International Journal of Cardiology 209 (2016) 114–117

Contents lists available at ScienceDirect

International Journal of Cardiology

journal homepage: www.elsevier.com/locate/ijcard

Correspondence

Detectable interleukin-9 plasma levels are associated with impaired cardiopulmonary functional capacity and all-cause mortality in patients with chronic heart failure

Alberto M. Marra [a], Michele Arcopinto [b], Andrea Salzano [c], Emanuele Bobbio [c], Salvatore Milano [d], Gabriella Misiano [e], Francesco Ferrara [f], Olga Vriz [g], Raffaele Napoli [c], Vincenzo Triggiani [h], Pasquale Perrone-Filardi [i], Francesco Saccà [j], Francesco Giallauria [c], Andrea M. Isidori [k], Carlo Vigorito [c], Eduardo Bossone [f], Antonio Cittadini [c,l,*]

[a] IRCCS S.D.N., Via Gianturco 113, 80143 Naples, Italy
[b] Department of Cardiac Surgery, IRCCS Policlinico San Donato Milanese, Milan, Italy
[c] Department of Translational Medical Sciences, Federico II University, Naples, Italy
[d] Sezione Biochimica Clinica e Medicina Molecolare Clinica, Dipartimento di Biopatologia e Biotecnologie Mediche, Università degli Studi di Palermo, Italy
[e] Sezione di Patologia Generale, Dipartimento di Biopatologia e Biotecnologie Mediche, Università degli Studi di Palermo, Italy
[f] Department of Cardiology and Cardiac Surgery, University Hospital "Scuola Medica Salernitana", Salerno, Italy
[g] Department of Emergency and Cardiology, "S. Antonio" Community Hospital, San Daniele del Friuli (UD), Italy
[h] Interdisciplinary Department of Medicine, Endocrinology and Metabolic Diseases, University of Bari, Italy
[i] Department of Advanced Biomedical Sciences, Section of Cardiology, Federico II University, Naples, Italy
[j] Department of Neurological Sciences, Federico II University, Naples, Italy
[k] Department of Experimental Medicine, "Sapienza" University of Rome, Italy
[l] Interdisciplinary Research Centre in Biomedical Materials (CRIB), University of Naples, Naples, Italy

ARTICLE INFO

Article history:
Received 10 January 2016
Accepted 1 February 2016
Available online 3 February 2016

Keywords:
Chronic heart failure
Risk stratification
Cytokines
Interleukin-9
Peak oxygen consumption
Outcomes

Inflammatory activation plays a pivotal role in chronic heart failure (CHF) through the increased expression of pro-inflammatory cytokines [1]. Decreased plasma levels of Interleukin (IL-) 5, IL-7 and Interferon-γ (IFN-γ) and increased levels of IL-9 have been already described in CHF [2], and a negative correlation was also reported between IL-9 and left ventricular ejection fraction (LVEF) [2]. Yet, there are only limited data exploring the association between cytokines and functional capacity in CHF and their prognostic role [3]; therefore, primary end-point of the current study was to evaluate all-cause mortality according to changes in cytokines plasma levels in CHF patients. For this purpose, 75 CHF patients (mean age ± standard deviation (SD) = 65.7 ± 9.5 yrs. 15 F/60 M), with reduced (<35%) LVEF, (mean = 32.7 ± 4.3%), clinically stable for at least 6 months, were recruited in 3 outpatient CHF clinics (Naples, Salerno and San Daniele del Friuli) and followed up for 55 + 24 months (median 50 months). Exclusion criteria were significant comorbidities (active cancer, advanced liver cirrhosis, and end-stage renal failure, autoimmune disease, allergies). At baseline, plasma concentration of 27 cytokines and growth factors (interleukin IL-1b; IL-1 receptor a; IL-2; IL-4; IL-5; IL-6; IL-7; IL-8; IL-9; IL-10; IL-12 p70; IL-13; IL-15; IL-17; eotaxin; FGFb; G-CSF; GM–CSF; IFN-γ; IP-10; MCP-1; MIP-1a; MIP-1b; PDGF;TNF-α; VEGF and RANTES) were assayed using Bio-Plex protein array systems (Bio-Rad, Hercules, CA), based on xMAP technology (Luminex, Austin, TX) in the CHF patients and in 24 age- and sex-matched healthy controls. Cytokines and growth factors levels were measured by an experienced core lab (G.M. and S.M.) to minimize inter- and intra-assay variability. All patients underwent physical examination, Doppler-echocardiography and Cardiopulmonary Exercise Stress Testing (CPET) as described elsewhere [4,5]. Optimal therapy was administered with 98% of patients taking ACE-I or ARB, 96% β-blockers, and 37% aldosterone receptor antagonists.

Medical records or telephone interviews with the patients' relatives confirmed all deaths. Data are expressed as mean ± SD. Unpaired Student's t-test, Mann–Whitney U-test, and χ^2 test were used as required. The Kaplan–Meier curves and the log-rank test were used to compare the cumulative rates of all-cause death between different subgroups. Univariate Cox proportional hazards model was used for testing the association between demographic, clinical and biochemical variables and patient survival. Multivariate analysis was used to identify independent predictors of death from all cause; covariates showing

* Corresponding author at: Department of Translational Medical Sciences, "Federico II" University-School of Medicine, Via Pansini 5, 80131 Naples, Italy.
E-mail address: antonio.cittadini@unina.it (A. Cittadini).

http://dx.doi.org/10.1016/j.ijcard.2016.02.017

significant association with the outcome in the univariate analysis were added to the model. Significance was set at 0.05. Data were analyzed by using the SPSS 16.0 package (SPSS Inc., Chicago, IL). Institutional ethics committees approved the study protocol. The study purpose was explained to the patients and written informed consent was obtained accordingly. Fig. 1 shows cytokine levels in CHF and controls. Higher IL-8, IL-9 and Eotaxin and lower IL-6, PDGF and MIP-1b levels were observed in CHF patients. No significant differences were found with regards to other cytokines (data not shown). As reported in Table 1, CHF patients with detectable IL-9 (≥7 pg/ml) levels showed impaired CEPT parameters compared to controls (Peak VO_2 15.5 ± 4.2 *vs.* 18.2 ± 3.3 ml/Kg/min, p = 0.004; VE/VCO_2 slope: 32.3 ± 7.1 *vs.* 28.7 ± 6.7 ml/Kg/min, p = 0.03; Peak workload 91.1 ± 26.9 *vs.* 100.9 ± 25.5 W, p = 0.003) and higher NYHA class (2.5 ± 0.7 *vs.* 2.1 ± 0.6, p:=0.04). During follow-up, 28 patients died (37.3%), 18 with detectable IL-9 (51%) and 10 (25%) with undetectable IL-9. Twenty-one deaths (14 in the detectable group and 7 in the undetectable group) were due to cardiovascular events (13 for heart failure, 5 sudden death, 2 myocardial infarction, 1 stroke) while 7 were non-cardiac deaths. The Kaplan–Meier survival curves and log-rank test comparing the two groups stratified according to IL-9 detectability are shown in Fig. 2. CHF patients with detectable IL-9 showed worse prognosis compared to those with undetectable values (log rank 6.32, p: 0.013). No significant prognostic differences were observed with regard to the other cytokine levels. Univariate Cox proportional hazards analysis found that higher NYHA class, diabetes, NT-proBNP levels, renal function indexes and peak VO_2 were also predictors of all-cause mortality among CHF patients (Table 2). After adjusting for all covariates significantly associated to all-cause mortality at univariate analysis, multivariate analysis showed that IL-9 detectability maintained its predictive value and was independently associated with all-cause mortality (hazard ratio = 2.36, 95% confidence interval 1.42–5.23, p = 0.01).

Table 1
Baseline characteristics of general population and according to Interleukin-9 (IL-9) plasma levels.

Variable	All patients (n = 75)	Undetectable IL-9 (n = 40)	Detectable IL-9 (n = 35)	p value*
Age-years	65.7 ± 9	64.7 ± 10	67.5 ± 9	0.35
BMI-m^2/Kg	27.5 ± 4	27.9 ± 3	27.1 ± 4	0.28
Female sex (%)	15 (19.7%)	6 (17.1%)	9 (22.5%)	0.77
T2DM prevalence (%)	23 (30.6%)	10 (28.6%)	13 (32.5%)	0.91
Ischemic etiology prevalence (%)	46 (60.5%)	21 (60%)	25 (62.5%)	0.89
NYHA class	2.3 ± 1	2.1 ± 1	2.5 ± 1	**0.04**
NTproBNP (pg/ml)	2756 ± 2920	2797 ± 2491	2727 ± 3269	0.91
eGFR (ml/min)	79.9 ± 20	78.1 ± 19	81.4 ± 20	0.51
LVEF (%)	32.7 ± 4	32.4 ± 5	33.1 ± 4	0.49
Peak VO_2 (ml/Kg/min)	16.75 ± 4	18.2 ± 3	15.5 ± 4	**0.004**
VE/VCO2 slope	30.6 ± 7	28.7 ± 7	32.3 ± 7	**0.03**
Peak Workload	91.1 ± 27	100.9 ± 25	82.6 ± 24	**0.003**

Data are shown as mean ± SD; * = detectable *vs.* undetectable; T2DM = Type 2 Diabetes Mellitus; NYHA = New York Heart Association; NTproBNP = N-terminal of the pro-hormone brain natriuretic peptide; LVEF: left ventricular ejection fraction; eGFR = estimated glomerular filtration rate according to Cockroft-Gault formula; VO_2 = oxygen consumption; VCO_2 = carbon dioxide production; VE = ventilation per min.

Primary end-point of the current study was fulfilled, being detectable IL-9 plasma levels associated to worse outcome among CHF patients. Consistently, the presence of plasma IL-9 was also associated with impaired cardiopulmonary functional capacity in these patients. In addition,

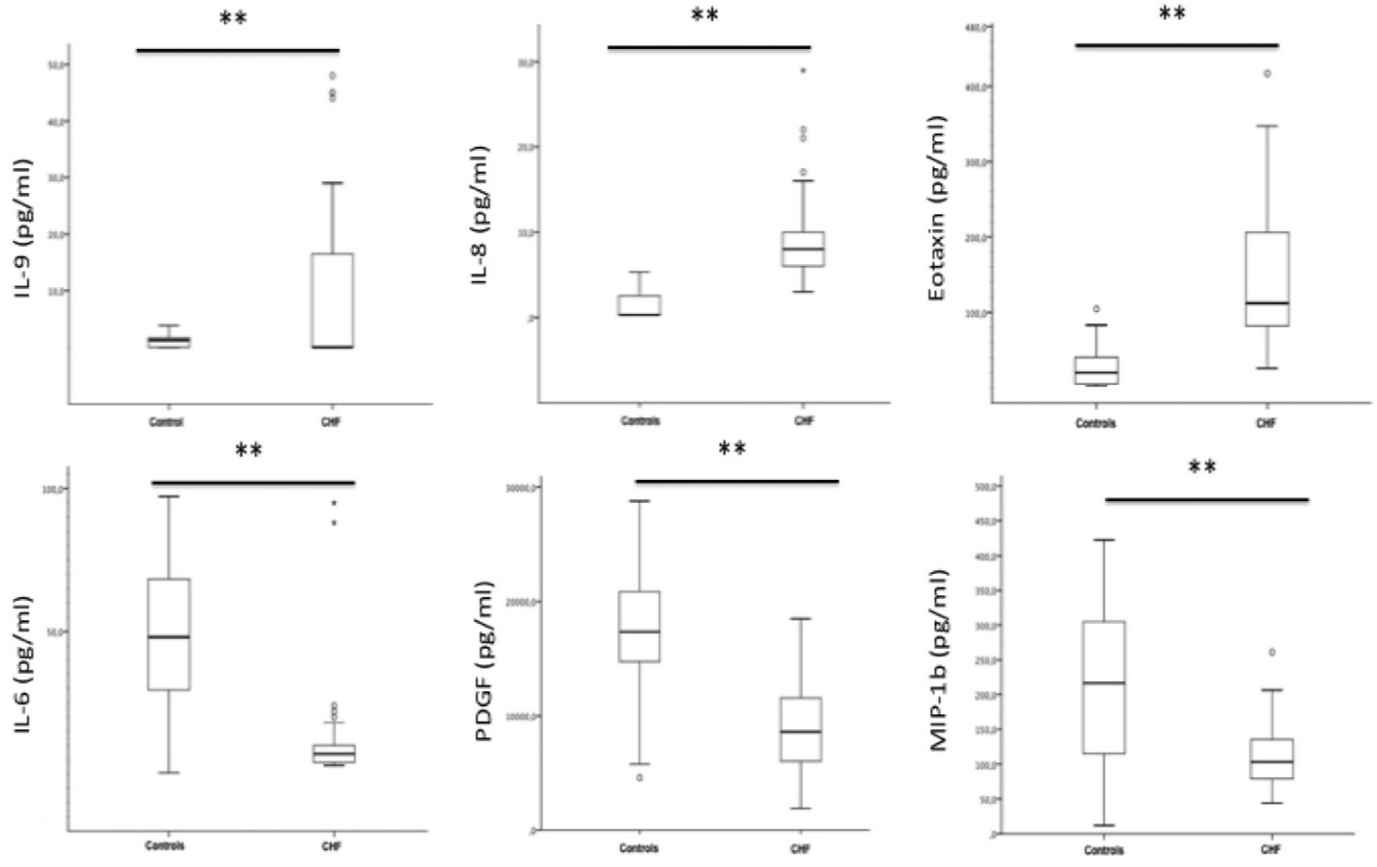

Fig. 1. Cytokine plasma levels in CHF and controls.