

The clinical significance of plasma neopterin in heart failure with preserved left ventricular ejection fraction

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Abstract

Aims Although inflammation plays an important role in the pathogenesis of heart failure (HF), the precise pathophysiological role of inflammation in HF with preserved left ventricular ejection fraction (HFpEF) still remains unclear. Hence, we examined the clinical significance of plasma neopterin, an inflammatory biomarker, in HFpEF patients.

Methods and results In the present study, we recruited consecutive HFpEF patients hospitalized in Kumamoto University Hospital, and further measured plasma neopterin by high-performance liquid chromatography and serum derivatives of reactive oxidative metabolites (DROM), a new biomarker of reactive oxygen species. Compared with risk factors (number of patients, age, sex, and equal incidence of diabetes mellitus, hypertension, and dyslipidemia) -matched non-HF patients ($n=68$), plasma neopterin levels, but not serum high-sensitivity C-reactive protein levels, were significantly increased in patients with HFpEF ($n=68$) ($P<0.001$ and $P=0.15$, respectively), accompanied by an elevation in serum DROM levels ($P<0.001$). Plasma neopterin levels in New York Heart Association (NYHA) class III/IV HFpEF patients were significantly higher than in NYHA class II patients ($P<0.004$). Furthermore, plasma ln-neopterin levels had significant and positive correlation with ln-DROM values ($r=0.57$) and parameters of cardiac diastolic dysfunction [the ratio of early transmitral flow velocity to tissue Doppler early diastolic mitral annular velocity ($r=0.34$), left atrial volume index ($r=0.17$), and B-type natriuretic peptide ($r=0.38$)]. Kaplan–Meier analysis showed that the high-neopterin group (>51.5 nM: median value of neopterin in HFpEF patients) had a higher probability of cardiovascular events than the low-neopterin group (log-rank test, $P=0.003$).

Conclusions Plasma neopterin levels significantly increased in HFpEF and correlated with the severity of HF. Furthermore, high neopterin were significantly correlated with future cardiovascular events, indicating that measurement of plasma neopterin might provide clinical benefits for risk stratification of HFpEF patients.

Keywords Heart failure with preserved left ventricular function; Inflammation; Neopterin; Reactive oxygen species

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Introduction

Inflammation plays an important role in the pathogenesis of heart failure (HF), and C-reactive protein (CRP), a representative inflammatory biomarker, has been shown to be an independent predictor of HF hospitalization after acute myocardial infarction (MI).¹ However, the precise pathophysiological role of inflammation in HF with preserved left ventricular (LV) ejection fraction (HFpEF), which has a poor prognosis equivalent to HF with

reduced LVEF, still remains unclear. Using Dahl salt-sensitive hypertensive rats (DS rats), which are useful experimental models of HFpEF, we have previously shown the close association of LV macrophage infiltration with reactive oxygen species (ROS) in HFpEF.² Furthermore, we previously reported that pentraxin (PTX) 3, a member of the PTX superfamily including CRP, is a significant inflammatory marker in patients with HFpEF.³

On the other hand, plasma neopterin is mainly secreted by activated macrophages, and is known to be an indicator of inflammation. Moreover, neopterin was reported to enhance

macrophage cytotoxicity through its interactions with ROS, and to promote ROS-induced apoptosis of vascular smooth muscle cells and atherosclerosis.⁴ As well as one other inflammatory biomarker, CRP,¹ plasma neopterin was demonstrated as an independent predictor of HF hospitalization after acute MI.⁵ However, the precise role of neopterin in HFpEF remains totally unknown.

In this study, we hypothesized that plasma neopterin is closely associated with pathophysiology of HFpEF, and sought to examine the clinical significance of plasma neopterin levels in symptomatic HFpEF patients with New York Heart Association (NYHA) functional class II–IV. Because baseline characteristics of all non-HF and HFpEF patients were quite different, we aimed to match risk factors for our study groups and thus to investigate whether plasma neopterin levels are affected by HFpEF. We divided patients into the risk factor-matched HFpEF group ($n=68$) and the risk factor-matched non-HF group ($n=68$) using nearest neighbour matching, no replacement, and one-to-one pair matching the following risk factors: number of patients, age, sex, and equal incidence of diabetes mellitus, current smoking, hypertension, dyslipidemia, and coronary artery disease (CAD).

Methods

We performed a prospective cohort study to investigate the clinical significance of plasma neopterin in HFpEF patients. In the present study, we recruited consecutive HFpEF patients hospitalized in Kumamoto University Hospital from July 2010 to January 2013. We further examined blood biomarkers [levels of plasma neopterin and serum derivatives of reactive oxidative metabolites (DROM, Diacron srl, Grosseto, Italy); normal range: 250–300 unit called the Carratelli unit (U. CARR), a new biomarker of ROS], and cardiac function by ultrasound cardiography at stable condition after the optimal therapy according to guidelines for the treatment of acute HF by The Japanese Circulation Society. We defined HFpEF clinically according to the criteria of the European Working Group to HFpEF⁶: (i) symptoms or signs of HF (NYHA functional class II–IV); (ii) normal or mildly reduced LV systolic function [LVEF $>50\%$ and LV end-diastolic volume index (LVEDVI) $<97\text{ mL/m}^2$]; and (iii) evidence of abnormal LV relaxation, filling, diastolic distensibility, and diastolic stiffness. In our study, we stratified the ratio of early transmitral flow velocity to tissue Doppler early diastolic mitral annular velocity (E/e') as ≥ 15 or >8 and <15 , and B-type natriuretic peptide (BNP) levels with a cutoff of 100 pg/mL. LVEF (the modified Simpson method), LVEDVI, LV stroke volume index (SVI), LV mass index (LVMI), and left atrial volume index (LAVI) (the prolate ellipse method) were measured by conventional echocardiography [Vivid 7 (GE-Vingmed Ultrasound, Horton, Norway) and Aplio XG (Toshiba, Tokyo, Japan)], and E/e' was

measured by tissue Doppler imaging using these modalities. Echocardiography was performed by experienced cardiac sonographers who had no knowledge of our study data under stable conditions after the optimal therapy for HF. Furthermore, the reproducibility and repeatability of the echo parameters were confirmed by two different experienced sonographers. Plasma neopterin levels were directly measured in duplicate by high-performance liquid chromatography with the electrochemical detection method, as previously described,⁷ and were compared between in HFpEF patients and in non-HF patients after matching risk factors. The reproducibility and repeatability of the methods of biochemical biomarkers measurement were strictly confirmed. We excluded patients who have the following reasons: severe valvular disease ($n=12$), severe collagen disease ($n=3$), active infective disease ($n=5$), history of malignancy ($n=16$), and the end stage of renal disease ($n=6$, eGFR; estimated glomerular filtration rate $<15\text{ mL/min/1.73 m}^2$). Furthermore, patients were followed-up until 1000 days after discharge or until the occurrence of cardiovascular events (cardiovascular death, acute coronary syndrome, or hospitalization for HF). We used the median value of plasma neopterin (51.5 nM) to divide HFpEF patients into low- and high-neopterin groups (Figure 1). The study protocol conformed to the principles of the Declaration of Helsinki. Written informed consent was obtained from all patients.

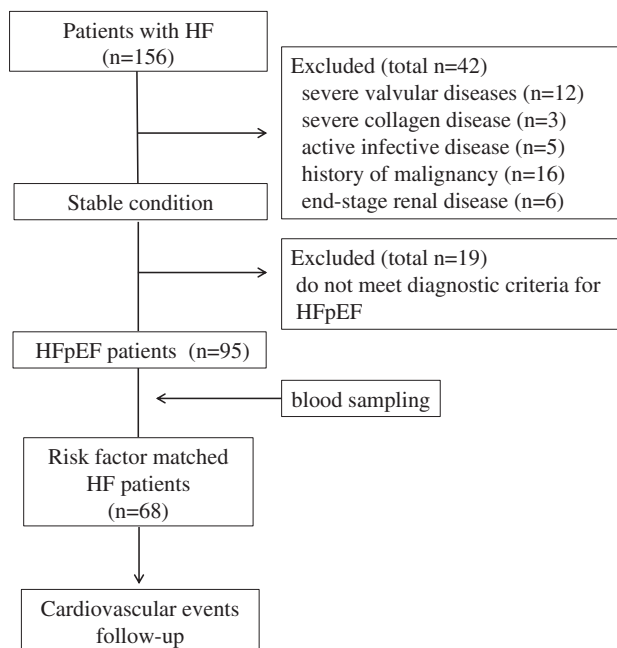
We have assessed day-to-day, intra-, and inter-observer reproducibility of echocardiography in our institutes by three methods: (i) the intra-class correlation (ICC), (ii) Cronbach's alpha, and (iii) the coefficient of variation (CV), and have confirmed that echocardiography measurements in our institutes have good reproducibility in ICC, Cronbach's alpha, and CV as well as previous studies reported.

Differences between two groups in risk factor matching data were tested with McNemar test for categorical variables. Differences in continuous variables in risk factor matching data were analysed by the paired t -test, or Wilcoxon signed-rank test, as appropriate. Kaplan–Meier analysis was performed by using the median value of neopterin in HFpEF patients and was compared such incidence by the log-rank test. A P value of <0.05 was considered statistically significant. Statistical analyses were performed using The Statistical Package for Social Sciences version 22 (IBM Japan, Ltd., Tokyo, Japan).

Results

Baseline characteristics of patients are shown in Table 1. Compared with risk factor-matched non-HF patients, plasma neopterin levels, but not serum high-sensitivity CRP levels, were significantly increased in patients with HFpEF [neopterin; 37.2 (30.6–46.1) nM vs. 51.5 (43.6–57.2) nM,

Figure 1 Flow chart showing the protocol used for this study.



$P < 0.001$, and high-sensitivity CRP; 0.2 (0.1–0.6) mg/L vs. 0.5 (0.2–0.9) mg/L, $P = 0.15$, respectively, *Table 1*]. Similarly with our previous clinical report,⁸ serum DROM levels were significantly increased in HFpEF patients [298.0 (272.0–370.8) U. CARR vs. 384.0 (348.5–426.0) U. CARR, $P < 0.001$, *Table 1*], and which were accompanied by an elevation in plasma neopterin levels in HFpEF patients. Compared with risk factor-matched non-HF patients, furthermore, the eGFR (69.1 ± 14.5 vs. 59.2 ± 19.8) and LVSVI (44.6 ± 5.7 mL/m² vs. 35.7 ± 6.9 mL/m², $P < 0.001$) values were significantly lower (all, $P < 0.001$, *Table 1*), and plasma BNP [28.1 (12.7–39.7) pg/mL vs. 75.4 (30.3–123.2) pg/mL, $P < 0.001$], LVEDVI (43.3 ± 9.0 mL/m² vs. 47.6 ± 14.1 mL/m², $P < 0.05$), LVMI (105.1 ± 18.1 g/m² vs. 146.8 ± 39.4 g/m², $P < 0.001$), LAVI values (24.2 ± 7.2 mL/m² vs. 35.9 ± 22.2 mL/m², $P < 0.001$), and E/e' levels (11.0 ± 3.8 vs. 17.2 ± 4.0 , $P < 0.001$) were significantly higher in HFpEF patients (*Table 1*). Plasma neopterin levels in HFpEF patients with NYHA class III/IV ($n = 31$) were significantly higher in those with NYHA class II ($n = 37$) [49.9 (43.3–52.8) nM vs. 56.0 (44.3–67.3) nM, $P < 0.004$, *Figure 2*], indicating significant correlation of neopterin in HFpEF with the severity of HF.

Furthermore, plasma ln-neopterin levels had strong and positive correlation with ln-DROM values in all 136 enrolled

Table 1 Baseline characteristics of 68 risk factor-matched non-heart failure patients and 68 risk factor-matched heart failure with preserved left ventricular ejection fraction patients

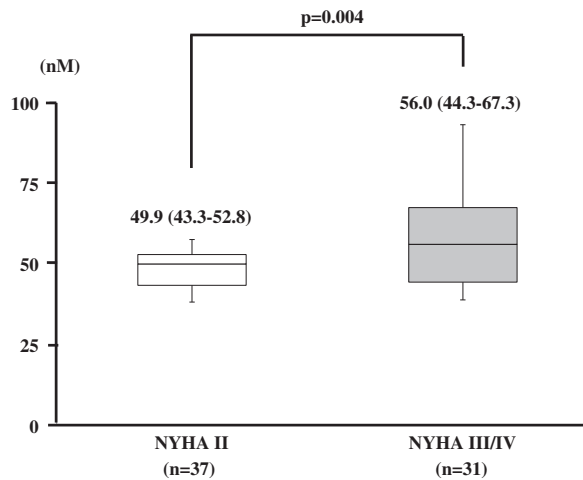
	All patients (n = 136)	Matched non-HF (n = 68)	Matched HFpEF (n = 68)	P value*
Age (years)	68.5 (8.8)	68.5 (8.4)	68.5 (9.2)	0.98
Sex (male, %)	58.8	58.8	58.8	1.0
BMI (kg/m ²)	23.5 (3.5)	22.8 (3.0)	24.3 (3.8)	0.01
CAD (yes, %)	62.5	61.8	63.2	0.86
Hypertension (yes, %)	85.3	85.3	85.3	1.0
DM (yes, %)	33.1	32.4	33.8	0.86
Current smoking (yes, %)	14.7	14.7	14.7	1.0
Dyslipidemia (yes, %)	86.8	86.8	86.8	1.0
Neopterin (nM)	45.0 (34.7–54.0)	37.2 (30.6–46.1)	51.5 (43.6–57.2)	<0.001
DROM (U. CARR)	361.5 (291.0–396.3)	298.0 (272.0–370.8)	384.0 (348.5–426.0)	<0.001
BNP (pg/mL)	37.2 (17.5–80.9)	28.1 (12.7–39.7)	75.4 (30.3–123.2)	0.005
eGFR (mL/min/1.73 m ²)	64.2 (18.0)	69.1 (14.5)	59.2 (19.8)	<0.001
Hs-CRP (mg/L)	0.4 (0.1–0.8)	0.2 (0.1–0.6)	0.5 (0.2–0.9)	0.15
LVEF (%)	62.4 (5.5)	63.0 (1.0)	61.9 (6.6)	0.23
LVEDVI (mL/m ²)	45.4 (12.0)	43.3 (9.0)	47.6 (14.1)	0.037
LVMI (g/m ²)	126.0 (37.0)	105.1 (18.1)	146.8 (39.4)	<0.001
LVSVI (mL/m ²)	40.2 (7.7)	44.6 (5.7)	35.7 (6.9)	<0.001
E/e'	14.1 (5.0)	11.0 (3.8)	17.2 (4.0)	<0.001
LAVI (mL/m ²)	30.1 (17.5)	24.2 (7.2)	35.9 (22.2)	<0.001
β-blockers (%)	52.2	30.9	73.5	<0.001
ACEI or ARB (%)	55.1	41.2	69.1	0.001
CCB (%)	53.7	54.4	52.9	0.86
HMG-CoA RI (%)	66.9	66.2	67.6	0.86
MR blockers (%)	12.5	5.9	19.1	0.02
Loop diuretics (%)	10.3	4.4	16.2	0.02

ACEI, angiotensin-converting enzyme inhibitors; ARB, angiotensin II receptor blockers; BMI, body mass index; BNP, B-type natriuretic peptide; CAD, coronary artery disease; CCB, calcium channel blockers; DM, diabetes mellitus; DROM, derivatives of reactive oxidative metabolites; E/e', the ratio of early transmitral flow velocity to tissue Doppler early diastolic mitral annular velocity; eGFR, estimated glomerular filtration rate; HF, heart failure; HFpEF, heart failure with preserved left ventricular ejection fraction; HMG-CoA RI, hydroxymethylglutaryl coenzyme A reductase inhibitors; hs-CRP, high-sensitivity C-reactive protein; LAVI, left atrial volume index; LVEDVI, left ventricular end-diastolic volume index; LVEF, left ventricular ejection fraction; LVMI, left ventricular mass index; LVSVI, left ventricular stroke volume index; and MR, mineralocorticoid receptor.

Data are mean (standard deviation), median (25th to 75th percentile range), or number (percentage).

*Compared between risk factor-matched non-HF patients and risk factor-matched HFpEF patients.

Figure 2 Association of neopterin levels with the severity of heart failure. We classified heart failure with preserved left ventricular ejection fraction patients according to the New York Heart Association functional class. Plasma neopterin levels were compared between 37 heart failure with preserved left ventricular ejection fraction patients with New York Heart Association II and 31 heart failure with preserved left ventricular ejection fraction patients with New York Heart Association III/IV.



patients ($P < 0.001$, $r = 0.57$, Figure 3A). In contrast, ln-neopterin values did not have a significant correlation with serum ln-high-sensitivity CRP levels ($P = 0.52$, $r = -0.06$, data not shown). In consideration of neopterin as the oxidized product of 7,8-dihydroneopterin, a pterin synthesized by activated macrophages,⁹ neopterin could be closely associated with ROS in HFpEF, independently of CRP. Moreover, ln-neopterin levels had significant correlation with cardiac diastolic function, indicated by increased LVMI, E/e' , and LAVI, and decreased SVI estimated by ultrasound cardiography ($P = 0.002$, $r = 0.26$, $P < 0.001$, $r = 0.34$, $P < 0.05$, $r = 0.17$, and $P = 0.003$, $r = -0.25$, respectively, Figure 4A–D), and weak

but significant positive correlation with plasma ln-BNP levels ($P < 0.001$, $r = 0.38$, Figure 3D), indicating close association of plasma neopterin with the severity of HFpEF.

During a mean of 23 months' follow-up (range, 1–50 months), 10 cardiovascular events were recorded (cardiovascular death; $n = 2$, acute coronary syndrome; $n = 2$, and hospitalization for HF; $n = 6$). Total cardiovascular events were significantly higher in the high-neopterin group than in the low-neopterin group. Kaplan–Meier analysis showed that the high-neopterin group ($n = 34$) had a higher probability of cardiovascular events than the low-neopterin group ($n = 34$) (log-rank test, $P = 0.003$, Figure 4) (Figure 5).

Discussion

Inflammation has been reported to be one of the major risk factors for the development of HFpEF, and a variety of inflammatory cytokines including interferon- γ and tumour necrosis factor- α (TNF- α) were found to be the most frequent inducers of neopterin synthesis.¹⁰ In the present study, we first demonstrated that plasma neopterin, but not high-sensitivity CRP, among inflammatory markers, was significantly increased by the presence of HFpEF, as well as a few previous reports which showed that blood neopterin levels were closely associated with other cardiovascular diseases. Therefore, it is possible that plasma neopterin levels could serve as more sensitive and specific markers of inflammation in HFpEF than commonly used conventional marker such as high-sensitivity CRP. In inflammatory process, neopterin is produced by activated macrophages via the guanosine triphosphate pathway, and can act as a pro-oxidant and promoting cell apoptosis. Because both oxidative stress and cell apoptosis in failing heart were closely associated with the pathogenesis and progression of HF, neopterin could be involved in the pathophysiology of HFpEF via ROS and apoptosis pathway. Using high salt-loaded DS rats, indeed, useful model of HFpEF, we previously

Figure 3 Correlations of the plasma ln-neopterin levels with serum ln-derivatives of reactive oxidative metabolites levels (A), and ln-B-type natriuretic peptide levels (B), respectively.

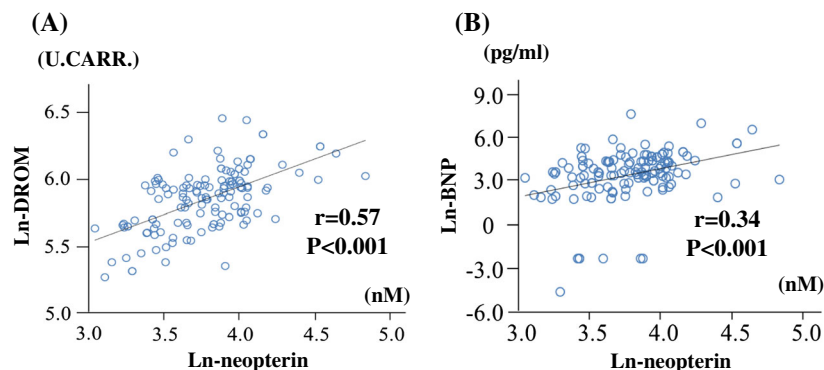


Figure 4 Correlations of the plasma Ln-neopterin levels with left ventricular mass index (A), the ratio of early transmitral flow velocity to tissue Doppler early diastolic mitral annular velocity (E/e') (B), left atrium volume index values (C), and left ventricular stroke volume index levels (D), respectively, estimated by echocardiography.

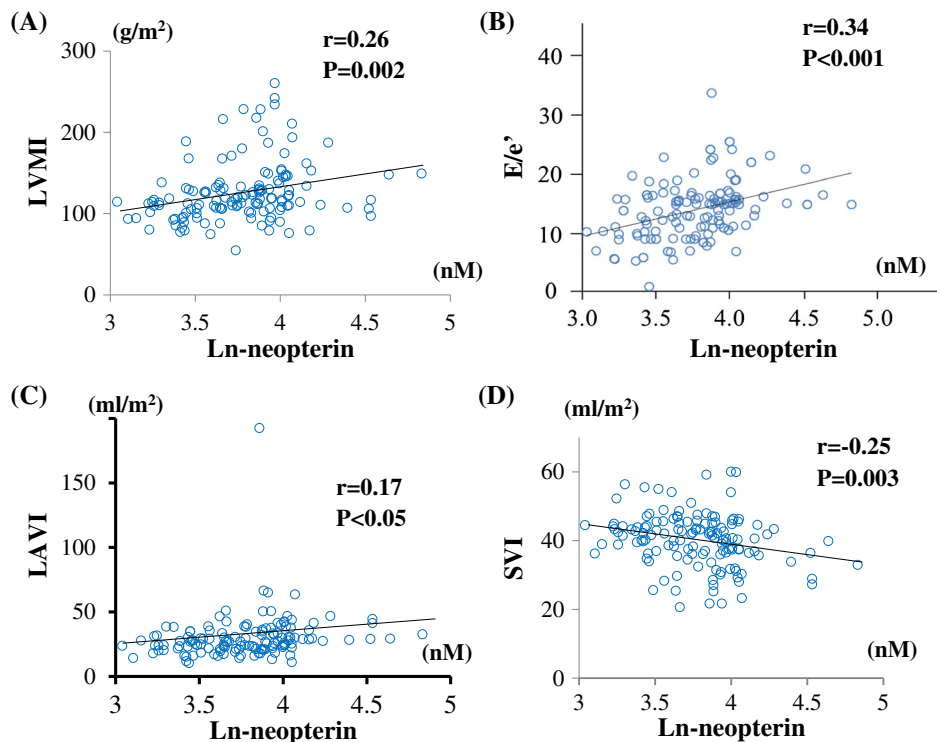
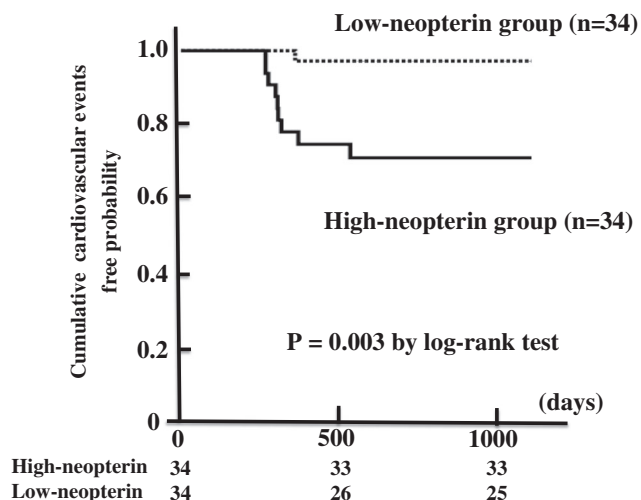


Figure 5 Kaplan–Meier analysis for the probability of cardiovascular events in heart failure with preserved left ventricular ejection fraction patients with low- or high-neopterin. Heart failure with preserved left ventricular ejection fraction patients were divided into two groups using the median value of plasma neopterin level (51.5 nM).



reported that activated macrophages were significantly increased, accompanied by ROS overproduction and myocardial apoptosis in LV.² In this study, plasma neopterin levels were

significantly correlated with the presence and NYHA-assessed severity of HFpEF. Also, the severity of HFpEF, indicated by elevated BNP values and increased LVMI, E/e' , and LAVI, and

decreased SVI by echocardiography, was closely associated with plasma neopterin, suggesting that neopterin might be a marker of not only inflammatory but also HF disease activity in HFpEF patients.

Several studies reported that the interaction between ROS and inflammation is involved in the pathophysiology of cardiovascular diseases, and we recently reported a positive correlation of DROM with high-sensitivity CRP in CAD patients.¹¹ However, the relative relationship between cardiac ROS and inflammation in HFpEF is not fully elucidated. In previous basic research using DS rats, Tian *et al.* showed that oxidative stress interacts with inflammatory cytokines,¹² and we also reported that angiotensin II-induced ROS overproduction directly activated cardiac macrophage infiltration via the activation of apoptosis signal-regulating kinase 1, a ROS-sensitive mitogen-activated protein kinase kinase.¹³ In the present study, we further demonstrated that In-neopterin was significantly correlated with In-DROM in HFpEF patients, which clinically confirmed that the possibility of close interaction of cardiac inflammation and ROS overproduction in the pathophysiology of HFpEF. In consideration of neopterin as pro-oxidant and triggering apoptosis in various cell types,⁹ 'a malignant cycle' between neopterin secretion and ROS production could be deeply involved in the pathophysiology of HFpEF. However, Williams *et al.* showed that this association between CRP and HF events was no longer significant in HFpEF patients, although elevated CRP levels predicted hospitalization for HF.¹⁴ Moreover, the present study showed that high-sensitivity CRP values were not significantly increased in HFpEF patients and correlated with plasma In-neopterin levels. As described above, we previously reported that PTX3, a novel inflammatory marker, TNF- α , and interleukin-6, but not high-sensitivity CRP, were significantly higher in HFpEF patients.³ Taken together these data, the production process of neopterin might be different from that of CRP in HFpEF patients. Hence, further investigations are needed to examine the difference of clinical significance between neopterin and CRP in the pathophysiology of HFpEF, and whether the measurement of a combination of ROS and inflammatory biomarkers can provide useful information for risk stratification of HFpEF patients in clinical practice.

Our study has several limitations. First, it was a single-centre design with a small patient population. Also, this study was observational and was not interventional by any cardiovascular drugs. Therefore, additional interventional studies in HFpEF patients in a large-scale population are necessary. Second, the present study was aimed at patients with HF, all of whom take a variety of cardiovascular agents. Hence, there could be selection biases because enrolled patients to this study had more cardiovascular agents than non-HF patients and plasma neopterin values in HFpEF patients could have been influenced by these medications.

Conclusions

The present clinical study first showed that plasma neopterin levels were significantly increased in HFpEF patients and were associated with serum DROM levels. Also, neopterin levels were significantly higher in HFpEF patients with NYHA III/IV than in those with NYHA II. These data suggested that neopterin is significantly associated with the presence and severity of HFpEF, and that the interaction between inflammation and ROS could be involved in the pathophysiology of HFpEF. Moreover, In-neopterin levels were significantly correlated not only with the In-DROM values but also the severity of HFpEF, indicated by the increase in E/e', LAVI, and In-BNP values. Cardiac diastolic dysfunction is known to be associated with adverse clinical outcome of HFpEF. Hence, this study indicated that the measurement of plasma neopterin levels could contribute to the risk stratification of HFpEF patients. In fact, Kaplan–Meier analysis showed a significantly higher probability of cardiovascular events in HFpEF patients with high neopterin levels than in those with low neopterin levels.

As described above, neopterin is described as the oxidation product of 7,8-dihydroneopterin,⁹ and 7,8-dihydroneopterin is a radical scavenger, thus protecting various cell types including macrophages.¹⁵ Because the significance of neopterin in HFpEF was demonstrated by clinical and basic reports including ours, any interventions to neopterin or/and 7,8-dihydroneopterin have possibilities to be a novel therapeutic strategy for HFpEF. Identification of effective therapeutic strategy for HFpEF has great clinical importance, so it is much required to elucidate whether the intervention to neopterin can improve cardiac diastolic dysfunction and subsequent occurrence of cardiovascular events in HFpEF patients.

Conflict of interest

The authors did not report any conflict of interest relevant to this article.

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References

1. Suleiman M, Khatib R, Agmon Y, Mahamid R, Boulos M, Kapeliovich M, Levy Y, Beyar R, Markiewicz W, Hammerman H, Aronson D. Early inflammation and risk of long-term development of heart failure and mortality in survivors of acute myocardial infarction: predictive role of C-reactive protein. *J Am Coll Cardiol* 2006; **47**: 962–968.
2. Yamamoto E, Kataoka K, Yamashita T, Tokutomi Y, Dong YF, Matsuba S, Ogawa H, Kim-Mitsuyama S. Role of xanthine oxidoreductase in reversal of diastolic heart failure by candesartan in salt-sensitive hypertensive rat. *Hypertension* 2007; **50**: 657–662.
3. Matsubara J, Sugiyama S, Nozaki T, Sugamura K, Konishi M, Ohba K, Matsuzawa Y, Akiyama E, Yamamoto E, Sakamoto K, Nagayoshi Y, Kaikita K, Sumida H, Kim-Mitsuyama S, Ogawa H. Pentraxin 3 is a new inflammatory marker correlated with left ventricular diastolic dysfunction and heart failure with normal ejection fraction. *J Am Coll Cardiol* 2011; **57**: 861–869.
4. Giese SP, Crone EM, Flavall EA, Amit Z. Potential to inhibit growth of atherosclerotic plaque development through modulation of macrophage neopterin/7,8-dihydroneopterin synthesis. *Br J Pharmacol* 2008; **153**: 627–635.
5. Nazer B, Ray KK, Sloan S, Scirica B, Morrow DA, Cannon CP, Braunwald E. Prognostic utility of neopterin and risk of heart failure hospitalization after an acute coronary syndrome. *Eur Heart J* 2011; **32**: 1390–1397.
6. Paulus WJ, Tschöpe C, Sanderson JE, Rusconi C, Flachskampf FA, Rademakers FE, Marino P, Smiseth OA, De Keulenaer G, Leite-Moreira AF, Borbély A, Edes I, Handoko ML, Heymans S, Pezzali N, Pieske B, Dickstein K, Fraser AG, Brutsaert DL. How to diagnose diastolic heart failure: a consensus statement on the diagnosis of heart failure with normal left ventricular ejection fraction by the Heart Failure and Echocardiography Associations of the European Society of Cardiology. *Eur Heart J* 2007; **28**: 2539–2550.
7. Carru C, Zinellu A, Sotgia S, Serra R, Usai MF, Pintus GF, Pes GM, Deiana L. A new HPLC method for serum neopterin measurement and relationships with plasma thiols levels in healthy subjects. *Biomed Chromatogr* 2004; **18**: 360–366.
8. Hirata Y, Yamamoto E, Tokitsu T, Kusaka H, Fujisue K, Kurokawa H, Sugamura K, Maeda H, Tsujita K, Yamamuro M, Kaikita K, Hokimoto S, Sugiyama S, Ogawa H. Reactive oxidative metabolites are associated with the severity of heart failure and predict future cardiovascular events in heart failure with preserved left ventricular ejection fraction. *Int J Cardiol* 2015; **179**: 305–308.
9. Schobersterger W, Hoffmann G, Hobisch-Hagen P, Bock G, Volkl H, Baier-Bitterlich G, Wirleitner B, Wachter H, Fuchs D. Neopterin and 7,8-dihydroneopterin induce apoptosis in the rat alveolar epithelial cell line L2. *FEBS Lett* 1996; **397**: 263–268.
10. Cospér PF, Harvey PA, Leinwand LA. Interferon- γ causes cardiac myocyte atrophy via selective degradation of myosin heavy chain in a model of chronic myocarditis. *Am J Pathol* 2012; **181**: 2038–2046.
11. Hirata Y, Yamamoto E, Tokitsu T, Kusaka H, Fujisue K, Kurokawa H, Sugamura K, Maeda H, Tsujita K, Kaikita K, Hokimoto S, Sugiyama S, Ogawa H. Reactive oxygen metabolites are closely associated with the diagnosis and prognosis of coronary artery disease. *J Am Heart Assoc* 2015; **4**: e001451. doi: 10.1161/JAHA.114.001451
12. Tian N, Moore RS, Braddy S, Rose RA, Gu JW, Hughson MD, Manning RD Jr. Interactions between oxidative stress and inflammation in salt-sensitive hypertension. *Am J Physiol Heart Circ Physiol* 2007; **293**: H3388–H3395.
13. Yamamoto E, Kataoka K, Shintaku H, Yamashita T, Tokutomi Y, Dong YF, Matsuba S, Ichijo H, Ogawa H, Kim-Mitsuyama S. Novel mechanism and role of; angiotensin II-induced vascular endothelial injury in hypertensive diastolic heart failure. *Arterioscler Thromb Vasc Bio* 2007; **27**: 2569–2575.
14. Williams ES, Shah SJ, Ali S, Na BY, Schiller NB, Whooley MA. C-reactive protein, diastolic dysfunction, and risk of heart failure in patients with coronary disease: Heart and Soul Study. *Eur J Heart Fail* 2008; **10**: 63–69.
15. Giese SP, Duggan S, Rait C, Platt A. Protein and thiol oxidation in cells exposed to peroxyl radicals, is inhibited by the macrophage synthesised pterin 7,8-dihydroneopterin. *Biochim Biophys Acta* 2002; **1591**: 139–145.