Impact of plasma xanthine oxidoreductase activity in patients with heart failure with preserved ejection fraction

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Abstract

Aims Reactive oxygen species are reportedly involved in the mechanism underlying heart failure with preserved ejection fraction (HFrEF); however, the disease pathophysiology remains poorly understood. Xanthine oxidoreductase (XOR), the rate-limiting enzyme of purine metabolism, plays an important role in uric acid production and generates reactive oxygen species. However, the impact of plasma XOR activity on the clinical outcomes of patients with HFrEF remains unclear. The aim of this study was to investigate whether plasma XOR activity is associated with major adverse cardiovascular events (MACEs) in patients with HFrEF.

Methods and results The plasma XOR activity was measured in 257 patients with HFrEF, who were then divided into three groups according to the activity levels: low XOR group (<33 pmol/h/mL, n = 45), normal XOR group (33–120 pmol/h/mL, n = 160), and high XOR group (>120 pmol/h/mL, n = 52). During the median follow-up period of 809 days, there were 74 MACEs. Kaplan–Meier analysis revealed that the high XOR group was at the highest risk for MACEs. Multivariate analysis by Cox’s proportional hazard regression approach showed that high XOR activity was significantly associated with MACEs, after adjustment for confounding factors. The patients were also divided into four groups according to the absence/presence of high XOR activity and/or hyperuricaemia. According to the multivariate Cox regression analysis, high XOR activity was associated with MACEs, regardless of the hyperuricaemia status.

Conclusions Elevated plasma XOR activity is significantly associated with adverse clinical outcomes in patients with HFrEF.

Keywords Xanthine oxidoreductase; Heart failure with preserved ejection fraction

Introduction

The increasing proportion of patients with heart failure with preserved ejection fraction (HFrEF) relative to that of patients with heart failure with reduced ejection fraction (HFrEF) has been recognized as a public health problem.1,2 In contrast to HFrEF, an effective evidence-based therapy for HFrEF has not been established.3 It was reported that the mortality rates were similar between patients with HFrEF and those with HFrEF.4

Although the pathophysiology of HFrEF remains incompletely understood, coexisting systemic pro-inflammatory conditions, such as hypertension, diabetes mellitus, obesity, and the metabolic syndrome, are thought to contribute to its development.5 Induced systemic microvascular endothelial inflammation results in the production of reactive oxygen species (ROS) and subsequently endothelial dysfunction. In HFrEF, ROS decrease both the bioavailability of nitric oxide in coronary microvascular endothelial cells and the activity
of protein kinase G activity in adjacent cardiomyocytes, causing left ventricular (LV) dysfunction.6

Xanthine oxidoreductase (XOR), the rate-limiting enzyme of purine metabolism, plays a pivotal role in producing uric acid (UA) production and generates ROS.7 It has been shown that UA itself causes inflammation and ROS generation in endothelial cells.8 Although a previous study has shown that hyperuricaemia is strongly associated with adverse clinical outcomes in patients with HFP EF,9 the association between XOR activity and clinical outcomes in these patients remains unclear. Therefore, the aim of this study was to investigate whether plasma XOR activity is associated with cardiovascular events in patients with HFP EF.

**Methods**

**Study subjects**

The study subjects were made up of 257 patients with HFP EF, who were admitted to Yamagata University Hospital for the diagnosis and/or treatment of heart failure (HF). The diagnosis of HFP EF was based on the Framingham criteria, that is, the clinical diagnosis of HF according to two cardiologists and the echocardiographic finding of an LV ejection fraction of ≥50%.10,11 Patients with acute coronary syndrome (ACS) within 3 months preceding admission, active hepatic diseases, pulmonary diseases, and malignant diseases were excluded. The major risk factors of HF, such as hypertension, dyslipidaemia, diabetes mellitus, obesity, and smoking (both current and past smokers), were assessed. Clinical data on age, gender, and New York Heart Association (NYHA) functional class were obtained from the patients’ medical records or history of medical therapy, and medications were assessed at discharge. The investigation conforms with the principles outlined in the Declaration of Helsinki. The study protocol was approved by the Institutional Ethics Committee of Yamagata University School of Medicine, and all patients provided their written informed consent for study participation.

**Xanthine oxidoreductase activity assay**

Blood samples were collected in the early morning, within 24 h after hospital admission. The samples were centrifuged at 3000 g for 15 min at 4 °C, and the obtained plasma was stored at −80 °C until analysis. The XOR activity assay was performed using a stable isotope-labelled substrate and liquid chromatography–triple quadrupole mass spectrometry (Sanwa Kagaku Kenkyusho Co., Ltd., Mie, Japan).12 The patients were divided into three XOR groups according to their levels of XOR activity: low XOR group (<33 pmol/h/mL, n = 45), normal XOR group (33–120 pmol/h/mL, n = 160), and high XOR group (>120 pmol/h/mL, n = 52).13

Additionally, to assess the clinical impact of the co-morbidity of hyperuricaemia with high XOR activity on adverse outcomes in patients with HFP EF, the patients were divided into four groups according to the presence of high XOR activity and/or hyperuricaemia. Hyperuricaemia was defined as a serum UA levels of >7 mg/dL in both genders, according to the Japanese guidelines for the management of hyperuricaemia and gout.14

**Endpoint and follow-up period**

All patients were prospectively followed up for a median period of 809 days (inter-quartile range, 458–1444 days). The endpoints were major adverse cardiovascular events (MACEs), namely, rehospitalization for HF, ACS, and cardiac death (defined as death due to progressive HF, ACS, or sudden cardiac death).

**Statistical analysis**

The results are expressed as the mean ± standard deviation (SD) for continuous variables and percentages for categorical variables. Skewed values are presented as the median and inter-quartile range. The correlation between plasma XOR activity and UA level was analysed by single linear regression analysis. The t-test and χ² test were used to compare the continuous and categorical variables, respectively. If the data were not normally distributed, the Mann–Whitney U test was employed. Differences among groups were analysed by analysis of variance. Cox’s proportional hazard analysis was used to determine the independent predictors for MACEs. Significant variables from the univariate analysis were then entered into the multivariate analysis. Event-free survival curves were constructed according to the Kaplan–Meier method and compared using log-rank tests. Receiver operating characteristic curves for the MACEs were constructed and used as a measure of the predictive accuracy of plasma XOR activity for such events. In addition, the net reclassification index (NRI) and integrated discrimination index (IDI) were calculated to determine the quality of improvement of the corrected reclassification following the addition of plasma XOR activity to the baseline model, which was based on the age, NYHA functional class, UA, estimated glomerular filtration rate (eGFR), log brain natriuretic peptide (BNP), and loop diuretics use variables. P values of <0.05 were considered statistically significant. All statistical analyses were performed with a standard software package (JMP Version 12, SAS Institute, Cary, NC, USA; EZR, Saitama Medical Center, Jichi Medical University, Shimotsuke, Japan).

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Results

Comparisons of clinical characteristics among the xanthine oxidoreductase groups

The baseline characteristics of the patients according to the XOR groups are shown in Table 1. Of the 257 patients eligible for study inclusion, 45 had low XOR activity, 160 had normal XOR activity, and 52 had high XOR activity. Patients in the high XOR group were younger and had higher body mass index values than the patients in the other groups. Patients in the low XOR group had a more severe NYHA functional class, lower levels of eGFR and haemoglobin, and a higher use of loop diuretics than the individuals in the other groups. There were no significant differences among the three groups in terms of gender, prevalence of hypertension, dyslipidaemia, diabetes mellitus, and atrial fibrillation, and echocardiographic parameters. The UA levels tended to be higher in the high XOR group, but the difference did not reach statistical significance. However, as shown in Figure 1, there was a weak positive correlation between the XOR activity and UA levels ($R = 0.147$, $P = 0.018$). Because UA levels can be affected by the reduction in renal function and the use of diuretics, we performed the analysis by excluding the patients with chronic kidney disease Stages 3b–5 (eGFR < 45 mL/min/1.73 m$^2$) or high-dose ($\geq 40$ mg/day) loop diuretics use. There was no significant correlation between the XOR activity and UA levels ($R = 0.129$, $P = 0.077$; Supporting Information, Figure S1).

Table 1. Comparison of clinical characteristics among three groups based on XOR activity

| Variables                      | Low XOR (n = 45) | Normal XOR (n = 160) | High XOR (n = 52) | $P$ value |
|--------------------------------|----------------|---------------------|------------------|----------|
| Age (years)                    | 78 ± 10        | 71 ± 11             | 69 ± 12          | <0.001   |
| Male, n (%)                    | 21 (47)        | 93 (58)             | 28 (54)          | 0.385    |
| BMI (kg/m$^2$)                 | 20.2 ± 3.4     | 22.0 ± 4.0          | 23.1 ± 4.4       | 0.015    |
| Hypertension, n (%)            | 35 (78)        | 121 (76)            | 37 (71)          | 0.734    |
| Dyslipidaemia, n (%)           | 14 (31)        | 33 (21)             | 14 (27)          | 0.296    |
| Diabetes mellitus, n (%)       | 8 (18)         | 38 (24)             | 15 (29)          | 0.436    |
| Atrial fibrillation, n (%)     | 26 (58)        | 86 (54)             | 35 (67)          | 0.222    |
| NYHA III-IV, n (%)             | 24 (53)        | 49 (31)             | 19 (37)          | 0.022    |
| **Echocardiographic data**     |                |                     |                  |          |
| IHD/VHD/DCM/Others             | 7/11/11/16     | 23/46/18/73         | 14/13/3/22       | 0.085    |
| **Blood examination**          |                |                     |                  |          |
| eGFR (mL/min/1.73 m$^2$)       | 53.7 ± 24.3    | 69.1 ± 22.1         | 66.8 ± 22.1      | <0.001   |
| UA (mg/dL)                     | 6.0 ± 2.4      | 6.1 ± 2.1           | 6.7 ± 2.1        | 0.161    |
| Hb (g/dL)                      | 10.9 ± 1.8     | 12.1 ± 2.0          | 12.8 ± 2.3       | <0.001   |
| BNP (pg/mL)                    | 361 (190–635)  | 184 (86–484)        | 267 (111–698)    | 0.079    |
| **Medications**                |                |                     |                  |          |
| ACEIs and/or                   | 29 (64)        | 108 (68)            | 34 (65)          | 0.912    |
| ARBs, n (%)                    | 28 (62)        | 85 (53)             | 29 (56)          | 0.551    |
| Beta-blockers, n (%)           | 33 (73)        | 78 (49)             | 27 (52)          | 0.011    |
| Loop diuretics, n (%)          | 8 (27)         | 11 (12)             | 3 (10)           | 0.127    |
| XOR inhibitors, n (%)          | 10 (22)        | 25 (16)             | 9 (17)           | 0.598    |

ACEIs, angiotensin-converting enzyme inhibitors; ARBs, angiotensin II receptor blockers; BMI, body mass index; BNP, brain natriuretic peptide; DCM, dilated cardiomyopathy; eGFR, estimated glomerular filtration rate; Hb, haemoglobin; IHD, ischaemic heart disease; LAD, left atrial diameter; LVEDD, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association; UA, uric acid; VHD, valvular heart disease; XOR, xanthine oxidoreductase.

Data are expressed as mean ± standard deviation, n (%), or median (inter-quartile range).

Plasma xanthine oxidoreductase activity and major adverse cardiovascular events in patients with heart failure with preserved ejection fraction

During the follow-up period, there were 74 MACEs, namely, 39 rehospitalizations for HF, 14 ACS, and 21 cardiac deaths. As shown in Figure 2A, Kaplan–Meier analysis revealed that the high XOR group had the highest risk for MACEs, whereas there was no difference in the occurrence of these events between the low and normal XOR groups. Similarly, Supporting Information, Figure S2A showed that the high XOR group had higher risk for MACEs than the other groups, even if excluding the patients with lower levels of eGFR or the use of diuretics.

Both univariate and multivariate Cox proportional hazard regression analyses were conducted to identify predictors of MACEs in patients with HfPEF. The univariate analysis revealed that the plasma XOR activity was significantly
associated with MACEs in these patients [hazard ratio (HR), 1.320 per 1 SD increase; 95% confidence interval (CI), 1.134–1.498; \( P < 0.001 \)]. Furthermore, the patients’ age, NYHA functional class, eGFR, BNP, UA level, and use of loop diuretics were also related to MACEs (Table 2). The multivariate analysis showed that the plasma XOR activity was an independent risk factor for MACEs after adjustment for confounding factors (HR, 1.253 per 1 SD increase; 95% CI, 1.079–1.454; \( P = 0.003 \); Table 2). By contrast, the UA levels were not an independent predictor of MACEs (Table 2). In addition, the multivariate analysis revealed that the high XOR group was at a higher risk for MACEs after adjustments for age, NYHA functional class, eGFR, log BNP, UA level, and loop diuretics use compared with the low XOR group (HR, 3.6; 95% CI, 1.683–8.120; \( P < 0.001 \)) and normal XOR group (HR, 3.3; 95% CI, 1.923–5.455; \( P < 0.001 \); Figure 2B). These results were more prominent for the patients without renal failure or diuretics use (Supporting Information, Figure S2B).

**Impact of hyperuricaemia on clinical outcomes in patients with heart failure with preserved ejection fraction**

To clarify the impact of the co-morbidity of hyperuricaemia with high XOR activity on the clinical outcomes of patients with HFpEF, the patients were divided into four groups according to the absence (−) or presence (+) of high XOR activity and/or hyperuricaemia: (i) high XOR activity (−) and hyperuricaemia (−), \( n = 146 \); (ii) high XOR activity (−) and hyperuricaemia (+), \( n = 59 \); (iii) high XOR activity (+) and hyperuricaemia (−), \( n = 31 \); and (iv) high XOR activity (+) and hyperuricaemia (+), \( n = 21 \). As shown in Table 3, regardless of the high XOR activity status, patients with hyperuricaemia had lower levels of eGFR and higher uses of loop diuretics and XOR inhibitors than those without hyperuricaemia. The group with the high XOR activity and hyperuricaemia had the highest prevalence of atrial fibrillation and tended to have higher BNP levels than the other groups. There were no significant differences among the four groups in terms of the prevalence of hypertension, diabetes mellitus, and dyslipidaemia, and NYHA functional class.

Kaplan–Meier analysis showed that the group with the co-morbidity had the highest risk for MACEs (Figure 3A). As shown in Figure 3B, patients with hyperuricaemia but without high XOR activity tended to have a high risk for MACEs, albeit the difference did not reach statistical significance. By contrast, the patients with high XOR activity had a higher risk for MACEs than those without high XOR activity, regardless of whether hyperuricaemia was present or not. These results were similar, even if excluding the patients with lower levels of eGFR or diuretics use (Supporting Information, Figure S3A and S3B).

**Fig 1**  Correlation between plasma xanthine oxidoreductase (XOR) activity and serum uric acid.

**Fig 2**  Impact of xanthine oxidoreductase (XOR) activity on clinical outcomes in heart failure with preserved ejection fraction. (A) Kaplan–Meier curves for major adverse cardiovascular events based on XOR activity. (B) Multivariate Cox regression analysis for predicting major adverse cardiovascular events in patients with heart failure with preserved ejection fraction.
Table 3  Comparison of clinical characteristics among four groups according to the presence of high XOR activity and/or hyperuricaemia

| Variables                                      | High XOR (−) and hyperuricaemia (−) (n = 146) | High XOR (−) and hyperuricaemia (+) (n = 59) | High XOR (+) and hyperuricaemia (−) (n = 31) | High XOR (+) and hyperuricaemia (+) (n = 21) | P value |
|------------------------------------------------|-----------------------------------------------|----------------------------------------------|-----------------------------------------------|-----------------------------------------------|---------|
| Age (years)                                     | 72 ± 11                                       | 74 ± 10                                      | 69 ± 10                                       | 68 ± 14                                       | 0.088   |
| Male, n (%)                                      | 74 (51)                                       | 40 (68)                                      | 14 (45)                                       | 14 (67)                                       | 0.058   |
| BMI (kg/m²)                                      | 21.8 ± 4.1                                    | 21.3 ± 3.8                                   | 23.3 ± 4.6                                    | 22.9 ± 4.4                                    | 0.222   |
| Hypertension, n (%)                              | 112 (77)                                      | 44 (75)                                      | 19 (61)                                       | 18 (86)                                       | 0.212   |
| Dyslipidaemia, n (%)                             | 37 (25)                                       | 10 (17)                                      | 9 (29)                                        | 5 (24)                                        | 0.516   |
| Diabetes mellitus, n (%)                         | 37 (25)                                       | 9 (15)                                       | 8 (26)                                        | 7 (33)                                        | 0.278   |
| Atrial fibrillation, n (%)                       | 73 (50)                                       | 39 (66)                                      | 18 (58)                                       | 17 (81)                                       | 0.016   |
| NYHA III–IV, n (%)                               | 47 (32)                                       | 26 (44)                                      | 12 (39)                                       | 7 (33)                                        | 0.437   |
| Aetiology                                        | IHD/VHD/DCM/Others                            | 21/36/26/63                                  | 9/21/3/26                                     | 8/8/2/13                                      | 0.127   |
| Echocardiographic data                           |                                              |                                              |                                              |                                              |         |
| LVEDD (mm)                                       | 50 ± 9                                        | 50 ± 9                                       | 49 ± 6                                        | 49 ± 7                                        | 0.882   |
| LVEF (%)                                         | 64 ± 9                                        | 66 ± 9                                       | 63 ± 10                                       | 64 ± 8                                        | 0.481   |
| LAD (mm)                                         | 44 ± 9                                        | 45 ± 10                                      | 45 ± 6                                        | 49 ± 9                                        | 0.245   |
| Blood examination                               |                                              |                                              |                                              |                                              |         |
| eGFR (mL/min/1.73 m²)                            | 69.1 ± 22.0                                   | 57.3 ± 25.0                                  | 70.8 ± 20.7                                   | 61.0 ± 23.4                                   | 0.004   |
| UA (mg/dL)                                       | 5.0 ± 1.2                                     | 8.6 ± 1.9                                    | 5.2 ± 1.0                                     | 8.9 ± 1.5                                     | <0.001  |
| Hb (g/dL)                                        | 12.0 ± 2.0                                    | 11.6 ± 2.1                                   | 12.5 ± 2.1                                    | 13.4 ± 2.5                                    | 0.005   |
| BNP (pg/mL)                                      | 217 (80–477)                                  | 283 (105–769)                                | 238 (73–1492)                                 | 579 (172–1766)                                | 0.091   |
| Medications                                      |                                              |                                              |                                              |                                              |         |
| ACEIs and/or ARBs, n (%)                         | 99 (68)                                       | 38 (64)                                      | 17 (55)                                       | 17 (81)                                       | 0.240   |
| Beta-blockers, n (%)                             | 85 (58)                                       | 28 (47)                                      | 15 (48)                                       | 14 (67)                                       | 0.298   |
| Loop diuretics, n (%)                            | 70 (48)                                       | 41 (69)                                      | 12 (39)                                       | 15 (71)                                       | 0.004   |
| XOR inhibitors, n (%)                            | 5 (4)                                         | 16 (29)                                      | 1 (3)                                         | 4 (19)                                        | <0.001  |
| Statins, n (%)                                   | 27 (18)                                       | 8 (14)                                       | 7 (23)                                        | 2 (10)                                        | 0.505   |

ACEIs, angiotensin-converting enzyme inhibitors; ARBs, angiotensin II receptor blockers; BMI, body mass index; BNP, brain natriuretic peptide; DCM, dilated cardiomyopathy; eGFR, estimated glomerular filtration rate; Hb, haemoglobin; IHD, ischaemic heart disease; LAD, left atrial diameter; LVEDD, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association; UA, uric acid; VHD, valvular heart disease; XOR, xanthine oxidoreductase.

Data are expressed as mean ± standard deviation, n (%), or median (inter-quartile range).
Improvement of the prognostic value by the addition of plasma xanthine oxidoreductase activity to the baseline model

To investigate whether the model fit and discrimination were improved by the addition of plasma XOR activity to the baseline model, the improvements of the C index, NRI, and IDI were evaluated. The baseline model takes the patients’ age, NYHA functional class, UA level, eGFR, log BNP, and use of loop diuretics into account. The addition of XOR activity to the baseline model significantly improved the C index (0.710 vs. 0.758, P = 0.017; Table 4), as well as the NRI (0.499; 95% CI, 0.244–0.754; P < 0.001) and IDI (0.062; 95% CI, 0.024–0.100; P = 0.002) (Table 4).

Discussion

Main findings

The main findings of the present study were as follows: (i) high XOR activity was an independent risk factor for MACEs in patients with HFP EF, whereas low XOR activity was not associated with poor clinical outcomes; (ii) high XOR activity was significantly associated with MACEs, regardless of whether hyperuricaemia was present or not; and (iii) the prediction model with XOR activity included improved the prognostic value for patients with HFP EF.

Impact of xanthine oxidoreductase activity and uric acid levels on clinical outcomes in heart failure with preserved ejection fraction

Impaired LV relaxation and diastolic dysfunction are reported to be involved in the genesis of symptoms in patients with HFP EF.15,16 Previous studies have shown that endothelial dysfunction is related to the progression of LV diastolic dysfunction.17–19 Coronary microvascular endothelial dysfunction is thought to play a key role in the pathophysiology of HFP EF. Furthermore, systemic endothelial dysfunction contributes to an increase in peripheral arterial stiffness and generates an earlier wave reflection, which can augment the central blood pressure and lead to the development of HFP EF.20

Xanthine oxidoreductase exists in two interconvertible forms: xanthine dehydrogenase (XDH) and xanthine oxidase (XO). Whereas XDH reacts preferentially with nicotinamide adenine dinucleotide, XO consumes molecular oxygen and thus generates ROS, such as the superoxide anion (O2•−).21 In the inflammatory state, XDH is induced in endothelial cells

Table 4 Statistics for model fit and improvement with the addition of XOR activity on the prediction of major adverse cardiovascular events

|                  | C index (P value) | NRI (95% CI, P value) | IDI (95% CI, P value) |
|------------------|------------------|-----------------------|-----------------------|
| Baseline model   | 0.710            | Reference             | Reference             |
| + XOR activity   | 0.758 (P = 0.017)| 0.499 (0.244–0.754, P < 0.001) | 0.062 (0.024–0.100, P = 0.002) |

IDI, integrated discrimination index; NRI, net reclassification index; CI, confidence interval; XOR, xanthine oxidoreductase. Baseline model includes age, New York Heart Association functional class, log brain natriuretic pepti, estimated glomerular filtration rate, uric acid, and loop diuretics use.
and released to the circulation. The circulating XDH is then rapidly converted to XO by either reversible sulfhydryl oxidation or irreversible proteolytic modification.\textsuperscript{22} XO-derived O\textsubscript{2} can react with nitric oxide, producing peroxynitrate (ONOO\textsuperscript{−}), which is a strong oxidizing mediator of endothelial cell injury.\textsuperscript{23} In addition, ONOO\textsuperscript{−} has the potential to convert XDH into XO, leading to a further increase in ROS generation.\textsuperscript{24} We had previously demonstrated the association between plasma XOR activity and coronary artery spasm, which is thought to be related to endothelial dysfunction.\textsuperscript{25} Furthermore, the plasma XOR activity was associated with the severity of coronary artery spasm, being increased with a higher disease severity. Thus, XOR-derived ROS might be one of the causes of endothelial dysfunction and subsequent HFpEF development.

It has been reported that UA transporters are expressed in both renal tubular cells and endothelial cells.\textsuperscript{8} Despite the existing controversy over whether UA causes endothelial dysfunction, many previous studies have demonstrated that patients with hyperuricaemia also had dysfunction of the endothelium.\textsuperscript{26–28} Furthermore, it has been demonstrated that hyperuricaemia was associated with the incidence of HF in patients with arterial hypertension and of MACEs in patients with HFpEF.\textsuperscript{9,29} However, the present study showed that high XOR activity—but not hyperuricaemia—was an independent risk factor for MACEs in patients with HFpEF. Remarkably, it was reported that UA levels are associated with poor clinical outcomes in patients with HF without chronic kidney disease, because hyperuricaemia represents increased XOR activity.\textsuperscript{30} There is the possibility that the contribution of UA to the clinical outcomes of HF partially reflects that of the XOR activity. Considering these results, it can be presumed that the XOR activity rather than UA contributes to the pathophysiology of HFpEF. By contrast, some previous studies reported that UA is an inhibitor of XOR.\textsuperscript{31,32} Because UA is known to have an antioxidative effect and be protective against ROS, the contribution of UA to cardiovascular disease is controversial.\textsuperscript{33}

**Clinical implications**

Although XOR inhibitors have been reported to improve endothelial function in patients with chronic HF,\textsuperscript{34,35} their potential effects in patients with HFpEF have not been elucidated. We had previously reported that both low and high XOR activities were significantly associated with adverse clinical outcomes in patients with chronic HF, including HFpEF and HFrEF.\textsuperscript{13} However, in the present study, low XOR activity was not associated with MACEs in patients with HFpEF. Givertz et al.\textsuperscript{36} demonstrated that XOR inhibitors failed to improve clinical outcomes in patients with symptomatic HF. Those authors had enrolled patients who had LV ejection fraction levels of ≤40\% and serum UA levels of ≥9.5 mg/dL. In addition, most of the patients had used a high dose of diuretics (median furosemide equivalent dose of 120 mg/day) and had a co-morbidity of chronic kidney disease. As the XOR activity was not evaluated in that study, patients with low XOR activity in spite of a high UA levels might have been included. There is a possibility that XOR inhibitors become less effective or harmful to such patients.

Xanthine oxidoreductase inhibition has been demonstrated to improve endothelial function by reducing ROS, but not by lowering the UA level.\textsuperscript{37} Considering that high (but not low) XOR activity was found to be associated with MAC es in patients with HFpEF, XOR inhibitors could have beneficial effects in improving clinical outcomes in HFpEF if administered to patients with high XOR activity. Further investigations are needed to determine whether XOR inhibitors are effective for the treatment of HFpEF.

**Limitations**

The current study had several limitations. First, as this study involved only a single centre and had a relatively small sample size, the generalizability of our results is limited. The proportion of patients with normal XOR activity was larger than those with abnormal XOR activities, and unbalanced sample size of those groups can affect statistical analysis. Second, because we did not assess directly XOR activity directly in coronary endothelial cells, we could not determine the direct contribution of endothelial XOR to coronary endothelial dysfunction. Finally, because we did not measure the endothelial function in the patients, we could not evaluate the association between coronary endothelial function and the pathophysiology of HFpEF.

**Conclusions**

This study revealed that elevated plasma XOR activity is significantly associated with adverse clinical outcomes in patients with HFpEF. Therefore, the levels of plasma XOR activity can be used to predict cardiovascular events in patients with this disease.

Elevated plasma XOR activity was significantly associated with adverse clinical outcomes in patients with HFpEF.

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Conflict of interest

None declared.

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References

1. Steinberg BA, Zhao X, Heidenreich PA, Peterson ED, Bhatt DL, Cannon CP, Hernandez AF, Fonarow GC. Trends in patients hospitalized with heart failure and preserved left ventricular ejection fraction: prevalence, therapies, and outcomes. Circulation 2012; 126: 65–75.

2. Owan TE, Hodge DO, Herges RM, Jacobsen SJ, Roger VL, Redfield MM. Trends in prevalence and outcome of heart failure with preserved ejection fraction. N Engl J Med 2006; 355: 251–259.

3. Senni M, Paulus WJ, Gavazzi A, Fraser AG, Diez J, Solomon SD, Smiseth OA, Guazzi M, Lam CS, Maggioni AP, Tsuche C, Metra M, Hummel SL, Edelmann F, Ambrosio G, Stewart Coats AJ, Filippatos GS, Gheorghiade M, Anker SD, Levy D, Pfeffer MA, Stough WG, Pieske BM. New strategies for heart failure with preserved ejection fraction: the importance of targeted therapies for heart failure phenotypes. Eur Heart J 2014; 35: 2797–2815.

4. Bhatia RS, Tu JV, Lee DS, Austin PC, Fang J, Haozzi A, Gong Y, Liu PP. Outcome of heart failure with preserved ejection fraction in a population-based study. N Engl J Med 2006; 355: 260–269.

5. Redfield MM. Heart failure with preserved ejection fraction. N Engl J Med 2016; 375: 1868–1877.

6. Paulus WJ, Tsuche C. A novel paradigm for heart failure with preserved ejection fraction: comorbidities drive myocardial dysfunction and remodeling through coronary microvascular endothelial inflammation. J Am Coll Cardiol 2013; 62: 263–271.

7. Battelli MG, Bolognesi A, Polito L. Pathophysiology of circulating xanthine oxidoreductase: new emerging roles for a multi-tasking enzyme. Biochim Biophys Acta 2014; 1842: 1502–1517.

8. Maruhashi T, Hisatome I, Kihara Y, Higashi Y. Hyperuricemia and endothelial function: from molecular background to clinical perspectives. Atherosclerosis 2018; 278: 226–231.

9. Palazzuoli A, Ruocco G, De Vivo O, Nuti R, McCullough PA. Prevalence of hyperuricemia in patients with acute heart failure with either reduced or preserved ejection fraction. Am J Cardiol 2017; 120: 1146–1150.

10. McKee PA, Castelli WP, McNamara PM, Kannel WB. The natural history of congestive heart failure: the Framingham study. N Engl J Med 1971; 285: 1441–1446.

11. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JGF, Coats AJS, Falk V, Gonzalez-Juanatey JR, Harjola VP, Jankowska EA, Jessup M, Linde C, Nihoyannopoulos P, Parissis JT, Pieske B, Riley JP, Rosano GMC, Ruilope LM, Ruschitzka F, Rutten FH, van der Meer P. 2016 ESC guidelines for the diagnosis and treatment of acute and chronic heart failure: the Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure of the ESC. Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. Eur Heart J 2016; 37: 2129–2200.

12. Murase T, Nampei M, Oka M, Miyachi A, Nakamura T. A highly sensitive assay of human plasma xanthine oxidoreductase activity using stable isotope-labeled xanthine and LC/TQMS. J Chromatogr B Analyt Technol Biomed Sci 2016; 1039: 51–58.

13. Otaki Y, Watanabe T, Kinoshita D, Yokoyama M, Takahashi T, Toshima T, Sugai T, Murase T, Nakamura T, Nishiyama S, Takahashi H, Arimoto T, Shishido T, Miyamoto T, Kubota I. Association of plasma xanthine oxidoreductase activity with severity and clinical outcome in patients with chronic heart failure. Int J Cardiol 2017; 228: 151–157.

14. Yamanaka H. Japanese guideline for the management of hyperuricemia and gout: second edition. Nucleosides Nucleotides Nucleic Acids 2011; 30: 1018–1029.

15. Lam CS, Roger VL, Rodeheffer RJ, Bursi F, Borlaug BA, Ommen SR, Kass DA, Redfield MM. Cardiac structure and ventricular-vascular function in persons with heart failure and preserved ejection fraction from Olmsted County. Minnesota Circulation 2007; 115: 1982–1990.

16. Borlaug BA, Jaber WA, Ommen SR, Lam CS, Redfield MM, Nishimura RA. Diastolic relaxation and compliance reserve during dynamic exercise in heart failure with preserved ejection fraction. Heart 2011; 97: 964–969.

17. Sorop O, Heinonen I, van Kranenburg M, van de Wouw J, de Beer VJ, Nguyen ITN, Ocavio Y, van Duin RWB, Stam R, van Geuns RJ, Wielopolski PA, Krestin GP, van den Meiracker AH, Verjans R, van Bilzen M, Danser AHJ, Paulus WJ, Cheng C, Linke WA, Joles JA, Verhaar MC, van der Velden J, Merkus D, Duncker DJ. Multiple common comorbidities produce left ventricular diastolic dysfunction associated with coronary microvascular dysfunction, oxidative stress, and myocardial stiffening. Cardiovasc Res 2018; 114: 954–964.

18. Ma LN, Zhao SP, Gao M, Zhou QC, Fan P. Endothelial dysfunction associated with left ventricular diastolic dysfunction in patients with coronary heart disease. Int J Cardiol 2000; 72: 275–279.

19. Leung M, Phan Y, Leung DY. Endothelial function and left ventricular diastolic functional reserve in type 2 diabetes mellitus. Open Heart 2014; 1: e000113.

20. Marti CN, Gheorghiade M, Kalogeropoulos AP, Georgiopoulou VV, Quyyumi AA, Butler J. Endothelial dysfunction, arterial stiffness, and heart failure. J Am Coll Cardiol 2012; 60: 1455–1469.

21. Battelli MG, Polito L, Bolognesi A. Xanthine oxidoreductase in atherosclerosis pathogenesis: not only oxidative stress. Atherosclerosis 2014; 237: 562–567.

22. Cantu-Medellin N, Kelley EE. Xanthine oxidoreductase-catalyzed reactive species generation: a process in critical need of reevaluation. Redox Biol 2013; 1: 353–358.

23. Forstermann U, Xia N, Li H. Roles of vascular oxidative stress and nitric oxide in the pathogenesis of atherosclerosis. Circ Res 2017; 120: 713–735.
24. Sakuma S, Fujimoto Y, Sakamoto Y, Uchiyama T, Yoshioka K, Nishida H, Fujita T. Peroxynitrite induces the conversion of xanthine dehydrogenase to oxidase in rabbit liver. *Biochem Biophys Res Commun* 1997; 230: 476–479.

25. Watanabe K, Shishido T, Oraki Y, Watanabe T, Sugai T, Toshima T, Takahashi T, Yokoyama M, Kinoshita D, Murase T, Nakamura T, Wanezaki M, Tamura H, Nishiyama S, Takahashi H, Arimoto T, Yamauchi S, Yamanaka T, Murase T, Nakamura T, Watanabe M. Increased plasma xanthine oxidoreductase activity deteriorates coronary artery spasm. *Heart Vessels* 2019; 34: 1–8.

26. Mercuro G, Vitale C, Cerquetani E, Zoncu S, Deidda M, Fini M, Rosano GM. Effect of hyperuricemia upon endothelial function in patients at increased cardiovascular risk. *Am J Cardiol* 2004; 94: 932–935.

27. Tomiyama H, Higashi Y, Takase B, Node K, Sata M, Inoue T, Ishibashi Y, Ueda S, Shimada K, Yamashina A. Relationships among hyperuricemia, metabolic syndrome, and endothelial function. *Am J Hypertens* 2011; 24: 770–774.

28. Zoccali C, Maio R, Mallamaci F, Sesti G, Perticone F. Uric acid and endothelial dysfunction in essential hypertension. *J Am Soc Nephrol* 2006; 17: 1466–1471.

29. Gu J, Fan YQ, Zhang HL, Zhang JF, Wang CQ. Serum uric acid is associated with incidence of heart failure with preserved ejection fraction and cardiovascular events in patients with arterial hypertension. *J Clin Hypertens (Greenwich)* 2018; 20: 560–567.

30. Filippatos GS, Ahmed MI, Gladden JD, Mujib M, Aban IB, Love TE, Sanders PW, Pitt B, Anker SD, Ahmed A. Hyperuricaemia, chronic kidney disease, and outcomes in heart failure: potential mechanistic insights from epidemiological data. *Eur Heart J* 2011; 32: 712–720.

31. Radi R, Tan S, Prodanov E, Evans RA, Parks DA. Inhibition of xanthine oxidase by uric acid and its influence on superoxide radical production. *Biochim Biophys Acta* 1992; 1122: 178–182.

32. Tan S, Radi R, Gaudier F, Evans RA, Rivera A, Kirk KA, Parks DA. Physiologic levels of uric acid inhibit xanthine oxidase in human plasma. *Pediatr Res* 1993; 34: 303–307.

33. Glantzounis GK, Tsimoyiannis EC, Kappas AM, Galaris DA. Uric acid and oxidative stress. *Carr Pharm Des* 2005; 11: 4145–4151.

34. Farquharson CA, Butler R, Hill A, Belch JJ, Struthers AD. Allopurinol improves endothelial dysfunction in chronic heart failure. *Circulation* 2002; 106: 221–226.

35. George J, Carr E, Davies J, Belch JJ, Struthers A. High-dose allopurinol improves endothelial function by profoundly reducing vascular oxidative stress and not by lowering uric acid. *Circulation* 2006; 114: 2508–2516.

36. Givertz MM, Anstrom KJ, Redfield MM, Deswal A, Haddad H, Butler J, Tang WH, Dunlap ME, LeWinter MM, Mann DL, Felker GM, O’Connor CM, Goldsmith SR, Offli EO, Saltzberg MT, Margulies KB, Cappola TP, Konstam MA, Semigran MJ, McNulty SE, Lee KL, Shah MR, Hernandez AF. Effects of xanthine oxidase inhibition in hyperuricemic heart failure patients: the Xanthine Oxidase Inhibition for Hyperuricemic Heart Failure Patients (EXACT-HF) Study. *Circulation* 2015; 131: 1763–1771.