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Neopterin levels are independently associated with cardiac remodeling in patients with chronic heart failure

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ABSTRACT

Objectives: Neopterin, a marker of inflammation and monocyte activation, is found increased in patients with heart failure (HF). This study investigates whether neopterin levels correlate with left ventricular (LV) remodeling and brain natriuretic peptide (BNP), a marker of cardiac stress, in chronic HF (CHF) patients with different severity of disease.

Design and methods: The relationship between neopterin and LV dimensions, NT-proBNP, and pro-inflammatory cytokines were studied in 98 CHF patients, while nineteen healthy subjects were enrolled as controls. Nineteen (19%) patients were in NYHA class I, 38 (39%) in NYHA class II, 27 (28%) in NYHA class III, and 14 (14%) in NYHA class IV.

Results: Neopterin levels were higher in CHF patients than in age- and gender-matched healthy controls, and related with indexed LV end-diastolic volume (LVEDVi). Prospectively CHF patients were separated into tertiles of low, medium and high neopterin levels. Among patients, male gender, LVEDVi, diuretic treatment, NYHA class I, NT-proBNP and IL-8 levels were significant determinants of urine neopterin levels by bivariate analysis. Neopterin levels were associated only to LV remodeling, as assessed by LVEDVi, and IL-8 levels, a crucial monocyte chemoattractant, by multivariate ordinal regression analysis.

Conclusions: The relationship between elevated neopterin levels and LV enlargement in CHF patients suggests a crucial role of monocyte activation in the development of cardiac dysfunction in CHF patients. Assessment of neopterin levels is a potential biomarker to evaluate the progression of LV remodeling in CHF patients.

Introduction

Although the neurohormonal mechanisms are known to be critically involved in the development of chronic heart failure (CHF), a state of immune activation with persistent expression of pro-inflammatory cytokines has also been demonstrated to contribute to the exacerbation of HF [1,2]. Several studies suggest that activated monocytes are involved in the release of inflammation factors that may contribute to progression of cardiac dysfunction [3,4] and to matrix remodelling in acute myocardial infarction (AMI) [5]. Production of pro-inflammatory cytokines, such as interleukin (IL)-6, IL-8 and tumor necrosis factor alpha (TNF)-α, potentially affecting left ventricular (LV) remodeling [6], has been also attributed to secretion by mononuclear cells. High circulating levels of chemoattractant of monocytes, such as IL-8, were observed in congestive HF patients [7] and in CHF patients in New York Heart Association (NYHA) class IV [8].

Neopterin, an aromatic pteridine mainly synthesized by activated monocytes, is a marker of inflammation, immune system activation and an active participant in cardiovascular diseases [9]. Its assessment is proposed as potential tool useful for risk stratification of patients with AMI [10], for prediction of 1-year LV remodeling in patients with ST-segment elevation AMI [11], and as independent predictor of HF hospitalization after an acute coronary syndrome [12]. High serum neopterin levels were found in CHF patients with elevated NYHA class [13] and were associated with high rates of cardiac events in HF patients [14]. In patients with critical limb ischemia, the cardiac rhythm disturbances and ischemic electrocardiographic changes

Abbreviations: AMI, acute myocardial infarction; CHF, chronic heart failure; CI, confidence interval; CV, coefficent of variation; eGFR, estimated glomerular filtration rate; HPLC, high-pressure liquid chromatography; IDC, idiopathic dilated cardiomyopathy; IL, interleukin; LV, left ventricular; LVEDVi, left ventricular end-diastolic volume index; LVESVi, left ventricular end-systolic volume index; Neo/Cr, neopterin to creatinine ratio; NT-proBNP, NT-pro-Brain natriuretic peptide, NYHA, New York Heart Association; OR, odds ratio; MMPs, metalloproteinases; sICAM, soluble intracellular adhesion molecule; TNF, tumor necrosis factor.

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were related to inflammatory mediators, specifically to circulating neopterin levels that correlated with echocardiographic signs of congestive HF [15].

These findings strongly suggest that enhanced activation of the monocyte-macrophage system may play a role in LV remodeling in CHF patients, probably by secretion of pro-inflammatory cytokines. Thus the purposes of this study were: 1) to assess whether neopterin levels were associated with LV remodeling and circulating levels of pro-inflammatory cytokines, and 2) to compare the neopterin levels with NT-pro-Brain natriuretic peptide (NT-proBNP), recognized as cardiac stress marker, in relation to LV dimensions, in CHF patients with different clinical severity of HF.

Materials and methods

Patients

We studied 98 patients with myocardial dysfunction secondary to ischemic or idiopathic dilated cardiomyopathy (IDC), who had been admitted for routine re-evaluation or for treatment of advanced HF at the Cardiovascular Department of Niguarda Hospital from June 2010 to November 2011. None of the patients included in the study had acute myocarditis, primary pulmonary hypertension, irreversible renal or hepatic failure due to hepatic-renal chronic disease, uncontrolled diabetes mellitus, severe peripheral vascular or cerebrovascular disease, coexisting active neoplasia, pregnancy and alcohol/drug abuse. At the time of enrollment, baseline clinical and cardiac imaging data were collected. Urine and peripheral blood samples were drawn and immediately processed, and plasma and urine aliquots were frozen at −80 °C for measurement of biomarkers.

Nineteen healthy subjects with normal regional and global LV function were enrolled as control group.

The study protocol was approved by the Local Ethics Committee. All subjects gave written informed consent to participate in the study.

Definitions of clinical and instrumental measures

Clinical cardiac derangement was defined according to the NYHA classification provided separately by two attending cardiologists (MF and AV). Full agreement in class definition was attained in all cases. Transthoracic echocardiograms were performed with a Vivid 7 (GE Healthcare, London, United Kingdom) or iE33 (Philips Healthcare, Best, Netherlands); LV end-systolic and end-diastolic volumes (LVESV and LVEDV, respectively) were measured from four-chamber and two-chamber apical views means of Simpson's method. For LV volume analysis, intraobserver and interobserver variability were 6% and 8%, respectively.

Renal function was assessed by estimated glomerular filtration rate (eGFR) using the abbreviated MDRD formula [16].

Biochemical assays

Blood and urine samples were drawn in the morning in fasting state to determine levels of inflammatory mediators [plasma IL-6, IL-8, soluble intracellular adhesion molecule (sICAM)-1, serum C-reactive protein (CRP), and urine neopterin] and of serum NT-proBNP by technicians unaware of clinical status of patients.

Plasma IL-6, IL-8 and sICAM-1 levels were measured by enzyme-linked immunosorbent assays (R&D Systems, Minneapolis, MN-USA).

Serum CRP concentrations were measured using a Roche/Hitachi 917 Analyzer by high-sensitive immuno-nephelometric method (Roche Diagnostic GmbH, Mannheim, Germany).

Urinary neopterin concentrations were measured by an isocratic high-pressure liquid chromatography (HPLC) method and were normalized by urine creatinine concentrations (Neo/Cr). Briefly, urine samples, stored at −20 °C were thawed and centrifuged; the surnatant was then adequately diluted with chromatographic mobile phase (15 mM of K_2HPO_4, pH 3.0). Neopterin and creatinine levels were measured using a Kontron instrument (pump 422-S, autosampler 465) coupled to a fluorimetric detector (JASCO FP-1520, $\lambda_{ex}=355$ nm and at $\lambda_{em}=450$ nm) for neopterin detection and to a UV–VIS detector (BIO-RAD 1706, $\lambda=240$ nm) for creatinine determination. Neopterin and creatinine separations were performed at 50 °C on a 5 μm Discovery C18 analytical column (250×4.6 mm I.D., Supelco, Sigma-Aldrich) at flow rate of 0.9 mL/min. The calibration curves were linear over the range of 0.125–1 μmol/L and of 1.25–10 mmol/L for neopterin and creatinine levels, respectively.

Serum NT-proBNP levels were measured by the ADVIA Centaur assay (Siemens Healthcare Diagnostics).

Intra-assay and inter-assay coefficients of variation (CV) of investigated biomarkers are reported in Table 1.

Statistical analysis

Data are expressed as median (25th–75th percentile) or frequency (percentage). Categorical variables were compared using the chi-square test or Fisher exact test when appropriate. Continuous variables were compared between groups using Student's *t*-test or the Mann–Whitney *U*-test, according to whether variables were normally distributed or not, as tested by the Kolmogorov–Smirnov test. Prospectively CHF patients were distributed into three groups according to the tertile of Neo/Cr levels. Ordinal logistic regression analysis was used to examine the association between neopterin tertiles (dependent variable) and clinical, echocardiographic and inflammatory variables only in HF patients. We established a multivariate ordinal logistic model which included only variables that were significantly related to neopterin (significance level set at $p<0.05$). Results are presented as odds ratio (OR), and their 95% confidence interval (CI). A two tailed $p<0.05$ was considered statistically significant.

Results

Characteristics findings in patients with LV dysfunction and controls

The population in this study has been described in Table 2. Briefly an average age of the patients was 56 years, 17% were female. Fifty-six patients (57%) had a diagnosis of post-ischemic LV dysfunction. Nineteen (19%) patients were in NYHA class I, 38 (39%) patients were in NYHA class II, 27 (28%) in class III, and 14 (14%) in class IV. Cardiac volumes were higher in patients compared to healthy subjects. Moreover, forty-two patients (43%) showed LVEDV index (LVEDVi) ≥97 mL/m^2, reflecting severe grade of LV remodeling

Table 1
Coefficients of variation of intra- and inter-assays.

Parameter	Control Levels	Intra-assay			Inter-assay		
		Concentration of controls	CV (%)	n	Concentration of controls	CV (%)	N
IL-6 (pg/mL)	Low	1.2	5.8	20	1.5	9.6	20
	High	10.0	3.0	20	13.0	7.7	20
IL-8 (pg/mL)	Low	3.5	6.6	20	4.2	11.5	20
	High	22.0	4.5	20	25.0	6.3	20
sICAM-1 (ng/mL)	Low	4.5	3.6	20	4.5	6.8	40
	High	20.0	5.0	20	20.0	4.4	40
CRP (mg/dL)	Low	0.16	1.0	21	0.15	3.3	21
	High	1.84	0.5	21	3.91	1.9	21
Neo/Cr (μmol/mol)	Low	100.0	0.3	20	100.0	1.8	15
	High	300.0	0.2	20	300.0	1.4	15
NT-proBNP (ng/L)	Low	82.0	2.6	21	77.0	1.8	60
	High	2318.0	1.2	21	2105.0	1.2	60

CRP = C-reactive protein; CV = coefficient of variation; IL = interleukin; Neo/Cr = neopterin normalized by creatinine; NT-proBNP = N terminal pro-brain natriuretic peptide; sICAM-1 = soluble intercellular adhesion molecule type I.

Table 2
Clinical and biochemical characteristics between patients and controls.

	Controls ($n=19$)	CHF patients ($n=98$)	p
Age, years	50 (33–61)	56 (49–63)	0.076
Male, *n* (%)	13 (68)	81 (83)	0.204
BMI, kg/m^2	25.8 (24.7–27.8)	25.3 (23.4–29.4)	0.726
LVEDVi, mL/m^2	47 (42–56)	91 (64–117)	<0.001
LVESVi, mL/m^2	17 (15–19)	69 (56–93)	<0.001
NYHA class, *n* (%)			
III + IV	0 (0)	41 (42)	<0.001
Therapy, *n* (%)			
ACEi	4 (21)	62 (63)	0.001
β-Blocker	2 (11)	77 (79)	<0.001
Statin	2 (11)	56 (57)	<0.001
Antiplatelets	0 (0)	61 (63)	<0.001
Diuretic	1 (5)	71 (72)	<0.001
Smoking, *n* (%)	2 (11)	11 (11)	1.000
Hypertension, *n* (%)	7 (37)	34 (35)	1.000
Diabetes mellitus, *n* (%)	4 (21)	25 (25)	0.779
eGFR, mL/min/1.73 m^2	100 (84–124)	81 (68–104)	0.028
NT-proBNP, ng/L	19 (12–54)	910 (291–2824)	<0.001
Inflammatory variables			
C-reactive protein, mg/dL	0.25 (0.10–0.45)	0.20 (0.10–0.50)	0.809
IL-6, pg/mL	0.53 (0–0.72)	1.80 (0.17–3.22)	<0.001
IL-8, pg/mL	2.9 (2.5–3.5)	3.9 (2.8–5.1)	0.038
sICAM-1, ng/mL	154 (122–185)	188 (139–242)	0.021
Neo/Cr, μmol/mol	120 (100–210)	183 (142–250)	0.001

Data are expressed as median (25th–75th percentile) or frequency (percentage). ACEi = angiotensin converting enzyme inhibitor; BMI = Body mass index; eGFR = estimated glomerular filtration rate; IL = interleukin; LVEDVi = left ventricular end-diastolic volume index; LVESVi = left ventricular end-systolic volume index; Neo/Cr = neopterin normalized by creatinine; NT-proBNP = N terminal pro-brain natriuretic peptide; NYHA = New York Heart Classification; sICAM-1 = soluble intercellular adhesion molecule type I.

[17], as well as NT-proBNP levels were higher in CHF patients than in controls (Table 2).

All patients were taking optimal tolerated medical treatment including ACE-inhibitors, β-blockers, diuretics, and statins.

Controls' median age was 50 years (33–61), 6 subjects (32%) were female. All control subjects had a LVEF > 55%. Smoking habit, hypertension, and diabetes mellitus were similarly represented in controls and HF patients, while renal function, evaluated by eGFR, was lower in patients than in controls.

Neo/Cr levels were higher in patients than in controls. The levels of other investigated inflammatory variables were higher in CHF patients compared to controls, with the exception of serum CRP levels, that were similar between groups (Table 2). Only Neo/Cr levels correlated significantly with LVEDVi values ($R=0.29$, $p=0.006$).

Neopterin, clinical and echocardiographic variables in CHF patients

Patients were distributed according tertiles of Neo/Cr levels as described in Table 3. The Neo/Cr levels range from 66 to 155 μmol/mol in the first tertile, from 157 to 225 μmol/mol in the second tertile, and from 226 to 811 μmol/mol in the third tertile. The median Neo/Cr level of patients in the first tertile was comparable to that of controls, while the median Neo/Cr levels of patients in the second and third tertiles were higher with respect to that of controls ($p=0.002$ and $p<0.001$, respectively).

Increasing Neo/Cr levels were significantly associated with LVEDVi and NT-proBNP (Table 3), with the highest tertile of Neo/Cr levels seen with the highest LVEDVi and NT-proBNP levels. Patients with severely abnormal LV dimension (LVEDVi ≥ 97 mL/m^2) were 8 (25%) in the first tertile, 17 (50%) in the second tertile, and 17 (53%) in the third tertile of Neo/Cr levels (81% of patients with severely abnormal LV dimension were in the second and third tertile).

Statin treatment, and the frequencies of male gender and NYHA class I, was significantly decreased according to the increment of Neo/Cr levels, while diuretic treatment was significantly increased according to the increment of Neo/Cr levels by bivariate analysis (Table 3).

Etiology, cardiovascular risk factors and renal function, assessed by creatinine clearance, were not associated to Neo/Cr levels.

Table 3
Bivariate ordinal logistic regression analysis vs tertiles of Neopterin.

	[a]Tertiles of Neo/Cr levels			OR	95% CI		*P*
	I ($n=32$)	II ($n=34$)	III ($n=32$)				
Age, years	54 (47–64)	57 (49–64)	57 (50–63)	1.003	0.972	1.035	0.851
Male, *n* (%)	30 (94)	28 (82)	23 (72)	0.316	0.115	0.871	0.026
BMI, kg/m^2	27 (23–30)	28 (24–30)	25 (22–28)	0.965	0.892	1.043	0.365
LVEDVi, mL/m^2	76 (51–102)	97 (76–117)	99 (80–154)	1.012	1.004	1.020	0.004
LVESVi, mL/m^2	63 (47–82)	71 (57–90)	72 (57–122)	1.009	0.999	1.019	0.102
NYHA class, *n* (%)							
I	12 (38)	5 (15)	2 (6)	0.077	0.019	0.321	<0.001
II	11 (34)	16 (47)	11 (34)	0.298	0.089	0.993	0.049
III	6 (19)	11 (32)	10 (31)	0.419	0.119	1.472	0.175
IV	3 (9)	2 (6)	9 (28)	1.000	-	-	
IHD, *n* (%)	19 (59)	23 (68)	14 (44)	0.615	0.293	1.290	0.199
Therapy, *n* (%)							
ACEi	19 (59)	22 (65)	21 (66)	1.223	0.574	2.605	0.603
B-Blocker	25 (78)	26 (77)	26 (81)	1.145	0.471	2.781	0.765
Statin	23 (72)	22 (65)	11 (34)	0.302	0.140	0.654	0.002
Antiplatelets	19 (59)	25 (74)	17 (55)	0.869	0.408	1.853	0.717
Diuretic	17 (53)	27 (79)	27 (84)	3.501	1.478	8.293	0.004
Smoking, *n* (%)	5 (16)	2 (6)	4 (13)	0.749	0.236	2.381	0.624
Hypertension, *n* (%)	12 (38)	8 (24)	14 (45)	1.285	0.596	2.771	0.522
Diabetes mellitus, *n* (%)	7 (22)	9 (27)	9 (28)	1.275	0.552	2.944	0.569
eGFR, mL/min/1.73 m^2	85 (76–113)	78 (62–105)	75 (66–104)	0.991	0.981	1.001	0.110
NT-proBNP, ng/L	294 (126–904)	888 (505–2870)	2296 (820–3879)	1.000	1.000	1.001	0.001
Inflammatory variables							
C-reactive protein, mg/dL	0.10 (0.10–0.30)	0.20 (0.10–0.60)	0.30 (0.20–0.80)	2.158	1.082	4.301	0.029
IL-6, pg/mL	1.47 (0.71–1.77)	2.04 (1.50–3.58)	2.97 (1.39–6.11)	1.048	0.996	1.103	0.079
IL-8, pg/mL	3.67 (2.42–4.23)	4.10 (2.79–5.02)	4.70 (3.10–7.60)	1.273	1.071	1.512	0.006
sICAM-1, ng/mL	162 (137–199)	208 (148–250)	221 (103–257)	1.003	0.999	1.007	0.172

Data are expressed as median (25th–75th percentile). For abbreviations see Table 2.
[a] Tertiles of Neo/Cr levels: I, (first) tertile: 126 (107–142) μmol/mol; II (second) tertile: 183 (166–215) μmol/mol; III (third) tertile: 321 (249–469) μmol/mol.

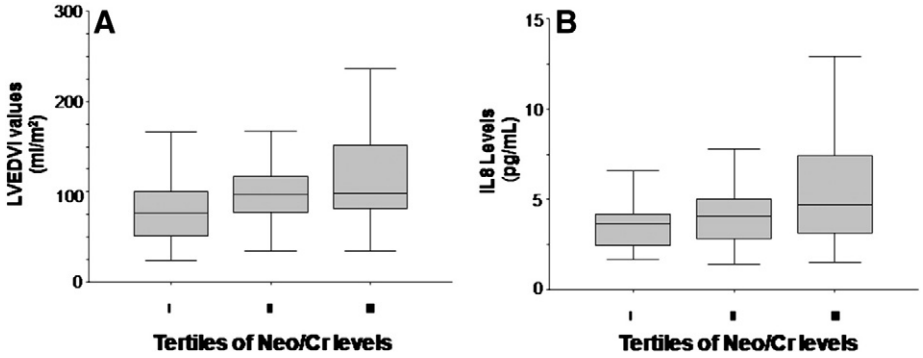

Fig. 1. Grade of LVEDVi (A) and IL-8 levels (B) according to tertiles of Neo/Cr levels in chronic heart failure patients. Tertiles of Neo/Cr levels (μmol/mol): I, 126 (107–142); II, 183 (166–215); III: 321 (249–469).

Neopterin and inflammatory variables in HF patients

Among inflammatory variables, only serum CRP and plasma IL-8 levels increased according to neopterin levels, while sICAM-1 levels were comparable among CHF patients with different neopterin levels (Table 3). The IL-6 levels increased according to neopterin levels but only with a trend by bivariate analysis (Table 3).

Multivariate analysis

All the variables that reached the significance level $p<0.05$ were entered into the final multivariable ordinal regression model. The only parameters independently associated with neopterin levels were LVEDVi values (OR 1.014, 95% CI 1.002–1.026, $p=0.024$, Fig. 1A), IL-8 levels (OR 1.353, 95% CI 1.008–1.815, $p=0.044$, Fig. 1B), and male gender (OR 0.257, 95% CI 0.067–0.977; $p=0.046$).

Discussion

The findings from our study indicate that, in CHF patients characterized from different clinical severity of HF and grade of LV remodeling, higher neopterin levels are independently associated with increased dimensions of cardiac volume, echocardiographically defined by LVEDVi values. Among inflammatory variables, only IL-8 levels resulted associated with neopterin levels in CHF patients.

Neopterin is a marker of macrophage/monocyte activation with a putative physiological role in enhancing macrophage cytotoxicity through its interactions with reactive oxygen, nitrogen, chloride species [18], and by its influence on myeloperoxidase activity [19]. Neopterin has shown to promote oxidative stress-triggered apoptosis of vascular smooth muscle cells and to promote plaque growth [20], even suggesting its role in the inflammatory cascade promoting atherogenesis [21,22]. In isolated perfused rat hearts neopterin infusion favors negative effects on cardiac performance by an enhancement of oxidative stress, as demonstrated by co-treatment with the antioxidant N-acetylcysteine that attenuated the adverse effect of neopterin [23]. Previous study has demonstrated an inverse correlation between neopterin levels and LV ejection fraction in patients with chronic stable angina [24]. In our CHF patients we demonstrate a relationship between urine neopterin levels and LV remodeling, in particular with diastolic enlargement of cardiac volume, independently from etiology and clinical status of HF patients (NYHA class). Furthermore, patients with severely abnormal LV diastolic dimension [17] were greater associated with higher levels of neopterin. In addition, CHF patients with the greatest concentration of circulating neopterin also showed the highest levels of IL-8, a chemokine crucial on monocyte recruitment and attraction on endothelial cells. Therefore the presence of high levels of IL-8 and of LVEDVi, in association with the severity of the levels of neopterin, support a heightened state of immune activation in CHF patients and the pathological role of monocyte on LV remodeling. As observed even in acute coronary syndrome [12], C-reactive protein and NT-proBNP levels weren't associated with neopterin in our CHF patients. These data support the potential contribution of the determination of neopterin for the monitoring of ventricular remodeling with respect to C-reactive protein, a non-specific inflammatory biomarker, and NT-proBNP, that reflects the wall stress of left ventricle.

Noteworthy, in the HF disease the activation of metalloproteinases (MMPs) and alteration of extracellular matrix components are considered crucial mechanisms involved in cardiac structural changes that deteriorates into a dilated eccentric pattern [2]. An abnormal balance between MMPs activity and their tissue inhibitors, or an increased activity of MMPs, appear to be involved in collagen scaffold degradation, LV dilation, and to compromise cardiac systolic function [25]. Several studies have even suggested that oxidative stress acts properly on MMPs metabolism, favoring progression of cardiac remodeling [26,27].

Previous studies have demonstrated that neopterin concentration, in patients at risk for atherosclerosis or with cancer, relates to their antioxidant status [28,29]. Moreover, neopterin levels have been found to relate with homocysteine, a pro-oxidant aminothiol deleterious for vascular function [30]. Baseline serum neopterin and MMP-9 concentrations are found associated with rapid progression of coronary artery disease in patients with chronic stable angina pectoris [31]. As a matter of fact inducible MMP-9, a crucial MMP involved in LV remodeling, is also expressed in the failing myocardium by infiltrating inflammatory cells, such as monocytes [32]. In addition, in chronic renal failure the dysregulation of MMPs and their inhibitors were found associated to increased synthesis of neopterin [33]. Therefore, the association found between LVEDVi and neopterin levels in CHF patients is suggestive of an involvement of monocyte activation in LV remodeling probably by oxidative stress induced-MMPs activation.

Neopterin monitoring in body fluids provide information on current state of cellular immune response and frequently help to predict progression of several diseases with intensified monocyte/macrophage activity [18,34]. In IDC patients, abnormal neopterin levels were associated with the development of progressive HF and death, suggesting that neopterin levels are related to HF severity [35]. Other study reported that plasma levels of neopterin might predict adverse cardiovascular events in patients with coronary artery disease, acute coronary syndromes or severe peripheral artery disease [9]. In keeping with these reports, we provide evidence that neopterin levels are associated to LVEDVi values in CHF patients supporting neopterin determination as useful tool to evaluate the progression of LV remodeling. Moreover, in the present study, neopterin levels were measured in biological samples easily accessible, such as samples of urine, and assessed by a fast and cheap HPLC technique, with a procedure that lasts less than 15 minutes/sample. Therefore, the measurement of neopterin, as inflammatory and

oxidative stress marker, may be clinically helpful to ameliorate risk stratification and to follow disease progression in CHF patients.

Study limitations

Failure to ascertain the levels of metalloproteinases is a limitation of the study. The assessment of MMPs levels according to neopterin levels might provide more accurate information on the role of neopterin in LV remodeling. However, circulating levels of MMPs do not always reflect the expression of cardiac MMPs.

Conclusions

In conclusion, an independent association between urine neopterin levels and LV end-diastolic volume values was observed in CHF patients with different HF clinical severity. The increment of IL-8 levels, according with neopterin levels, suggests that monocyte activation is involved in adverse LV remodeling. The clinical utility of neopterin assessment in monitoring HF progression merits evaluation. Long-term evaluation of LV remodeling progression might identify neopterin as useful prognostic indicator.

Authors' disclosures or potential conflicts of interest

No authors declared any potential conflicts of interest.

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