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Clinical science

Elevated serum CA72-4 predicts gout flares during urate lowering therapy initiation: a prospective cohort study

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Abstract

Objective: Gout flares during urate-lowering therapy (ULT) initiation are common, but predictors of these flares are poorly understood. The aim of this study was to determine whether serum CA72-4 is an independent predictor for gout flares during ULT initiation.

Methods: A prospective cohort study was conducted between March 2021 and January 2022. Men with gout, at least one gout flare in the past year, and at least three serum CA72-4 measurements in the previous six months were enrolled. Participants were grouped according to their highest recorded serum CA72-4 levels (above or within the normal range). All participants took oral febuxostat 20 mg daily without flare prophylaxis therapy, and attended face-to-face visits every four weeks until 24 weeks. The incidence of gout flare was compared between the two groups. Backward stepwise logistic regression analyses were used to identify risk factors associated with flares. Receiver operating characteristic curve analysis was used to evaluate prediction efficacy.

Results: A total of 193 completed the study (79 with high CA72-4; 114 with normal CA72-4). The cumulative incidence of at least one gout flare was 48.1% (62.1% in the high CA72-4 group, 38.4% in the normal CA72-4 group, $P = 0.001$), and recurrent (≥2) flares was 33.0% (47.1% in the high CA72-4 group, 23.2% in the normal CA72-4, $P < 0.001$). High CA72-4, disease duration, intra-articular tophus size, glucose, high-density lipoprotein-cholesterol and ESR were independent risk factors for gout flares. Serum CA72-4 alone predicted recurrent flares with an area under the curve of 0.63 (95% CI = 0.54, 0.71), and 0.78 (95% CI = 0.71, 0.85) when combined with other independent variables.

Conclusion: High serum CA72-4 predicts the risk of gout flares during ULT initiation.

Trial registration: ChiCTR; https://www.chictr.org.cn/; ChiCTR2100043573.

Keywords: CA72-4, gout flare, prediction

Rheumatology key messages

- Elevated baseline serum CA72-4 was independently associated with recurrent flares.
- Serum CA72-4 alone predicted recurrent flares with an AUC of 0.63, and 0.78 when combined with the other five clinical variables.
- Monitoring the dynamic changes of CA72-4 may allow more rational use of anti-inflammatory prophylaxis.

Introduction

Gout flares occur in over one half of patients with gout at the time of initiating urate-lowering therapy (ULT) [1]. The risk of these flares can be reduced with gradual dose escalation of urate-lowering medication and use of anti-inflammatory prophylaxis for the first 3–6 months of ULT [2, 3]. However, many gout patients have contraindications or side effects to anti-inflammatory medications (colchicine, NSAIDs, oral steroids) for flares [4]. Except for intensity of serum urate lowering, risk factors for gout flares during initiation of ULT

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are not well defined. Identifying which patients are at higher risk for these flares may allow more rational use of anti-inflammatory prophylaxis.

CA72-4, a glycoprotein identified by two monoclonal antibodies CC49 and B72.3, is a biomarker for gastrointestinal, pancreatic, ovary, breast and non-small cell lung cancer [5–7]. Emerging studies showed that serum CA72-4 is also elevated in several inflammatory diseases including gout and FMF [8–11]. The epitope antigen of CA72-4 is the sialosyl-a(2–6)-N-acetylgalactosamine-a-serine structure, which is carried by the unknown mucin core protein [12, 13].

There is increasing evidence that CA72-4 may be a useful biomarker in gout diagnosis and prognosis. Two limited observational studies reported that serum CA72-4 was elevated in patients with gout [8, 9]. Our group reported the highest level of serum CA72-4 in patients with gout among 37 diseases [14]. In an observational study in 833 gout patients (in comparisons with 120 hyperuricemia, 1292 non-gouty arthritis and 541 heathy controls), we reported that serum CA72-4 was specifically elevated in gout and that high CA72-4 level (exceeding the upper limit of normal) was a strong predictor of gout flares over a six-month period [11]. A key outstanding question following this study was whether serum CA72-4 is an independent predictor for gout flare, or whether other confounding variables account for the observed association.

To address if serum CA72-4 is an independent predictor for gout flare, we conducted a 24-week prospective cohort study of gout patients initiating ULT with fixed low-dose febuxostat.

Methods

Study design and procedures

A prospective ULT cohort study was conducted with the primary aim to determine whether serum CA72-4 is an independent predictor for gout flare. Men with gout visiting the clinic were screened consecutively. Eligible patients were recruited and underwent a two-week washout period. All participants were then treated with fixed low-dose febuxostat and attended face-to-face visits every 4 weeks until 24 weeks. Gout flare incidence over the preceding 4 weeks was recorded at each study visit. A gout flare was defined as an episode with more than two signs/symptoms of joint swelling, tenderness and redness, which was deemed (by the participants and/or the investigator) to require treatment with an anti-inflammatory medication [2, 15].

This study was approved by the Ethics Committee of the Affiliated Hospital of Qingdao University and registered in Chinese Clinical Trial Registration Center (#ChiCTR2100043573). All participants provided written informed consent.

Participants

Eligibility criteria were male sex, age $\geq$18 years old, meeting the 2015 American College of Rheumatology/European League Against Rheumatism classification criteria for gout [16], serum CA72-4 measurement at least three times during the preceding 6 months, and at least one gout flare within the past 12 months. As serum CA72-4 levels can fluctuate over time, this approach was taken to identify patients with a persistently normal CA72-4 level. Key exclusion criteria were baseline serum urate <7 mg/dl, estimated glomerular filtration (eGFR) <45 mL/min/1.73m^2, and transaminases >2-fold of the upper normal limit. In this study, participants with CA72-4 >6.9 U/ml (the upper limit of normal) at least once were included in the high CA72-4 group, and participants with all CA72-4 measurements within the normal range were included in the normal CA72-4 group.

Urate-lowering therapy

All participants underwent a two-week washout period with discontinuation of drugs affecting serum urate level or occasional ULT and anti-inflammatory drugs, and a low-purine diet at least 5 days before the baseline visit. Participants then took oral febuxostat 20 mg once every morning for 24 weeks. No anti-inflammatory gout flare prophylaxis was used. If a gout flare occurred during the trial period, participants were additionally given etoricoxib 120 mg per day for 3–5 days as a regular treatment. Polyene phosphatidylcholine was given for liver protection if the serum transaminases exceeded two times of the normal upper limit. Participants who discontinued medication for three consecutive days during the study were identified as withdrawn from the study.

Variables and data collection

Parameters obtained at the baseline visit included age, height, body weight, blood pressure, comorbidities, medication, gout history (age at onset, disease duration, family history and gout flares in the last 6 months), palpable and ultrasonographic tophi. Ultrasonography of the knees, ankles and feet were performed by the same physician. Crystal deposition or aggregation was recorded, including the double-contour sign, the number and size of tophi inside the articular cavity or para-articular. The diameter of the largest tophus was selected for evaluating size of the intra-articular tophi. BMI $\geq$28 kg/m^2 was defined as obesity [17, 18]. Laboratory assessment included serum CA72-4, serum urate, creatinine (Cr), glucose, triglyceride, total cholesterol, high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), alanine aminotransferase (ALT), aspartate aminotransferase (AST), CRP and ESR.

Serum CA72-4 was measured by an electrochemiluminescence method using Elecsys kits (CA72-4/11776258 122, Roche Diagnostics GmbH, Mannheim, Germany), CRP by the latex-enhanced turbidimetric method, and ESR by Widmanstaten natural sedimentation method. Biochemical parameters were measured by an automatic biochemical analyser (TBA-40FR, Toshiba Company, Tokyo, Japan). The eGFR was used to assess kidney function and calculated as follows: Cr $\leq$0.9 mg/dl, $eGFR = 141 \times (Cr/0.9)^{-0.411} \times (0.993)^{age}$; Cr >0.9 mg/dl, $eGFR = 141 \times (Cr/0.9)^{-1.209} \times (0.993)^{age}$ [19].

Sample size

According to our previous study, the incidence of gout flare was 70.91% in patients with high serum CA72-4 level and 60.81% in patients with normal level, difference in rate of gout flare was 10.10% [11]. Based on these data, it was estimated that 65 in the high CA72-4 group and 98 in the normal CA72-4 group (1:1.5 ratio) would provide >85% power at a significance level of 0.05. Considering an attrition rate of ~20%, at least 88 in the high CA72-4 group and 122 in the

normal CA72-4 group participants were needed, and finally 87 and 125 participants included in two groups, respectively.

Statistical analyses

All analyses were performed using SPSS 26.0 (IBM, Armonk, NY, USA). *P*-value <0.05 was considered statistically significant. Continuous variables were expressed as mean (S.D.) or median (IQR), and categorical variables were expressed as number (percentage). Independent sample *t*-test or Wilcoxon sign-rank test were used to compare continuous variables and chi-squared test was used for categorical variables between the two groups. The flare-free and recurrent gout flares-free survival proportions of participants in different groups were estimated by Kaplan–Meier curves. Generalized estimating equations (GEE) test was also used to test associations between flares and serum CA72-4.

A backward stepwise logistic regression was used to identify possible predictors of recurrent gout flares out of the following covariates based on their biological relevance: age, BMI, comorbidities, disease duration, positive family history, palpable tophus, intra-articular tophus size, at least one flare in the last 6 months, high CA72-4, serum urate, eGFR, glucose, triglyceride, total cholesterol, HDL-C, LDL-C, ALT, AST, CRP, ESR. Comorbidities, positive family history, palpable tophus, at least one flare in the last 6 months and high CA72-4 were treated as dichotomous variables and others were continuous variables. Variables with *P*-values <0.1 were accepted in the model. Results are expressed as odds ratio (OR) and 95% confidence interval (95% CI). Subsequently, the receiver operating characteristic (ROC) curve analyses were performed with each independent variable included in the multivariate logistic regression to evaluate the prediction efficacy of recurrent gout flares.

Results

Participants and baseline characteristics

The study commenced in March 2021, and the last participant was enrolled in July 2021. Follow-up was completed in January 2022. A total of 271 patients were screened and 212 eligible participants were enrolled; of these 87 were classified as high CA72-4 and 125 as normal CA72-4. There were 193 (91.0%) participants who completed the 24-week study (Fig. 1). Of the 19 participants who dropped out, the leading cause was withdrawal of consent, one in the high CA72-4 group was lost to follow-up, and two in the normal CA72-4 group was due to adverse effects of febuxostat.

Demographic and clinical characteristics were shown in Table 1. Participants were predominantly middle-aged with a mean (S.D.) age of 45.3 (12.4) years, and a mean (S.D.) BMI of 27.2 (3.6) kg/m^2. The median duration of gout was 7.0 years, 13.2% of participants had palpable tophi and 87.7% had at least one comorbidity. Most baseline features were comparable between the high and normal CA72-4 groups, except for more intra-articular tophus (70.1% *vs* 56.0%, $P = 0.037$), larger intra-articular tophus size (9.0 mm *vs* 7.0 mm, $P = 0.029$), more participants had at least one flare in the last 6 months (54.0% *vs* 40.0%, $P = 0.005$) and higher median CRP (1.6 mg/l *vs* 0.9 mg/l, $P = 0.001$) in the high CA72-4 group.

During the 24-week follow-up, there was no difference in adverse events between the two groups (Supplementary Data S1, available at *Rheumatology* online).

Gout flares according to CA72-4 groups

Over the 24 weeks of follow-up, the cumulative incidence of gout flares was 48.1% in all participants (62.1% in the high CA72-4 group, 38.4% in the normal CA72-4 group, $P = 0.001$). A single gout flare occurred in 15.1% participants (14.9% in the high CA72-4 group, 15.2% in the normal CA72-4 group, $P > 0.05$), and recurrent (≥2) gout flares in 33.0% participants (47.1% in the high CA72-4 group, 23.2% in the normal CA72-4 group, $P < 0.001$) (Fig. 2A, B).

The incidence of flares in the preceding 4 weeks reduced gradually in both groups (for the high CA72-4 group: 40.2% in week 4 and 20.3% in week 24; for the normal CA72-4 group: 22.4% in week 4 and 8.8% in week 24), and was higher in the high CA72-4 group than in the normal CA72-4 group at every visit, except for week 20 ($P < 0.01$ at the first 12 weeks, $P < 0.05$ at week 16 and week 24) (Fig. 2C). Recurrent flares were more common in the high CA72-4 group in the first 12 weeks (41.4% *vs* 15.2%, $P < 0.001$) and the last 12 weeks of follow-up (17.1% *vs* 6.0%, $P = 0.012$) (Fig. 2D). GEE analysis confirmed that high baseline CA72-4 was associated with gout flare over the 24-week period (B Coefficient = 0.970, $P = 0.025$).

Clinical variables and their associations with the incidence of recurrent gout flares

Table 2 reports the univariate analysis for recurrent gout flares as well as the multivariate logistic regression analysis. Starting with 20 covariates that might theoretically be predictors of recurrent gout flares, the stepwise logistic regression model reduced them to 7. Among these independent variables, high CA72-4 level (OR = 2.34, 95% CI = 1.14, 4.82), larger intra-articular tophus size (OR = 1.97, 95% CI = 1.19, 3.24), longer disease duration (OR = 1.08, 95% CI = 1.01, 1.16) and increasing ESR (OR = 1.06, 95% CI = 1.01, 1.12) were associated with an increased likelihood of recurrent gout flares, while increasing glucose (OR = 0.51, 95% CI = 0.27, 0.97) and HDL-C (OR = 0.13, 95% CI = 0.03, 0.61) were associated with its reduction ($P < 0.05$). In addition, at least one flare in the last 6 months was also included in the multiple regression model, but it was not statistically significant (OR = 1.87, 95% CI = 0.91, 3.82, $P = 0.087$).

During the 24-week period, the serum CA72-4 level in the high CA72-4 group remained higher than in the normal CA72-4 group ($P < 0.001$ at every visit, Fig. 3A). Serum urate reduced in both groups, with no between-group differences throughout the study period (Fig. 3B). For inflammatory markers, CRP was higher in the high CA72-4 group at the first 12 weeks (Fig. 3C), while ESR did not differ between the two groups (Fig. 3D). Intra-articular tophus size reduced in both groups ($P < 0.05$), and did not differ between groups at week 24 (5.0 mm *vs* 4.0 mm, $P > 0.05$) (Fig. 3E).

Prediction efficacy for recurrent flares

High CA72-4 (>6.9U/ml) could predict recurrent gout flares during initiation of ULT with the AUC of 0.63 (95% CI = 0.54, 0.71), with a sensitivity of 0.58 and a specificity of 0.68. When CA72-4 was included as a continuous variable in the ROC analysis, the cut-off value was 24.58,

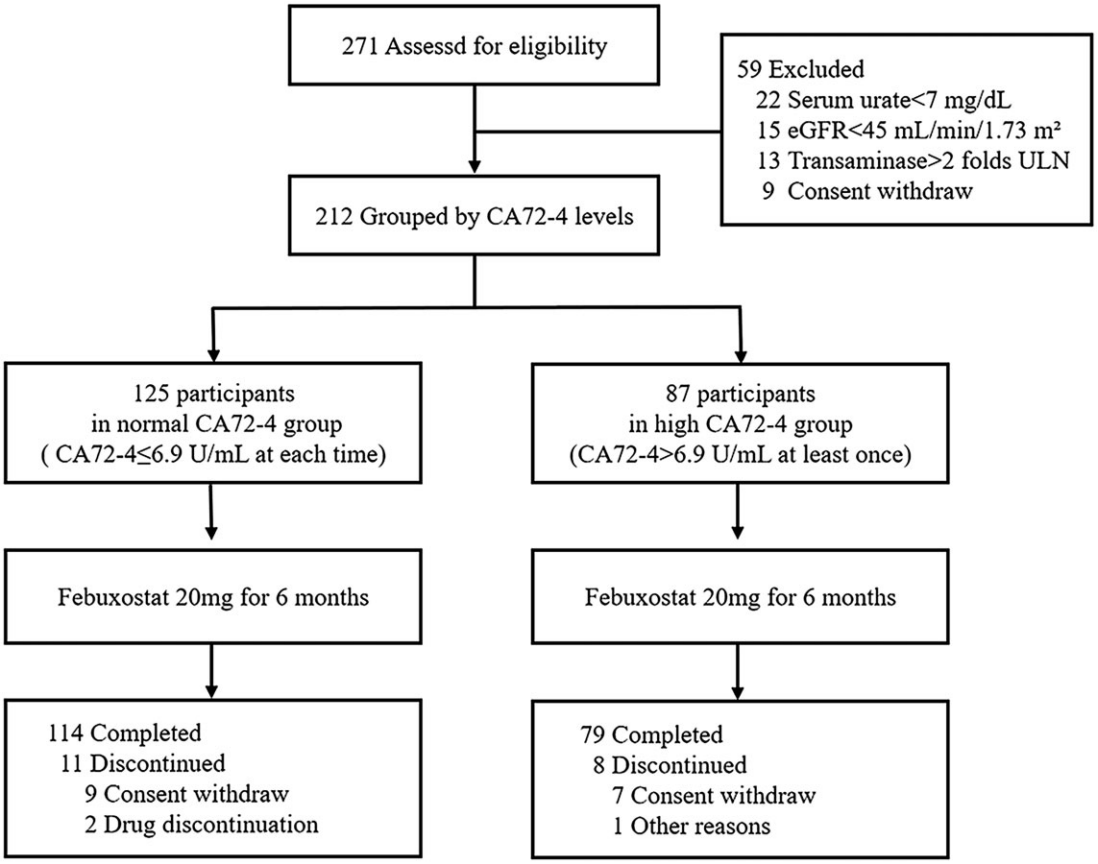

Figure 1. Flow chart

and the AUC was 0.67 (95% CI = 0.58, 0.75), with the sensitivity 0.32 and the specificity 0.96 (Supplementary Fig. S1. available at *Rheumatology* online). Considering the poor sensitivity of the latter, we chose high CA72-4 combined with other independent variables identified by the stepwise logistic regression model, including disease duration, intra-articular tophus size, glucose, HDL-C and ESR to predict recurrent gout flares, with the AUC of 0.78 (95% CI = 0.71, 0.85) (Fig. 4). Meanwhile, of these six independent variables, high CA72-4 had the highest AUC for future recurrent gout flares among easily measurable serum parameters, and was second only to intra-articular tophus size (Supplementary Table S1, available at *Rheumatology* online).

Discussion

Here, we report the first prospective cohort study to examine the association between serum CA72-4 and gout flares following ULT initiation. These findings provide evidence that baseline elevated serum CA72-4 is associated with gout flare incidence independent of other risk factors, and can assist with prediction of gout flares in the first 6 months of ULT. The ROC curve analysis of recurrent gout flares showed that AUC was 0.63 for serum CA72-4 >6.9 U/ml, and the AUC was 0.78 when combined with disease duration, intra-articular tophus size, glucose, HDL-C and ESR. These novel findings have identified patients who are most likely to flare during initiation of ULT, and suggest the potential to stratify patients who may benefit from gout flare prophylaxis drug treatments.

A variety of risk factors for recurrent flares have been reported, including periods of alcohol consumption, high diet-purine consumption, high serum urate, rapid drop of serum urate, longer disease duration, obesity and hypertriglyceridemia, male sex, and cardiometabolic comorbidities [20–26]. However, those risk factors, alone or in a composite manner, have not satisfactorily identified the patients with the highest frequency of flares over a prolonged period of time following ULT initiation [25]. A prior study developed a prediction model for gout flares in hospitalized patients with comorbid gout using nine clinical variables (pre-admission urate >0.36 mmol/l, tophus, no pre-admission ULT, no pre-admission gout prophylaxis, acute kidney injury, surgery, initiation or increase of gout prophylaxis, adjustment of ULT and diuretics prior to flare), and achieved adequate accuracy [27]. Given the information to date, and the assumption that aetiology of gout flare is heterogeneous, reliable markers of gout flare susceptibility remain an unmet need. In this study, participants with high concentration of baseline serum CA72-4 experienced more gout flares in terms of monthly incidence, cumulative incidence or recurrent gout during ULT initiation. Among all parameters with clinical importance, CA72-4 was an independent risk factor for recurrent gout flares, verified by the multivariate logistic model. The AUC was 0.63 for CA72-4 >6.9U/ml solely and up to 0.78 by combining CA72-4 >6.9U/ml with other five associated clinical variables. The findings suggest that serum CA72-4 is a potential marker for identifying those with recurrent gout flares who might benefit from anti-inflammatory prophylaxis treatment. A prospective controlled interventional study using anti-inflammatory

Table 1. Baseline characteristics

	Normal CA72-4 ($n = 87$)	High CA72-4 ($n = 125$)	*P*-value
Demographic and general characteristics			
Age (years)	44.0 (12.1)	47.3 (12.6)	0.057
Height (cm)	175.5 (6.5)	174.8 (5.1)	0.361
Body weight (kg)	84.5 (13.9)	82.3 (12.2)	0.230
BMI (kg/m^2)	27.4 (3.9)	26.9 (3.3)	0.336
Systolic BP (mmHg)	135.1 (15.8)	138.2 (17.3)	0.190
Diastolic BP (mmHg)	87.8 (10.6)	90.0 (11.5)	0.173
Comorbidities			
Obesity	48 (38.4%)	26 (29.9%)	0.201
Hypertension	34 (27.2%)	30 (34.5%)	0.256
Cardiovascular disease	2 (1.6%)	2 (2.3%)	0.722
Fatty liver	68 (54.4%)	50 (57.5%)	0.658
Hyperlipidemia	59 (47.2%)	43 (49.4%)	0.750
Diabetes	17 (13.6%)	12 (13.8%)	0.968
Kidney stones	16 (12.8%)	12 (13.8%)	0.834
Gout history and examination			
Onset age (years)	36.6 (10.4)	38.5 (11.1)	0.209
Disease duration (years)	6.0 (3.0-10.0)	8.0 (3.0-12.0)	0.193
Family history of gout	28 (22.4%)	21 (24.1%)	0.768
Palpable tophus	14 (11.2%)	14 (16.1%)	0.301
Intra-articular tophus	70 (56.0%)	61 (70.1%)	0.037
Intra-articular tophus size (mm)	8.0 (0.0-12.0)	9.0 (0.0-14.0)	0.029
At least one flare in the last 6 months	50 (40.0%)	47 (54.0%)	0.005
Laboratory measures			
CA72-4 (U/mL)	1.3 (1.0-1.8)	5.5 (3.2-9.2)	<0.001
Serum urate (mg/dL)	9.5 (1.3)	9.6 (1.5)	0.552
eGFR (mL/min/1.73 m^2)	88.4 (17.7)	83.8 (18.4)	0.072
Glucose (mmol/L)	5.9 (5.5-6.2)	5.8 (5.6-6.3)	0.685
Triglyceride (mmol/L)	1.7 (1.3-2.4)	1.9 (1.3-2.7)	0.521
Total cholesterol (mmol/L)	5.0 (1.0)	5.0 (0.9)	0.778
HDL-C (mmol/L)	1.2 (1.0-1.4)	1.2 (1.0-1.4)	0.279
LDL-C (mmol/L)	3.5 (0.9)	3.5 (0.8)	0.997
ALT (U/L)	25.0 (16.0-39.0)	24.0 (16.0-31.0)	0.282
AST (U/L)	20.0 (17.0-27.0)	20.0 (17.0-25.0)	0.866
CRP (mg/L)	0.9 (0.5-1.6)	1.6 (1.0-3.5)	0.001
ESR (mm/h)	6.0 (3.0-9.0)	7.0 (3.8-14.0)	0.064

Data are *n* (%), mean (S.D.), or median (IQR).
ALT: alanine aminotransferase; AST: aspartate aminotransferase; eGFR: estimated glomerular filtration rate; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol.

prophylaxis selectively in those with high baseline CA72-4 could test this hypothesis.

The variables contributing to gout flares in ROC analysis are clinically meaningful. The natural history of gout includes the progression from asymptomatic hyperuricemia to first flare, recurrent flares and chronic gout and tophi, especially when the disease is undertreated by ULT over time. MSU crystals induce the activation of NLRP3 inflammasome in macrophages and monocytes, triggering a gout flare and the recruitment of neutrophils. The formation of adequate neutrophil extracellular traps contributes to the resolution of a flare, as well as the formation of tophi [28]. Chronic inflammation induced by sustained flare or active tophi involves the activation of both innate immune and adaptive immune [29]. In that case, ESR may increase as reported by several studies [30]. In prolonged disease, both continuing flare activity and elevated ESR predicted those predisposed to more flares going forward. A reciprocal association between dyslipidaemia (hypertriglyceridemia and low HDL-C) and chronic low-grade inflammation has been established by several studies [31–33]. In this study, lower HDL-C predicted recurrent gout flares during ULT, indicating that dyslipidaemia might facilitate gouty inflammation, which corroborated a previous study [34]. Moreover, results of this study showed that tophus sizes were robust predictors for recurrent gout flares, consistent with previous studies [35, 36]. In this study, higher glucose levels are associated with lower incidence of recurrent gout flares, potentially because the majority of urate removal from the body is dependent on renal urate excretion, and a high urinary excretion rate of glucose level is inversely associated with serum urate level [37].

The reasons why serum CA72-4 is elevated in gout patients remain unclear. We speculate that increased CA72-4 in gout patients could be a consequence of urate priming or MSU crystal induced phagocyte or mucosal cell activation, as in other cases with innate immune activation induced by intrinsic abnormality like FMF, or an agonist such as ganoderma lucidium spore [8–11, 38]. CA72–4 is a saccharide structure of an unknown protein [12, 13]. Glycosylation is the most diverse and common posttranslational modification of proteins. In some inflammatory settings, cell membrane glycan structures often exhibit dramatic changes and incomplete synthesis followed by accumulation of precursor structures, which may be responsible for the epitope generation [14]. It appears that CA72-4 may be more predisposed than other cancer biomarkers to be elevated in those with active innate immune inflammatory states. CA72-4 is not only expressed in tumour tissues but also in normal tissues such as the secretory

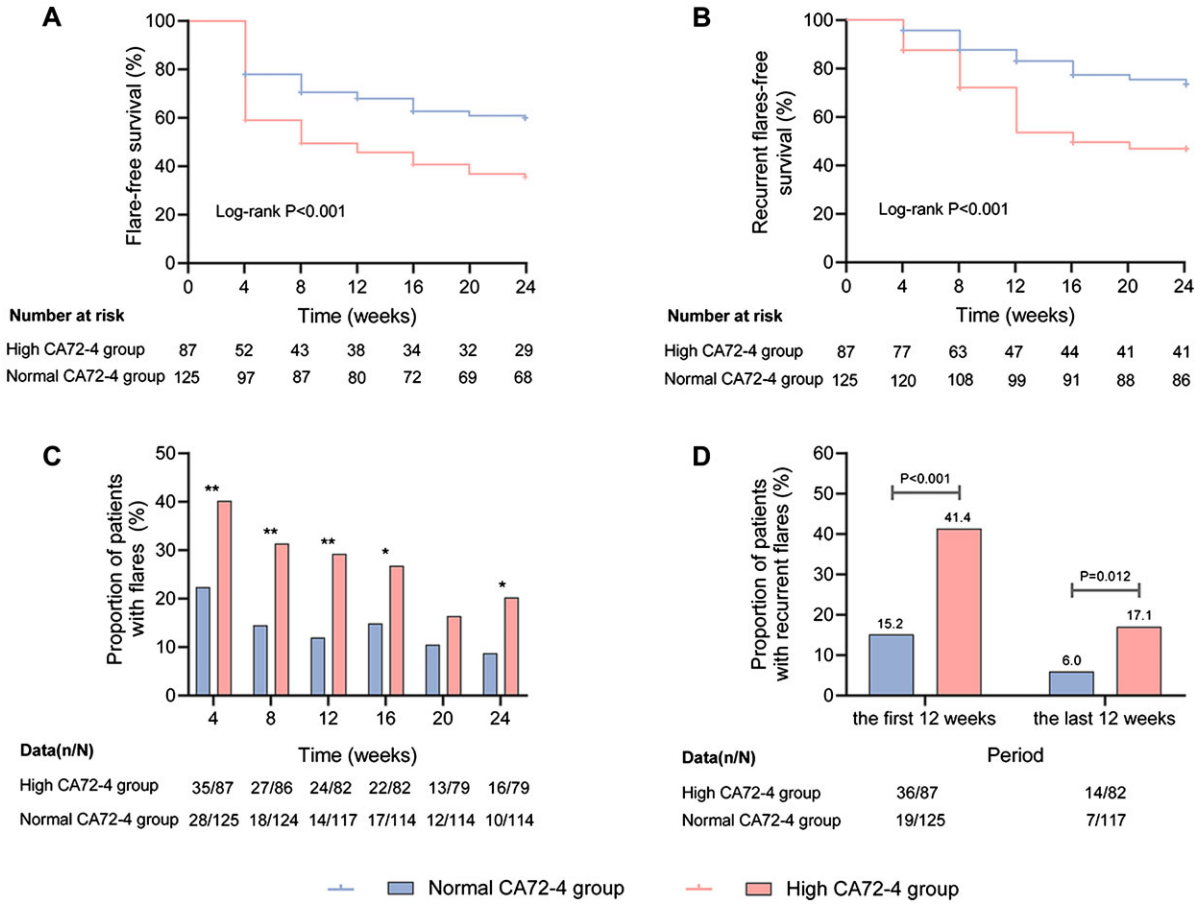

Figure 2. Gout flares in the high and normal CA72-4 group over the 24-week study period. (**A**) Kaplan Meier analysis of the accumulated flare-free survival curves. (**B**) Kaplan Meier analysis of the accumulated recurrent (≥2) flares-free survival curves. (**C**) The proportion of patients with flares. (**D**) The proportion of patients with recurrent (≥2) flares. *means comparisons between the two groups $P < 0.05$; **means comparisons between the two groups $P < 0.01$

Table 2. Logistic regression models for factors associated with recurrent flares

	Univariate analysis		Multivariate analysis			
			Model 1		Model 2	
	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
Age (years)	1.02 (0.99, 1.04)	0.235				
BMI (kg/m^2)	1.00 (0.92, 1.09)	0.958				
≥1 Comorbidities	1.63 (0.57, 4.71)	0.364				
Disease duration (years)	1.07 (1.01, 1.12)	0.013	1.01 (1.08, 1.15)	0.032	1.08 (1.01, 1.16)	0.026
Positive family history	1.24 (0.61, 2.53)	0.548				
Palpable tophus	2.84 (1.21, 6.67)	0.017				
Intra-articular tophus size (mm)	2.83 (1.55, 3.68)	<0.001	2.00 (1.22, 3.28)	0.006	1.97 (1.19, 3.24)	0.008
At least one flare in the last 6 months	1.97 (1.08, 3.59)	0.028	1.86 (0.92, 3.76)	0.085	1.87 (0.91, 3.82)	0.087
High CA72-4[a]	2.85 (1.54, 5.26)	0.001	2.41 (1.18, 4.91)	0.016	2.34 (1.14, 4.82)	0.021
Serum urate (mg/dL)	1.31 (1.05, 1.63)	0.017				
eGFR(mL/min/1.73m^2)	0.98 (0.97, 1.00)	0.034				
Glucose (mmol/L)	0.67 (0.41, 1.10)	0.116	0.55 (0.31, 0.98)	0.041	0.51 (0.27, 0.97)	0.040
Triglyceride (mmol/L)	1.23 (0.99, 1.53)	0.056				
Total cholesterol (mmol/L)	1.15 (0.84, 1.58)	0.391				
HDL-C (mmol/L)	0.25 (0.07, 0.87)	0.029	0.12 (0.03, 0.54)	0.006	0.13 (0.03, 0.61)	0.010
LDL-C (mmol/L)	1.05 (0.75, 1.49)	0.761				
ALT (U/L)	0.99 (0.97, 1.01)	0.377				
AST (U/L)	0.99 (0.96, 1.03)	0.740				
CRP (mg/L)	1.04 (0.98, 1.11)	0.204				
ESR (mm/h)	1.07 (1.02, 1.12)	0.003	1.06 (1.01, 1.11)	0.025	1.06 (1.01, 1.12)	0.020

Model 1 followed the backward stepwise selection rule. Model 2 was adjusted for the usage of anti-hypertensive drugs, glucose-lowering drugs, liver and kidney protecting drugs during the 24-week follow-up, and irregular ULT without a prescription in the preceding six months before enrolment.

[a] CA72-4 exceeded the normal upper limit at least once in the last 6 months.

95% CI: 95% confidence interval; ALT: alanine aminotransferase; AST: aspartate aminotransferase; eGFR: estimated glomerular filtration rate; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; OR: odds ratio.

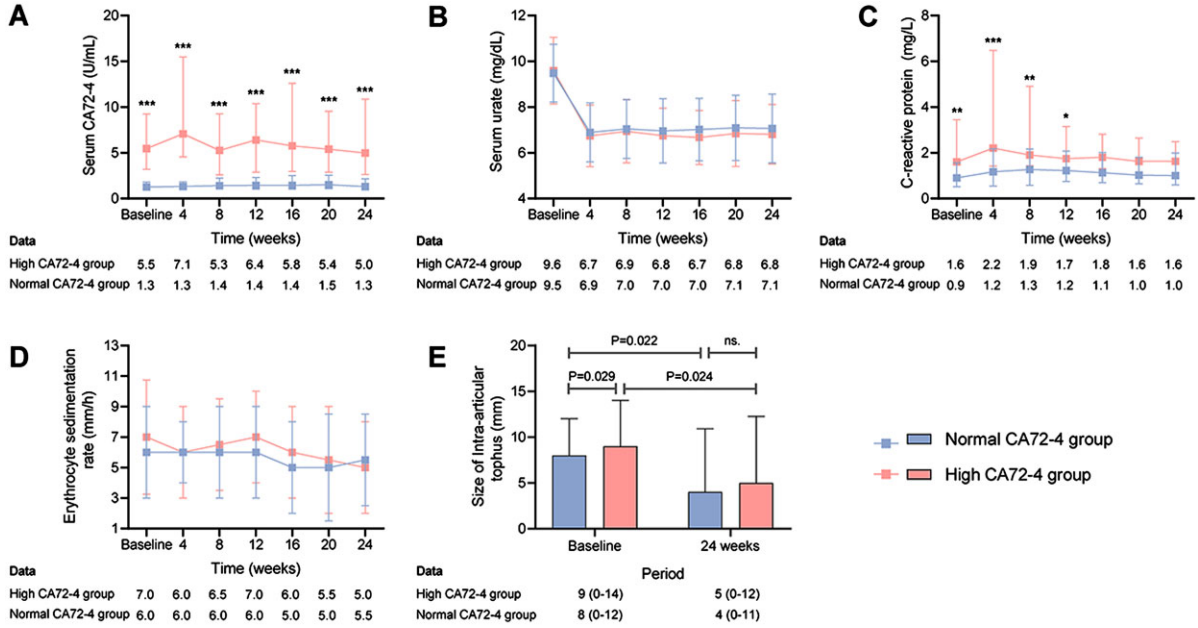

Figure 3. Other clinical and laboratory variables in the high and normal CA72-4 group over the 24-week study period. (**A**) Serum CA72-4. (**B**) Serum urate. (**C**) C-reactive protein. (**D**) Erythrocyte sedimentation rate. (**E**) Size of intra-articular tophus. Data are expressed as median (IQR), except serum urate as mean (S.D.). *means comparisons between the two groups $P<0.05$; **means comparisons between the two groups $P<0.01$; ***means comparisons between the two groups $P<0.001$

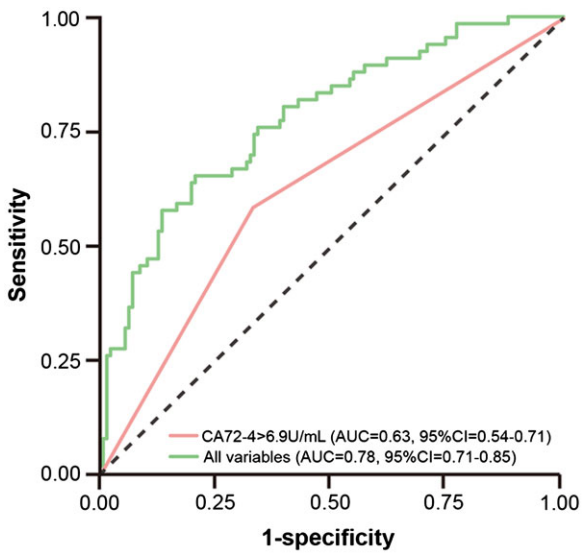

Figure 4. ROC curves for recurrent gout flares. All variables including CA72-4 >6.9U/mL, disease duration, intra-articular tophus size, glucose, high-density lipoprotein cholesterol and ESR. AUC: area under the curve; ROC: receiver operating characteristic.

endometrium and transitional colonic mucosa, which indicates that CA72-4 is not a unique product of cancer cells. It is still unclear what stimuli trigger CA72-4 production.

The main limitation of this study is that all subjects in the study were male and Chinese, so it is uncertain whether the association of CA72-4 with gout flares can be extended to females and other ethnicities. In addition, our ULT regimen used a standardized fixed low dose of febuxostat, rendering the control of serum urate levels incomplete in some participants. Therefore, the study findings may not be generalizable to patients treated with other febuxostat doses, other urate-lowering drugs, or with no urate lowering at all. Our study only recruited participants with relatively normal renal function, and the results may not be generalized to patients with severe kidney disease.

In conclusion, this study demonstrated that elevated serum CA72-4 predicts the risk of gout flares during ULT initiation, especially when combined with other variables. Monitoring the dynamic changes of CA72-4 may have potential to identify which patients may benefit from gout flare prophylaxis during ULT initiation.

Supplementary material

Supplementary data are available at *Rheumatology* online.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

Contribution statement

S.H., M.S., N.D. and C.L. contributed to study design. S.H., M.S., M.L., X.X., C.W., M.W., J.L., Z.R., H.L., A.J., W.S., X.L., Y.H., Z.L., H.Z., X.W., X.J. and C.L. contributed to study analysis. All authors contributed to interpretation of results. S.H., M.S., M.L., X.X., R.T. and N.D. drafted the manuscript, and all authors reviewed and revised the manuscript for content.

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