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Prognostic value of angiopoietin-2 in patients with chronic heart failure

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ABSTRACT

Purpose: The analysis of biomarkers with a prognostic value in chronic heart failure (CHF) is in constant progress. This study aimed to evaluate the short-term prognostic value of angiopoietin-2 (Ang2), galectin-3 (Gal-3), myeloperoxidase (MPO), endostatin (End), and pro-brain natriuretic peptide (pro-BNP) as a conventionally accepted prognosis biomarker in CHF patients.
Methods and results: 146 consecutive patients with CHF due to left ventricular systolic dysfunction (LVEF < 40% at echocardiography) were enrolled, and underwent serum/blood sample analysis after 12-h fasting. Within 1 year, 25 (17%) patients died (D) or underwent heart transplantation (HT). D + HT patients showed higher values of Ang2 (Log Ang2: 8.97 ± 0.52 vs. 8.45 ± 0.69, p = 0.0004), myeloperoxidase (MPO) (Log MPO: 5 ± 1.1 vs. 4.2 ± 1.3, p = 0.005) and pro-BNP (Log pro-BNP: 8.70 ± 0.9 vs. 7.45 ± 1.3, p < 0.00001). At univariate Cox regression, pro-BNP and Ang2 were the best predictors of 1-year mortality, with area under the curve (AUC) = 0.78 for pro-BNP (68% sensitivity and 82% specificity to predict outcome for a cut-off value of 5109 pg/mL, 95% confidence interval [CI] 0.70–0.85, p < 0.0001) and AUC = 0.73 for Ang2 (84% sensitivity and 61% specificity to predict outcome for a cut-off value of 5175 pg/mL, 95% CI 0.65–0.80, p < 0.0001). At multivariate analysis, pro-BNP was the only predictor of one-year D/HT.
Conclusion: In our series of CHF patients, Ang2 and pro-BNP showed the best predictive value for 1-year outcome, while only pro-BNP could independently predict D/HT.

1. Introduction

Starting from an initial damage (e.g. acute myocardial infarction, myocarditis) or chronic hemodynamic overload (e.g. hypertension, valvulopathies), a progressive impairment of systolic and/or diastolic performance of the left ventricle can develop. The right ventricle can also be involved. The impairment of myocardial function can stay still and hidden for years, the onset of symptoms being the final consequence of complex biochemical changes, at both local (heart and vessels) and systemic (blood) levels, over and above the pump deterioration in itself. In recent years, numerous data on molecular changes leading to the development of heart failure (HF) have emerged from in vitro as well as clinical studies [1–4,23]. As a whole, they indicate an increasing oxidative and inflammatory response to the initial damage, together with endothelial dysfunction, and activation of the fibrotic and angiogenic pathways along with progressive functional worsening. While the pathophysiological role and the prognostic value of markers of inflammation and fibrosis have been widely investigated [2–4], the current literature is scant on the clinical relevance of the molecular modifications involved in the angiogenic processes.

Therefore, the aim of this study was to evaluate the short-term prognostic value of pro-angiogenic factor angiopoietin-2 (Ang-2) as compared with the anti-angiogenic factor endostatin (End), galectin-3 (Gal-3) as a marker of fibrosis pathways activation, myeloperoxidase (MPO) as markers of inflammation and oxidative stress, and with pro-brain natriuretic peptide (pro-BNP) as a "haemodynamic" gold standard prognostic biomarker in CHF patients.

2. Methods

2.1. Patients and study design

This is a prospective observational study. We consecutively enrolled 146 consecutive patients with a diagnosis of chronic HF due to left ventricular systolic dysfunction (left ventricular ejection fraction [LVEF] < 40% at echocardiography). Echocardiographic measurements were performed as previously reported [5]. At enrolment all patients were in stable oral therapy. Patients with inflammatory/infectious disease, severe renal or hepatic function impairment, malignancies and

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psychiatric pathologies were excluded from the study. Informed consent was obtained from each subject prior to the study. The study was carried out in conformity with the 1975 Declaration of Helsinki, and performed according to the local Ethics Committee guidelines.

2.2. Blood/serum collection and analysis

Blood samples were collected after 12 h fasting using Becton Dickinson Vacutainer Cat Plus REF 367896 for serum (BD Diagnostics, Franklin Lakes, NJ, USA). Serum was then aliquoted and immediately frozen at −80 °C until analysis. Table 1 shows the lower detection limits and the method for enzyme-linked immunoassorbant assay (ELISA) quantification (Modular Analytics, Roche Diagnostics, Mannheim, Germany) for each of the molecules studied. Manufacturers' instructions were carefully followed for each of the ELISA kits used. Further details for each kit are available from the respective online datasheet (www.RnDsystems.com; www.roche.com; www.biomerieux.com).

2.3. Statistics

Continuous data are expressed as mean ± SD. Student's t-test for non-paired values was used to compare the means of groups for quantitative variables. For qualitative variables, the χ^2 test with Yates' correction or Fisher's exact test, if necessary, was employed. The level of statistical significance was set at a two-tailed p-value ≤ 0.05. Given the skewed data distribution, logarithmic transformation was applied, after imputation for missing data, to biomarkers Gal-3, MPO, endostatin, pro-BNP, Ang1 and Ang2 in order to obtain input variables approximately normal in distribution. Survival was estimated by the product-limit Kaplan–Meier method, considering death (D) or heart transplantation (HT) as events. The prognostic value of variables was determined using univariate Cox proportional-hazards regression analysis; multivariate regression analysis was finally performed to assess the best independent predictor.

Prognostic accuracy was evaluated using the receiver operating characteristic (ROC) area under the curve (AUC) according to Hanley and McNeil [6]. Cut point values for variables predicting outcome were generated with ROC at regular intervals, and the best threshold was automatically identified as the value that minimized the expression $[(1\text{-sensitivity})^2 + (1\text{-specificity})^2]$.

All calculations were performed using the STATA® 10 system (StataCorp, College Station, TX, USA).

3. Results

Demographics and general statistics in the total population, survivors and those with major events, are reported in Table 2, while univariate analysis results with hazard ratios (HR) are reported in Table 3. Within the first year of follow-up, 25 (17%) patients encountered a hard event (D or HT). Most patients with events were female (28% vs. 7%, p = 0.001), had a lower LVEF (25 ± 7% vs. 29 ± 8%, p = 0.04), a shorter deceleration time (DT) of left ventricular early filling (129 ± 33 vs. 177 ± 55 ms, p < 0.00001) and more severe mitral insufficiency (2.64 ± 1.0 vs. 2.11 ± 0.9, p = 0.01). When analyzing biomarker levels, D + HT patients showed higher values of Ang2 (Log-Ang2 8.97 ± 0.52 vs. 8.45 ± 0.69, p = 0.0004), MPO (Log-MPO 5 ± 1.1 vs. 4.2 ± 1.3, p = 0.005) and pro-BNP (Log-pro-BNP 8.70 ± 0.9 vs. 7.45 ± 1.3, p < 0.00001), had lower serum levels of sodium (Na 135.8 ± 2.8 vs. 138.7 ± 3.4 mEq/L, p = 0.0001), hemoglobin (Hb 12.34 ± 1.45 vs. 14.80 ± 1.58, p = 0.001), and a higher relative prevalence of neutrophils (70.0 ± 7.1 vs. 65.3 ± 8.1, p = 0.01). Finally, fewer patients with events were on active therapy with an angiotensin-converting enzyme (ACE)-inhibitor (68% vs. 92%, p = 0.001). The etiology of CHF did not influence the event of D/HT. Both pro-BNP and Ang2 were highly predictive of 1-year mortality (Cox analysis); when ROC curves were obtained (Fig. 1), pro-BNP showed a 68% sensitivity and 82% specificity, with a cut-off anti logarithmic value of 5109 pg/mL, 95% confidence interval [CI] 0.70–0.85, p < 0.0001, AUC = 0.78. The ROC curve for Ang2 showed an 84% sensitivity with 61% specificity, cut-off anti logarithmic value 5175 pg/mL, 95% CI 0.65–0.80, p < 0.0001, AUC = 0.73. Kaplan–Meier 1-year survival estimates for Ang2 and pro-BNP are also reported (Fig. 2). At multivariate regression analysis pro-BNP emerged as the only independent predictor of D/HT (HR = 1.98, p < .001).

Table 2
Patients' demographic, clinical characteristics, blood chemistry and biomarkers findings according to events at 1-year follow-up. Echocardiographic data are expressed as mean value ± SD or %. Biomarkers are expressed as mean ± SD after logarithmic transformation; routine blood data (sodium, potassium, creatinine, uric acid, hemoglobin) are expressed as mean ± SD; neutrophil percentages were obtained by a separate blood sample collected on the same morning day as the biomarkers.

	12 months follow-up			
	Survivors n.121	D + HT n.25	Overall patients n.146	p
Age (y)	63 ± 10.5	65.6 ± 10.3	63.5 ± 10.5	NS
Sex (m)	113 (93%)	18 (72%)	131 (90%)	.001
Weight (kg)	76 ± 15	65 ± 12	75 ± 12	.0008
ICM (%)	79 (66%)	14 (56%)	93 (65%)	NS
NYHA	2.3 ± .76	3.1 ± .76	2.4 ± .81	.0000
FU (days)	365	237.2 ± 132	343 ± 72.3	.0000
LVEDV (mL)	202.2 ± 87.2	191. ± 67.4	200.2 ± 84	NS
LVEF (%)	29 ± 8.94	25 ± 7.64	28.3 ± 8.8	.043
DT (ms)	177.9 ± 55.4	129.6 ± 33.2	169.7 ± 55.3	.0000
MR	2.11 ± .94	2.64 ± 1.03	2.20 ± .97	.014
Na (mEq/L)	138.7 ± 3.4	135.8 ± 2.84	138.24 ± 3.5	.0001
Hb (g/dL)	121.48 ± 1.58	12.34 ± 1.45	13.29 ± 1.62	.001
Neut (%)	65.3 ± 8.1	69.9 ± 7.1	66.1 ± 8.1	.01
Uric acid (mg/dL)	6.77 ± 2.13	6.52 ± 2.22	6.72 ± 2.14	NS
Creatinine (mg/dL)	1.32 ± .43	1.49 ± .46	1.35 ± .43	NS
K (mEq/L)	4.56 ± 2.56	4.51 ± .54	4.55 ± 2.34	NS
ACE-inhibitor (n/%)	111 (92%)	17 (68%)	128 (88%)	.001
Loop diuretics	111 (92%)	25 (100%)	136 (93%)	NS
Beta blockers	78 (64%)	14 (56%)	92 (63%)	NS
Digoxin	27 (22%)	8 (32%)	35 (24%)	NS
ICD	38 (31%)	7 (28%)	45 (31%)	NS
L-Ang1	9.53 ± .99	9.6 ± .49	9.54 ± .92	NS
L-Ang2	8.45 ± .69	8.97 ± .52	8.54 ± .69	.0004
L-proBNP (pg/mL)	7.45 ± 1.31	8.7 ± .93	7.67 ± 1.34	.0000
L-Gal 3 (ng/mL)	2.79 ± .39	2.93 ± .62	2.81 ± .44	NS
L-MPO	4.2 ± 1.29	5 ± 1.12	4.34 ± 1.29	.005
L-endostatin	4.13 ± .99	4.39 ± .82	4.18 ± .97	NS

Abbreviations: ICM = ischemic cardiomyopathy; LVEDV = left ventricular end diastolic volume; LVEF = left ventricular ejection fraction; DT = deceleration time of left ventricular early filling; MR = mitral regurgitation (0 = absent/trivial, 1 = fair, 2 = moderate, 3 = severe). Na = sodium; Hb = hemoglobin; Neut = % relative neutrophils; K = potassium; ICD = implanted cardiac defibrillator; L = logarithm.

Table 1
List of ELISA tests for biomarkers included in the study.

Molecules	Manufacturer (code)	Lower detection limit	Type of plasma/serum	Analytical Method
Galectin-3	Biomerieux (411191)	2.4 ng/mL	Serum	ELISA
Angiopoietin-1	R&D Systems (DANG10)	1,36 pg/mL	Serum	ELISA
Angiopoietin-2	R&D Systems (DANG20)	1,20 pg/mL	Serum	ELISA
Endostatin	R&D Systems (DNST0)	0.023 ng/mL	Serum	ELISA
Myeloperoxidase	R&D Systems (DMYE00B)	0.014 ng/mL	Serum	ELISA
NT-proBNP	Roche Diagnostics (03121640 122)	5 pg/mL	Serum	Modular Analytics