2. 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. The reported correlation coefficients were derived using a non-parametric method (Spearman's rank correlation). Correction for multiple correlation tests were performed using Holm's method. Survival curves were generated 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. A multivariate baseline model was fitted, including variables potentially associated with mortality in HF patients. This baseline regression model included age, NYHA class, LVEF. Model 1 was baseline model augmented with hs-cTnT, CRP, and NT-proBNP while model 2 included CTx/PIIINP ratio, sST2 and Gal-3. Model 3 was the baseline model adjusted by NT-proBNP and sST2. The last model (model 4) was the baseline model adjusted by NT-proBNP, CTx/PIIINP ratio and sST2. Because of skewed distributions, biomarker concentrations were log-transformed before modelling. Goodness of fit was assessed using Akaike's information criterion. Category-less (net reclassification improvement or NRI) was used to test the added usefulness of new biomarkers in Cox models. Comparison of two nested models was performed using the likelihood ratio test. Statistical analysis was performed using R 3.1.3 (R Development Core Team, Vienna, Austria).

3. Results

Of 182 consecutive patients considered for the study between May 2010 and February 2011, all biochemical measurements (adding the markers included in this study) and vital statuses were available for 164, which were included in our analysis. Over a median follow-up period of 42.3 months (range 12,3 to 47,1 months) there were 63 deaths (35%). The clinical and biochemical variables of survivors versus those of the deceased are reported in Table A given in supplementary appendix. At the start of the project, we included patients independently of their ejection fraction. However, of the 182 patients, a majority had a reduced left ventricular ejection fraction including 100 patients with ejection fraction < 40%, 39 from 40 to 50%, and 43 with ejection fraction > 50%.

The Kaplan-Meier analysis of cumulative rates of survival is shown in Fig. A available in supplementary appendix. Mortality increased in patients presenting sST2, Gal-3, PIIINP, CTx concentrations and CTx/PIIINP ratio concentrations higher than the median value (Fig. A, $p < .001$ for all).

In univariate Cox regression analysis over the 42 months, age, NYHA class and all the biomarkers except PINP (but including Gal-3, PIIINP, CTx and CTx/PIIINP ratio) were associated with all causes of mortality, as well as with cardiovascular mortality in particular (Table 1A).

In the multivariate baseline model, age remained independent predictors of all-cause and cardiovascular mortality (Table 1B and 1C) in all models. In model 1, age and NT-proBNP, were found associated with all cause and cardiovascular mortality, however, CRP was only associated to cardiovascular mortality. By contrast, in model 2, only CTX/PIIINP ratio and sST2 remained significant predictors. After the addition of NT-proBNP and sST2 to the baseline model (model 3), age and sST2 remained significant predictors. In the last model (model 4), age, CTx/PIIINP ratio, and sST2 remained significant predictors of all cause and cardiovascular mortality, while NT-proBNP was not. NT-proBNP and sST2 allow better predictions of the risk of death, as shown by a lower Akaike's Information Criterion and a higher C-statistic (model 3). This was confirmed by reclassification analysis (continuous NRI = 0.272, $p = .016$). Further addition of CTX/PIIINP ratio (model 4) led to even better prediction performances (C-statistic = 0.746, continuous NRI = 32.5%, $p = .049$ as compared to model with NT-proBNP and sST2). If model 4 was adjusted by CRP, only the CTx/PIIINP ratio and the sST2 remain significantly associated with all cause or cardiovascular mortality (data not shown).

A multimarker strategy based on Cox proportional hazard model has also been evaluated, analyzing the incremental value of CTx/PIIINP ratio over clinical parameters (age, NYHA and LVEF) and sST2. This model, including sST2, CTx/PIIINP ratio dichotomized as above (high) or below the median (low), performed better than a model including only sST2 (likelihood ratio test: $p = .003$) and better reflected the risk of mortality (category-less NRI = 35,6%, $p = 0{,}034$). Fig. 1 depicts the predicted *hazard ratio* (HR) (CI 95%) according to biomarkers levels. The additive value of ST2 and CTx/PIIINP ratio was significant for predicting worsened outcomes (HR 5.35, 95%CI 2.31–12.36, for high sST2 and high CTx/PIIINP ratio $p \leq .0001$, as compared to low sST2 and low Ctx/PIIINP ratio after adjustment for NYHA class, age, and LVEF, C-statistic = 0,72) (Fig. 1). Kaplan Meier analyses also showed significant separation of survival curves for patients with low and/or high levels of sST2 and CTx/PIIINP ratio for 3-year survival. Patients with both high ST2 and CTx/PIIINP ratio experienced a mortality rate of around 54.6% at 3 years (vs 8.7% in patients with both low sST2 and CTx/PIIINP ratio), indicating that assessment of both ST2 and fibrosis related markers was more effective at identifying a high risk subgroup that individual assessment of either marker.

4. Discussion

In the present study, we highlighted the risk stratification value of circulating biomarkers involved in collagen metabolism with sST2, Gal-3, PINP, PIIINP and CTx in a population with stable chronic HF. Association with all cause or cardiovascular mortality, after adjustment for clinical parameters and biomarkers, did not persist for Gal-3, whereas it remained significant for sST2 and CTx/PIIINP ratio, suggesting that high turnover of collagen is associated with poor prognosis. Our multimarker approach, demonstrated that collagen metabolism biomarkers particularly the CTx/PIIINP ratio add predictive value to sST2 and clinical data.

4.1. Collagen turnover products

Beyond close correlation between sST2 and CTx/PIIINP ratio (rho = 0.27, $p < .01$), their combination further improves risk stratification allowing discrimination of patients at high risk. High CTx/PIIINP ratio could suggest high levels of collagen turnover. Multivariate Cox regression analysis demonstrated an additive prognostic value of CTx/PIIINP ratio, independent of clinical parameters and other biological markers such as NT-proBNP, CRP, sST2 and Gal-3. Our results were comparable about a recent study of Lofsjorgard et al. on relationship between HF and markers of collagen degradation and synthesis. We found also the significant relationship between CTx/PIIINP ratio and NYHA class I, II, III and IV (Median [Q1:Q3] CTx/PIIINP ratio,-1.78 [−1.85: −1.74]; −1.46, [−1.85:-1.12]; −1.31 [−1.62: −0.884]); −1.1[−1.36:- 0.75; respectively) ($p < .001$). Following these data and ours, there is strong evidence to indicate that excessive degradation would be associated with a poor prognosis. The degradation of collagen fibers in a normal situation involves the action of MMPs. However, of note, the presence of excessive collagen turn over leads to cross linking collagen formation that are resistant to MMPs action. Therefore, the circulating serum CTx is of great interest because it is not formed from MMPs, unlike other markers of collagen

Table 1
(A) Univariate Cox regression analysis over 42 months for prediction of all-cause and cardiovascular mortality; (B) Multivariate Cox regression analysis after adjusting clinical parameters for prediction of (B) all-cause mortality and (C) cardiovascular mortality.

(A)

Variable	All-cause mortality		Cardiovascular mortality	
	HR [95% CI]	p	HR [95% CI]	p
Age	1.04 [1.02; 1.07]	< **0.001**	1.05 [1.01; 1.09]	**0.003**
Gender: Male	1.18 [0.68; 2.04]	0.540	0.99 [0.48; 2.03]	0.986
Dyslipidemia	1.22 [0.74; 2.01]	0.420	1.05 [0.53; 2.07]	0.879
NYHA class	2.15 [1.20; 3.84]	**0.010**	2.19 [0.99; 4.84]	**0.053**
LVEF	0.98 [0.96; 1.00]	0.192	0.98 [0.96; 1.01]	0.316
Hs-cTnT (log 10)	1.26 [1.10; 1.45]	**0.001**	1.33 [1.10; 1.60]	**0.003**
CRP (log 10)	1.39 [1.17; 1.64]	< **0.001**	1.54 [1.22; 1.94]	< **0.001**
NT-proBNP (log 10)	1.61 [1.34; 1.94]	< **0.001**	1.80 [1.38; 2.34]	< **0.001**
sST2 (log 10)	1.81 [1.44; 2.27]	< **0.001**	2.00 [1.48; 2.71]	< **0.001**
Galectin-3 (log 10)	2.13 [1.40; 3.25]	< **0.001**	2.55 [1.45; 4.47]	**0.001**
PINP (log 10)	1.30 [0.83; 2.02]	0.246	1.41 [0.77; 2.57]	0.258
PIIINP (log 10)	2.53 [1.58; 4.05]	< **0.001**	2.79 [1.49; 5.21]	**0.001**
CTx (log 10)	1.77 [1.39; 2.27]	< **0.001**	1.93 [1.39; 2.68]	< **0.001**
CTx/PIIINP. ratio	3.69 [2.17–6.25]	< **0.001**	4.79 [2.32–9.90]	< **0.001**

(B)

	Baseline Model		Model 1		Model 2		Model 3		Model 4	
Variable	HR [95% CI]	p	HR [95% CI]	p	HR [95% CI]	p	HR [95% CI]	p	HR [95% CI]	p
Age	1.04 [1.01–1.07]	0.001	1.03 [1.00–1.06]	0.007	1.04 [1.02–1.07]	< 0.001	1.04 [1.01–1.06]	0.002	1.04 [1.01–1.06]	0.001
NYHA class	1.78 [0.99–3.23]	0.054	1.59 [0.87–2.93]	0.131	1.04 [0.55–1.95]	0.897	1.07 [0.57–2.02]	0.811	1.02 [0.54–1.92]	0.936
LVEF	0.98 [0.96–1.00]	0.103	0.99 [0.97–1.01]	0.683	0.99 [0.97–1.01]	0.388	0.99 [0.97–1.01]	0.484	0.99 [0.97–1.01]	0.651
Hs-cTnT (log 10)			1.46 [1.16–1.84]	0.001						
CRP (log 10)			0.88 [0.72–1.08]	0.25						
NT-proBNP (log 10)			1.25 [1.05–1.49]	0.012			1.22 [0.98–1.52]	0.07	1.18 [0.95–1.48]	0.124
CTx/PIIINP ratio					1.96 [1.19–3.23]	0.008			1.95 [1.21–3.14]	0.006
sST2 (log 10)					1.74 [1.31–2.31]	< 0.001	1.62 [1.18–2.23]	0.003	1.53 [1.11–2.12]	0.009
Galectin-3 (log 10)					1.08 [0.63–1.83]	0.769				

(C)

	Baseline Model		Model 1		Model 2		Model 3		Model 4	
Variable	HR [95% CI]	p	HR [95% CI]	p	HR [95% CI]	p	HR [95% CI]	p	HR [95% CI]	p
Age	1.05 [1.01–1.09]	0.004	1.03 [1.00–1.07]	0.03	1.05 [1.01–1.09]	0.003	1.04 [1.01–1.08]	0.009	1.05 [1.01–1.08]	0.007
NYHA class	1.74 [0.77–3.93]	0.176	1.51 [0.66–3.48]	0.324	0.90 [0.38–2.15]	0.826	0.95 [0.40–2.24]	0.908	0.89 [0.37–2.13]	0.804
LVEF	0.98 [0.95–1.00]	0.192	0.99 [0.96–1.02]	0.83	0.99 [0.96–1.01]	0.5	0.99 [0.96–1.02]	0.596	0.99 [0.96–1.02]	0.742
Hs-cTnT (log 10)			0.89 [0.68–1.16]	0.394						
CRP (log 10)			1.33 [1.04–1.70]	0.021						
NT-proBNP (log 10)			1.56 [1.14–2.14]	0.005			1.28 [0.94–1.74]	0.11	1.22 [0.90–1.67]	0.192
CTx/PIIINP ratio					2.23 [1.14–4.38]	0.019			2.22 [1.17–4.22]	0.014
sST2 (log 10)					1.94 [1.31–2.86]	0.001	1.80 [1.16–2.79]	0.008	1.67 [1.07–2.61]	0.023
Galectin-3 (log 10)					1.11 [0.54–2.28]	0.769				

Model 1: Baseline model adjusted by hs-cTnT, CRP and NT-proBNP.
Model 2: Baseline model adjusted by CTx/PIIINP ratio, sST2 and Galectin-3.
Model 3: Baseline model adjusted by NT-proBNP and sST2.
Model 4: Baseline model adjusted by NT-proBNP, CTx/PIIINP ratio and sST2.
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; PINP: procollagen type I N-terminal propeptide; PIIINP: procollagen type III N-terminal propeptide; CTx: c-terminal telopeptide of collagen type I.

degradation such as CITP, but by the action from Cathepsin K. High levels of CTx could thus indicate action of Cathepsin K rather than MMPs and high collagen turn over [5]. The availability of circulating molecules in blood for use as biomarkers of myocardial fibrosis should not be underestimated. The association of biomarkers of collagen synthesis and degradation provides complementary information on the collagen homeostasis.

4.2. Cardiac fibrosis remodeling

Beyond conventional markers, sST2 has rapidly emerged as promising because of its pluripotent role in inflammation, mechanical strain, remodeling and fibrosis [6]. The role of sST2 for prognosis and risk stratification is well established in view of the numerous clinical studies (summarized in two meta-analyses, [7,8] and recent recommendations [9] on its use in clinical routine settings. Clinical studies on Gal-3 were, until recently, contradictory as to the association of Gal-3 with the severity and prognosis of HF. In addition, the relationship between plasma and myocardial Gal-3 levels in previous studies remains under debate. Our findings demonstrate first, even after adjustment for clinical variables and other well established and powerful biomarkers including NT-proBNP and fibrosis-related markers, sST2

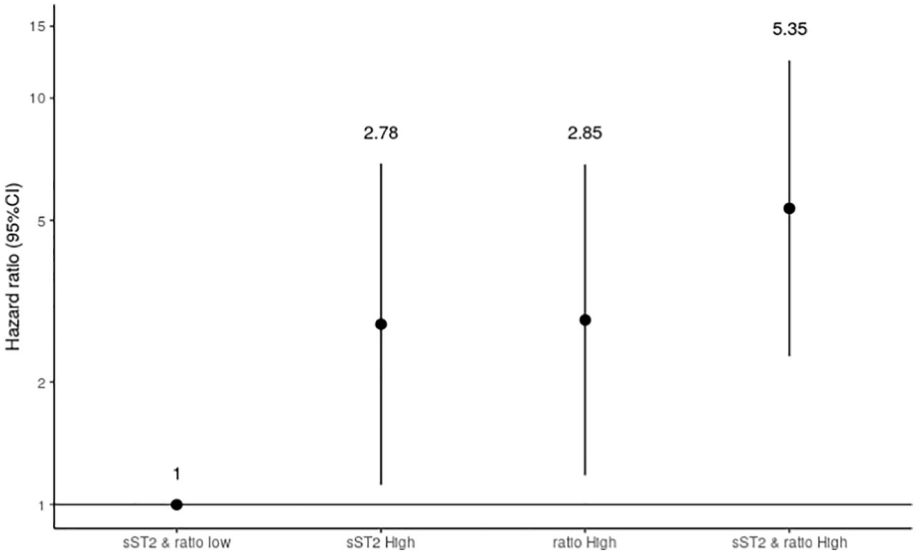

Fig. 1. Adjusted hazard ratio of death according to combined criteria as median of ST2, and CTx/PIIINP ratio.
ST2: suppression of tumorigenicity 2; PIIINP: procollagen type III N-terminal propeptide; CTx: c-terminal telopeptide of collagen type 1.

remains a strong prognosis biomarker in our population. sST2 has been shown to provide incremental prognostic value over NT-proBNP, and Gal-3. Second, in agreement with Besler's study [10], our results did not demonstrate incremental value of Gal-3 over clinical data and classical biomarkers in predicting mortality, nor a significant interaction with other markers, such as fibrosis-related markers or sST2. The current trend would be to consider Gal-3 as marker of HF risk in the general population [11–13] and would have a more important role in the beginning stage of HF, including early fibrosis and ventricular remodeling [14].

4.3. Multimarker strategy

Nevertheless, the greatest prognostic improvement was obtained with the combined addition of CTx/PIIINP ratio and sST2. This is a demonstration of the fact that several pathophysiological pathways of myocardial fibrosis, including synthesis, degradation and regulation processes, play an important role in determining outcomes in HF patients, and no specific pathway or biomarker can reflect all the aspects of HF.

These data could have potential clinical consequences/benefits in patients with chronic stable heart failure. Following guidelines improve outcomes [15], but physicians need in clinical practice tools to better adjudicate times and means. Based on an efficient biomarkers score, various populations could be distinguished, guiding the management: (1)The low-risk patients: This could justify a level 1 follow-up (every 6 months by the cardiologist) with annual assessment of the biomarker score and probably integrating a multiparameter score (including clinical and echography parameters), (2) The intermediate risk patients, (3) The very high risk patients: this could advocate for a strict follow-up with short-term reevaluation including frequent (monthly) consultation with the cardiologists, but also frequent reassessment of the biomarker score.

5. Limitations

The study was limited by its single-centre design and relatively small sample size. We only measured biomarkers at the time of recruitment to the study, and only one blood sample was available. In consequence, we did not evaluate the monitoring of the biomarkers, which can be also useful as monitoring markers. In addition, these biomarkers were not available in many centres, reducing the number of patients that could be included. In addition, the specified biomarkers were performed remotely of recruitment (4 years later) from frozen aliquots. However, the storage at-80 °C was immediately after sampling, an aliquot per marker with one freeze/thaw cycle, a single batch with same lot number of reagent, and the data published by previous studies has kept us safe from all trouble [16–19].

6. Conclusion

We reported that the CTx/PIIINP ratio indicating excessive turn over with high degradation rate may be considered an additional marker of mortality risk in patients with HF, and that a combined multimarker model including sST2 and CTx/PIIINP ratio identifies best risk stratification for all-cause mortality as well as cardiovascular mortality. Our data strengthen those of Lofjogard et al. [20,21]. The interpretation of serum levels of fibrosis-related biomarkers requires further investigation to confirm their association with myocardial fibrosis, in particular by using cardiac magnetic resonance, considered to be a non-invasive method able to evaluate the myocardial interstitial space. In addition, the likely therapeutic benefit from lowering fibrosis-related biomarkers remains to be demonstrated in interventional studies.

Acknowledgments

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Conflict of interest

The authors declare that they have no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cca.2018.08.042.

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