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ORIGINAL RESEARCH ARTICLE

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 (HFpEF); 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 HFpEF 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 HFpEF.

Methods and results The plasma XOR activity was measured in 257 patients with HFpEF, 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 HFpEF.

Keywords Xanthine oxidoreductase; Heart failure with preserved ejection fraction

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Introduction

The increasing proportion of patients with heart failure with preserved ejection fraction (HFpEF) 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 HFpEF has not been established.[3] It was reported that the mortality rates were similar between patients with HFpEF and those with HFrEF.[4]

Although the pathophysiology of HFpEF 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 HFpEF, 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 HFpEF,[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 HFpEF.

Methods

Study subjects

The study subjects were made up of 257 patients with HFpEF, who were admitted to Yamagata University Hospital for the diagnosis and/or treatment of heart failure (HF). The diagnosis of HFpEF 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 $\geq$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 HFpEF, 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 χ^2 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).

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 (≥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*).

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 was at 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

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
Aetiology				
IHD/VHD/DCM/Others	7/11/11/16	23/46/18/73	14/13/3/22	0.085
Echocardiographic data				
LVEDD (mm)	50 ± 8	50 ± 9	49 ± 6	0.717
LVEF (%)	66 ± 9	64 ± 9	63 ± 9	0.402
LAD (mm)	43 ± 10	45 ± 9	47 ± 8	0.153
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 (%)				
Beta-blockers, n (%)	28 (62)	85 (53)	29 (56)	0.551
Loop diuretics, n (%)	33 (73)	78 (49)	27 (52)	0.011
XOR inhibitors, n (%)	8 (27)	11 (12)	3 (10)	0.127
Statins, 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).

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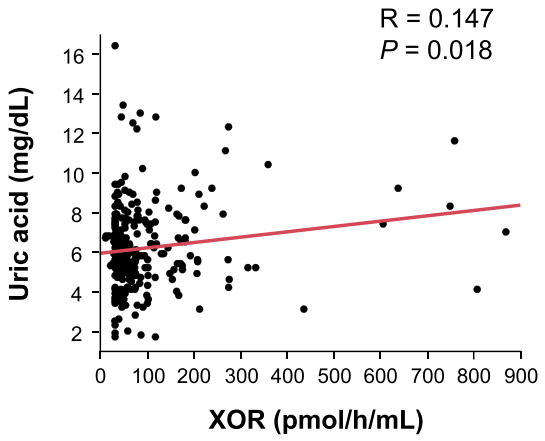
Fig 1 Correlation between plasma xanthine oxidoreductase (XOR) activity and serum uric acid.



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 in the patients (HR, 1.105 per 1 SD increase; 95% CI, 0.864–1.413; $P = 0.427$). 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 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.

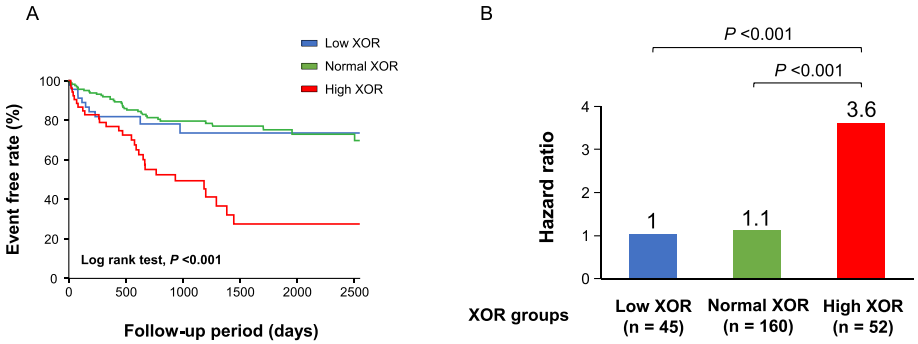


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Table 2 Univariate and multivariate Cox proportional hazard analyses of predicting major adverse cardiovascular events in patients with heart failure with preserved ejection fraction

	Univariate analysis			Multivariate analysis		
Variables	HR	95% CI	*P* value	HR	95% CI	*P* value
Age[a]	1.273	1.002–1.651	0.049	1.084	0.842–1.395	0.532
Male gender	1.293	0.815–2.084	0.277			
BMI[a]	0.989	0.738–1.313	0.941			
Hypertension	0.917	0.554–1.585	0.746			
Dyslipidaemia	0.962	0.543–1.616	0.887			
Diabetes mellitus	1.335	0.780–2.196	0.282			
Atrial fibrillation	1.165	0.734–1.879	0.519			
NYHA (III/IV vs. II)	3.493	2.198–5.616	<0.001	2.382	1.386–4.138	0.002
eGFR[a]	0.740	0.571–0.952	0.019	1.022	0.757–1.378	0.888
Log BNP[a]	1.828	1.413–2.394	<0.001	1.317	0.958–1.809	0.090
Hb[a]	0.876	0.702–1.101	0.253			
UA[a]	1.360	1.099–1.657	0.005	1.105	0.864–1.413	0.427
XOR[a]	1.320	1.134–1.498	<0.001	1.253	1.079–1.454	0.003
Loop diuretics use	2.110	1.313–3.468	0.002	1.159	0.684–2.003	0.586

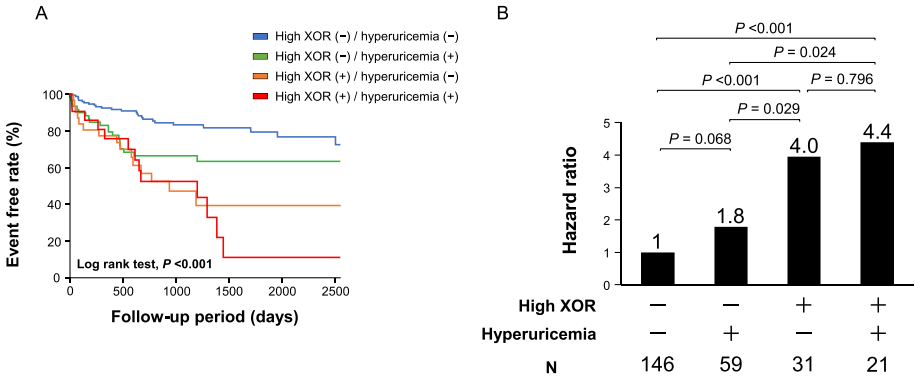
BMI, body mass index; BNP, brain natriuretic peptide; CI, confidence interval; eGFR, estimated glomerular filtration rate; Hb, haemoglobin; NYHA, New York Heart Association; UA, uric acid; XOR, xanthine oxidoreductase.
[a]Per 1 SD increase.

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/m^2)	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	6/5/1/9	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 m^2)	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).

Fig 3 Impact of the co-morbidity of hyperuricaemia with high xanthine oxidoreductase (XOR) activity on clinical outcomes in heart failure with preserved ejection fraction. (A) Kaplan–Meier curves for major adverse cardiovascular events according to the absence or presence of high XOR activity and/or hyperuricaemia. (B) Multivariate Cox regression analysis for predicting major adverse cardiovascular events in patients with heart failure with preserved ejection fraction.



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 HFpEF, 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 HFpEF.

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 HFpEF.[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 HFpEF. 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 HFpEF.[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 (O_2^-).[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.

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and released to the circulation. The circulating XDH is then rapidly converted to XO by either reversible sulfhydryl oxidation or irreversible proteolytic modification.[22] XO-derived O_2^- can react with nitric oxide, producing peroxynitrate ($ONOO^-$), which is a strong oxidizing mediator of endothelial cell injury.[23] In addition, $ONOO^-$ has the potential to convert XDH into XO, leading to a further increase in ROS generation.[24] We had previously demonstrated the association between plasma XOR activity and coronary artery spasm, which is thought to be related to endothelial dysfunction.[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.[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.[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.[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.[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.[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.[33]

Clinical implications

Although XOR inhibitors have been reported to improve endothelial function in patients with chronic HF,[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.[13] However, in the present study, low XOR activity was not associated with MACEs in patients with HFpEF. Givertz *et al.*[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.[35] Considering that high (but not low) XOR activity was found to be associated with MACEs 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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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Supporting Info Item
Figure S2. Supporting Info Item
Figure S3. Supporting Info Item

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