

## Awarded posters

# Plasma levels of matrix metalloproteinase-9 and matrix metalloproteinase-2 are increased in plasma of patients with heart failure

Paola Altieri, Sabina Nasti, Emanuela Devoto, Annamaria Nicolino, Silvia Ubaldi, Silvano Garibaldi, Giorgio Ghigliotti, Claudio Brunelli, Antonio Barsotti

*Laboratory of Cardiovascular Biology, Department of Internal Medicine, University of Genoa, Genoa, Italy*

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Address:

Dr.ssa Paola Altieri

*Laboratorio di Biologia  
Cardiovascolare  
Dipartimento di  
Medicina Interna  
Università degli Studi  
Viale Benedetto XV, 6  
16132 Genova  
E-mail: paobio@yahoo.it*

### Introduction

Previous studies in experimental models of heart failure (HF)<sup>1</sup> and in patients suffering from HF have suggested a role for persistent inflammatory activity associated with ventricular remodeling. Matrix metalloproteinases (MMPs) are well-characterized mediators of the inflammatory cascade. MMPs are an endogenous family of zinc-dependent enzymes that have been identified to be responsible for matrix remodeling in several disease states. MMP-9 and MMP-2 content and activity are increased in the failing human heart<sup>2</sup>. In addition to local inflammation, also the contribution of a systemic inflammatory response may mediate progression of HF. Accordingly, we have investigated whether MMP activity was increased in plasma of patients with HF, and we have compared it with the same activity in control subjects.

### Methods

**Population.** We have recruited for our observations 16 male patients with HF and 16 male healthy volunteers (controls). The clinical diagnosis of ischemic vs dilated cardiomyopathy in our patients with HF was based on clinical and angiographic criteria: history of prior myocardial infarction or significant coronary artery disease on coronary angiography. Severity of HF was assessed scoring functional class according to the NYHA classification. Demographics, cardiovascular risk factors and medications used were collected and recorded for all the participants in the study.

**Sample collection.** Blood samples for MMP assay were obtained from peripheral venous blood after 15 min of rest. The subjects were fasted for 10-12 hours before sample collection. Samples were put into EDTA tubes, the plasma was immediately separated, aliquoted at 100  $\mu$ l and frozen at -80°C until processing. Assays of fasting plasma concentrations of lipids, lipoproteins and of systemic markers of inflammation (von Willebrand factor, leukocytes, C-reactive protein) were performed by established procedures.

### Zymography and densitometric analysis.

MMP activity was detected by zymography<sup>3</sup> in EDTA plasma samples obtained. Plasma was analyzed on 9% SDS-PAGE in the absence of reducing agents. After an overnight electrophoretic run of gels and the following activation and staining, the resulting gelatinolytic bands were measured with the help of an image analyzer system (GeneGenius, Syngene, Cambridge, UK). Data were expressed as arbitrary units (AU) per milliliter of plasma. The coefficients of variation were: 5% (intraassay) and 10% (interassay). Recombinant MMP-9 and MMP-2 (Oncogene, Boston, MA, USA) were used as standard in the assays.

**Statistical analysis.** Results are expressed as mean  $\pm$  SD. MMP zymographic activities in patients with HF and in controls were compared with unpaired Student's t-test. Simple regression analysis was performed to examine the relationship between cardiovascular risk factors and zymographic activity in patients with HF.

Statview 4 program (Abacus Concepts, Berkeley, CA, USA) was used for the statistical analysis. A p value < 0.05 was considered statistically significant.

**Results**

**Clinical and biochemical features of the study sample.** Table I shows the clinical characteristics of HF patients and controls. As expected, patients with HF had higher values of leukocyte plasma levels, C-reactive protein, and von Willebrand factor, when compared to controls. Other baseline parameters and background medications for patients with HF are summarized in table II.

**Profiles of zymographic activity.** Plasma assayed in all the components of the study showed consistently three major gelatinolytic bands related to latent MMP-9, active MMP-9, and latent MMP-2. Levels of MMP-9 activity were increased by over 2-fold in patients with HF compared to controls with both the latent (p = 0.008) and the active (p = 0.003) form. Latent MMP-2 activity was also increased by 24% (p = 0.014) in HF patients, when compared to controls. A representative zymogram using gelatine as a proteolytic substrate in 5 healthy controls and in 5 HF patients is shown in figure 1. Data for levels of latent MMP-2 and for latent and active forms of MMP-9 activity are summarized in table III. Total number of

**Table I.** Clinical characteristics of the two study groups.

	HF patients (n=16)	Controls (n=16)
Age (years)	70 ± 2	62 ± 3
Body mass index (kg/m <sup>2</sup> )	25.8 ± 4.2	26.3 ± 6.1
Leukocytes (count/mm <sup>3</sup> )	8417 ± 2364*	6130 ± 1078
Current smokers (%)	32	38
History of hypertension (%)	70	57
C-reactive protein (mg/l)	19.2 ± 24.1*	4.4 ± 2.8
Total cholesterol (mmol/l)	191 ± 49	234 ± 40
LDL cholesterol (mmol/l)	140 ± 38	157 ± 33
Triglycerides (mmol/l)	137 ± 87	115 ± 48
von Willebrand factor (%)	221.8 ± 80.3*	126.5 ± 45.5

HF = heart failure. \* = p < 0.0001 vs controls.

**Table II.** Characteristics of patients with heart failure (HF).

	HF (%)
Postischemic etiology	50
History of prior myocardial infarction	47
NYHA class III	61
NYHA class IV	28
Diuretics	95
Digoxin	73
ACE-inhibitors	95
Beta-blockers	50
Nitrates	67

**Table III.** Metalloproteinase (MMP)-9 and MMP-2 activity: densitometric analysis.

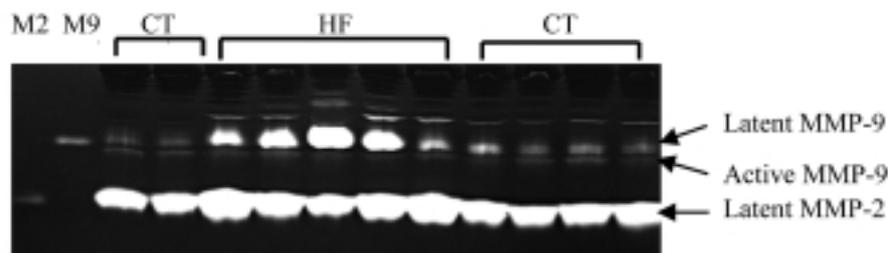
	Controls	HF
Latent MMP-9 (AU)	0.99 ± 0.63	2.02 ± 1.76*
Active MMP-9 (AU)	0.87 ± 0.51	1.76 ± 0.81**
Latent MMP-2 (AU)	10.07 ± 3.40	13.47 ± 3.82*

HF = heart failure; AU = arbitrary units. \* = p < 0.05; \*\* = p < 0.001.

leukocytes was associated with levels of active MMP-9 (r = 0.494, p = 0.04, simple regression analysis).

**Discussion**

The current study shows that MMP-9 in both the latent and active forms and latent MMP-2 are consistently increased in plasma of HF patients. MMP-2 is synthesized mainly by fibroblasts, endothelial cells and osteoblasts, whereas MMP-9 is produced by inflammatory cells, myoblasts, tumor cells, keratinocytes, and some epithelial cells. Thus, cells derived by the heart and by structures remote from the heart have the potential to be source for MMPs. Previous observations have demonstrated increased amount of MMPs in the failing human heart. With our observation, we wanted to address whether a systemic inflammatory component due to MMP was present in patients with HF at an advanced functional stage of their disease. New synthesis and/or release in plasma of MMPs may be triggered by sever-



**Figure 1.** Representative matrix metalloproteinase (MMP) gelatine zymography of plasma of patients with heart failure (HF) and controls (CT). Recombinant MMP-2 and MMP-9 were used in lane 1 and 2 respectively as standard. The arrows indicate the three specific gelatinolytic bands.

al proinflammatory moieties and cytokines. A likely mediator is tumor necrosis factor (TNF)-alpha. Specifically, TNF-alpha<sup>4</sup> can induce transcription factors which will bind to the MMP promoter. At the same time TNF-alpha induces release of MMP-9 zymogen in whole human blood by phagocytes<sup>5</sup>; in addition, MMP-like enzymes processed TNF-alpha from precursor to active form, amplifying inflammatory loops. HF shares with other chronic progressive and acute diseases, such as rheumatoid arthritis and sepsis, and the presence of raised levels of MMP-9. Thus, plasma levels of one or more MMPs may represent a sensitive and early marker to detect even a low-grade proinflammatory activity. One may postulate that in patients with HF repeated collection of blood samples over time to evaluate MMP levels may represent a potential tool to assess the efficacy of drugs directed to interfere with mediators of proinflammatory activity, and to address the role of inflammation at different stages of this syndrome.

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### References

1. Coker ML, Thomas CV, Clair MJ, et al. Myocardial matrix metalloproteinase activity and abundance with congestive heart failure. *Am J Physiol* 1998; 274 (Part 2): H1516-H1523.
2. Spinale FG, Coker ML, Heung LJ, et al. A matrix metalloproteinase induction/activation system exists in the human left ventricular myocardium and is upregulated in heart failure. *Circulation* 2000; 102: 1944-9.
3. Heussen CD, Dowdle EB. Electrophoretic analysis of plasminogen activators in polyacrylamide gels containing sodium dodecyl sulfate and copolymerized substrates. *Anal Biochem* 1980; 102: 196-202.
4. Levine B, Kalman J, Mayer L, Fillit HM, Packer M. Elevated circulating levels of tumor necrosis factor in severe chronic heart failure. *N Engl J Med* 1990; 323: 236-41.
5. Pugin J, Widmer MC, Kossodo S, Liang CM, Preas HL, Suffredini AF. Human neutrophils secrete gelatinase B in vitro and in vivo in response to endotoxin and proinflammatory mediators. *Am J Respir Cell Mol Biol* 1999; 20: 458-64.