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Original article

Serum CA72-4 is specifically elevated in gout patients and predicts flares

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Abstract

Objectives. Serum CA72-4 levels are elevated in some gout patients but this has not been comprehensively described. The present study profiled serum CA72-4 expression in gout patients and verified the hypothesis that CA72-4 is a predictor of future flares in a prospective gout cohort.

Methods. To profile CA72-4 expression, a cross-sectional study was conducted in subjects with gouty arthritis, asymptomatic hyperuricaemia, four major arthritis types (OA, RA, SpA, septic arthritis) and healthy controls. A prospective gout cohort study was initiated to test the value of CA72-4 for predicting gout flares. During a 6-month follow-up, gout flares, CA72-4 levels and other gout-related clinical variables were observed at 1, 3 and 6 months.

Results. CA72-4 was highly expressed in patients with gouty arthritis [median (interquartile range) 4.55 (1.56, 32.64) U/ml] compared with hyperuricaemia patients [1.47 (0.87, 3.29) U/ml], healthy subjects [1.59 (0.99, 3.39) U/ml] and other arthritis patients [septic arthritis, 1.38 (0.99, 2.66) U/ml; RA, 1.58 (0.95, 3.37) U/ml; SpA, 1.56 (0.98, 2.85) U/ml; OA, 1.54 (0.94, 3.34) U/ml; $P < 0.001$, respectively]. Gout patients with frequent flares (twice or more in the last year) had higher CA72-4 levels than patients with fewer flares (fewer than twice in the last year). High CA72-4 level (>6.9 U/ml) was the strongest predictor of gout flares (hazard ratio = 3.889). Prophylactic colchicine was effective, especially for patients with high CA72-4 levels ($P = 0.014$).

Conclusion. CA72-4 levels were upregulated in gout patients who experienced frequent flares and CA72-4 was a useful biomarker to predict future flares.

Key words: CA72-4, biomarker, gout, flare

CLINICAL SCIENCE

Rheumatology key message

- CA72-4 was specifically upregulated in patients with gouty arthritis, but not with other major arthritis types.
- High CA72-4 level (>6.9 U/ml) was the strongest predictor of gout flares.
- Prophylactic colchicine was effective, especially for gouty arthritis patients with high CA72-4 levels (>6.9 U/ml).

Introduction

Gouty arthritis is one of the most common types of inflammatory arthritis, and is characterized by abrupt self-limiting attacks of inflammation arising from monosodium urate crystal deposition in joints [1]. Hyperuricaemia is the prelude to gout. Only 10% of patients with hyperuricaemia develop gout, and a high serum uric acid (sUA) level is not always associated with gout flares [2]. In gouty arthritis, monosodium urate crystals induce nod-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome complex formation and the activation of IL-1β-dependent innate inflammatory processes [3]. However, the inflammasome complex and IL-1β-dependent inflammatory pathways are shared by gouty arthritis

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and other non-gout diseases, such as FMF, type 2 diabetes mellitus and Alzheimer's disease [4]. Although some 'triggers' of flares, including abrupt changes in sUA and cold, have been identified, biomarkers associated with gout inflammation are still unknown, which limits the prediction of gout flares [5].

CA72-4 is a commonly used cancer biomarker that was originally recognized as a tumour-associated glycoprotein (TAG-72) by two monoclonal antibodies, CC-49 and B72-3, in early studies [6, 7]. Subsequently, its epitope was identified as sialosyl-α(2–6)-*N*-acetylgalactosamine in contrast to peptide determinants [8, 9]. It was reported that CA72-4 is highly expressed in gastric, ovary, breast, colon, lung and pancreatic cancer tissues [10, 11]. In our dedicated gout clinic, CA72-4 levels were abnormally elevated in some gout patients during cancer screening, and almost all tumour diagnoses were subsequently excluded. Furthermore, one previous case report described aberrantly elevated serum CA72-4 levels in patients with gout flares [12]. However, there is no reasonable explanation for this phenomenon. Thus, we reviewed the serum CA72-4 levels in 37 diseases based on 38 526 laboratory tests in the Affiliated Hospital of Qingdao University, and found that the mean serum CA72-4 level was significantly higher in patients with gout than in patients with cancer [13]. Based on these findings, we designed a study to investigate the link between elevated serum CA72-4 and gouty arthritis, and to determine the potential role of CA72-4 in predicting flares.

Methods

Design

We initially conducted a cross-sectional study to profile serum CA72-4 expression levels in subjects with asymptomatic hyperuricaemia, gouty arthritis and non-gouty arthritis [RA, SpA, OA, septic arthritis (SA)] and healthy controls. We then performed a 6-month prospective observational cohort study of the gout patients to test and verify the hypothesis that CA72-4 is a predictor of future flares. During the 6-month follow-up, gout flares, CA72-4 levels and other gout-related clinical variables were observed at 1, 3 and 6 months. Other glycoprotein cancer markers including serum carcinoembryonic antigen (CEA), CA125 and CA19-9 were also examined in patients with gouty arthritis. The study was approved by the Ethics Committee of the Affiliated Hospital of Qingdao University and all study participants provided written informed consent.

Study population

Between June 2016 and May 2018, patients with gout or asymptomatic hyperuricaemia who visited the dedicated Gout Clinic at the Affiliated Hospital of Qingdao University were consecutively recruited, as were patients with RA, SpA, OA or SA who visited the Department of Rheumatology. The diagnoses were confirmed by senior gout experts and rheumatologists. Gout patients were diagnosed according to the 2015 ACR/EULAR gout classification criteria [14]. Asymptomatic hyperuricaemia was defined as fasting sUA >7 mg/dl detected at least twice on two separate days in patients on a normal purine diet (defined as avoiding alcohol and purine-rich food such as seafood and red meat, especially organ meat, but not purine-rich leafy green vegetables) without previous or current arthritis. RA patients included recent-onset and long-term patients who met the 2010 ACR/EULAR classification criteria [15]. SpA was diagnosed according to the Assessment of Spondyloarthritis International Society classification criteria [16]. Diagnoses of OA involving the hands and knees were established in accordance with two classification standards [17, 18]. SA was confirmed by synovial fluid germ culture or based on clinical assessments. Any patients manifesting sophisticated arthritis or with uncertain diagnosis according to the above criteria would not be recruited. Healthy controls, age- and gender-matched to gout patients, were recruited from the Health Examination Center at the hospital during the same period, and defined as persons with normal sUA, benign joint conditions and no diagnosis of cancer or arthritis. All subjects were aged $\geq$18 years but were $<$80 years (supplementary Table S1, available at *Rheumatology* online).

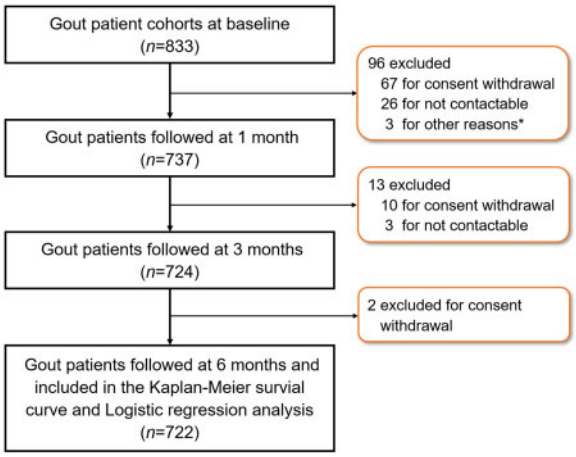
Informed consent and permission to use spare serum samples were obtained from all participants. All subjects had their serum CA72-4, sUA and CRP levels measured. Those with elevated CA72-4 were transferred to gastroenterologists and screened for neoplastic diseases. The exclusion criteria included: (i) presence or history of malignant disease; (ii) pregnant or lactating women; (iii) chronic renal failure (estimated glomerular filtration rate $<$15 ml/min/1.73 m^2); and (iv) hepatic dysfunction (alanine aminotransferase/total bilirubin greater than or equal to twice the upper limit of normal range).

Overall, 120 hyperuricaemia patients, 833 gout patients, 532 RA patients, 243 SpA patients, 474 OA patients, 43 SA patients and 541 healthy controls were enrolled. Patients who refused to follow the procedure, withdrew their consent or failed to meet the inclusion criteria for any other reason were excluded.

Follow-up procedures

The gout cohort of 833 patients was followed for an additional 6 months after enrolment as a prospective study to verify whether CA72-4 is a predictor of gout flares. Patients were followed at baseline (time of enrolment), and at 1, 3 and 6 months after enrolment (Fig. 1). All patients were treated with a standard protocol according to the 2012 ACR guidelines [19, 20]. Patients with a gout flare were treated with colchicine and/or NSAIDs and sodium bicarbonate. Patients in the intercritical phase or beyond 2 weeks after the flare were started on urate-lowering therapy (ULT) and prophylactic low-dose colchicine for 3 months. Patients who had no flares after 3 months of ULT ceased prophylactic

FIG. 1 Overall study design



Asterisk represents unable to commit time, poor health condition or subjected to surgery.

colchicine. All patients were required to keep diaries of flares and medication compliance. Gout flares, CA72-4 levels and other gout-related clinical variables, including medical history, presence or absence of tophi, BMI, sUA, CRP, transaminase, fasting glucose and estimated glomerular filtration rate, were collected at every visit. For the gout patients, serum CEA, CA125 and CA19-9 were also screened at baseline.

Outcome measurements

The serum samples were spare samples in the hospital laboratory reserved for biochemical purposes. All samples were stored at −80°C for future use. The levels of CA72-4, CEA, CA125 and CA19-9 were measured by an electrochemiluminescence method using Elecsys kits (CA72-4/11776258 122, CEA/11731629 322, CA125/11776223 190, CA19-9/11776193 122, Roche Diagnostics, GmhH-Mannheim, Germany). According to the kit instructions, the normal ranges of CA72-4, CEA, CA125 and CA 19-9 were 0–6.9 U/ml, 0–3.4 ng/ml, 0–35 U/ml and 0–39 U/ml, respectively. sUA and other biochemical parameters were detected by enzyme colorimetric methods. CRP was detected by a latex-enhanced turbidimetric assay using reagents and instrumentation provided by Goldsite (Shenzhen, China).

Statistical analysis

Baseline demographics and clinical features were summarized using standard descriptive statistics including the mean (S.D.) or median (interquartile range), frequency or percent as appropriate. Comparisons of baseline demographic and clinical measures between different groups were undertaken using χ^2 tests, one-way ANOVA and Kruskal–Wallis tests as appropriate. Multiple linear regression models were developed to adjust confounders of the relationships between argument variables and dependent variables. Logistic regression models were developed to find predictors for independent variables. To estimate the influences of baseline variables and drug use on future flares in the prospective cohort, a multivariate analysis was performed using the Cox proportional hazards model. The flare-free survival rates of patients with different CA72-4 levels (≤6.9 or >6.9 U/ml) were estimated by Kaplan–Meier curves. Receiver-operating characteristic curve analysis was used to test the performance of the gout flare prediction index. Two-sided tests with a 5% significance level ($P < 0.05$) were considered statistically significant. All statistical analyses were performed using SPSS version 19.0 (IBM, Armonk, NY, USA).

Results

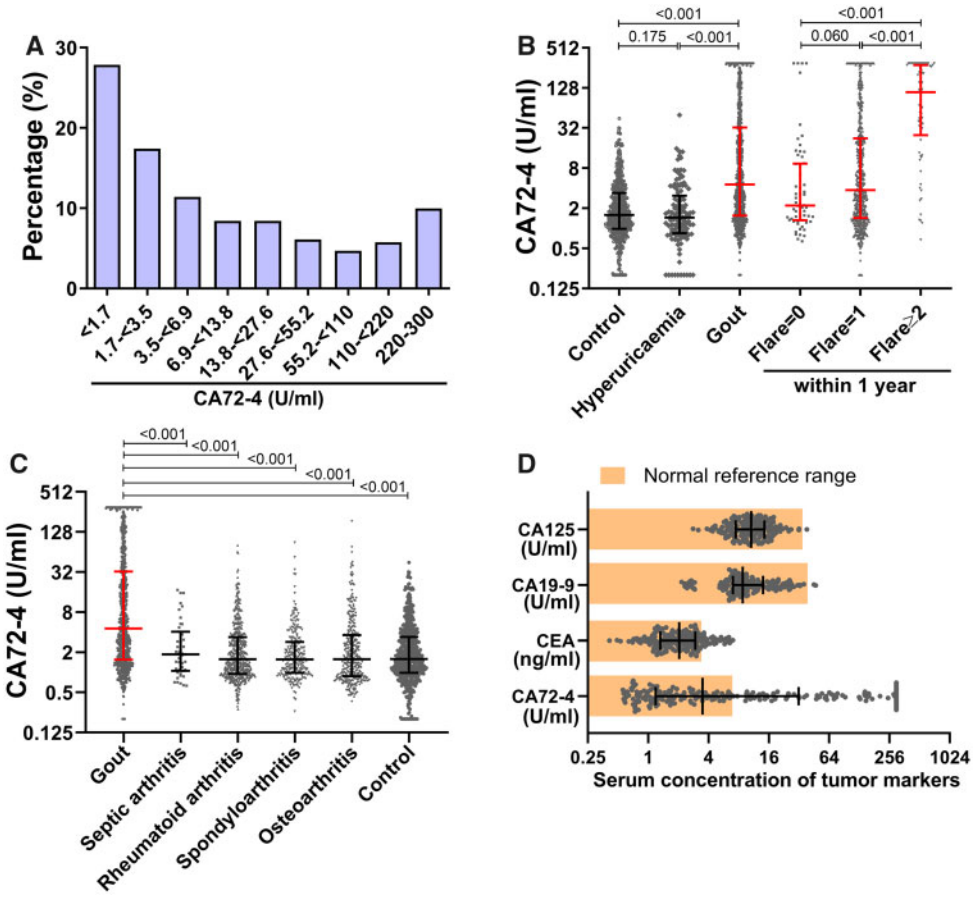
Profile of CA72-4 expression in the study population

Demographic data and baseline clinical characteristics among gouty and non-gouty arthritis, hyperuricaemia patients and healthy controls are shown in supplementary Table S1, available at *Rheumatology* online. The descriptive statistics of CA72-4 in gout patients indicated a non-normal distribution (Fig. 2A). The CA72-4 level in gout patients was significantly higher than that in healthy controls and hyperuricaemia patients ($P < 0.001$), and 42.7% of gout patients' CA72-4 levels exceeded the upper limit of the normal reference range (>6.9 U/ml) (Fig. 2A and B). Interestingly, patients who experienced only one flare in the last year before enrolment had similar CA72-4 levels to patients who did not have any flare, while patients with more frequent flares (two or more per year) [21, 22] had higher serum CA72-4 levels than patients who experienced fewer flares (none or one per year) (Fig. 2B). CA72-4 levels in asymptomatic hyperuricaemia patients and healthy subjects were within the normal reference range (Fig. 2B).

Most demographic and clinical variables were similar between the low (≤6.9 U/ml) and high (>6.9 U/ml) CA72-4 groups, except for patients with flare (60.81 *vs* 70.91%, $P = 0.002$), history of type 2 diabetes (4.66 *vs* 16.34%, $P < 0.001$), glucocorticoid use (14.83 *vs* 8.03%, $P = 0.001$) and fasting glucose (5.28 ± 0.81 *vs* 5.96 ± 1.46 mmol/l, $P < 0.001$) at baseline (Table 1). After adjustment by multiple regression analysis, only flare frequency and fasting glucose were independently associated with CA72-4 (supplementary Table S2, available at *Rheumatology* online).

By analysing the results of laboratory tests in patients with other common forms of arthritis (RA, OA, SpA, SA), we found the median (interquartile range) CA72-4 levels in these four types of non-gouty arthritis [RA: 1.58 (0.95–3.37) U/ml; OA: 1.54 (0.94–3.34) U/ml; SpA: 1.56 (0.98–2.85) U/ml; SA: 1.38 (0.99–2.66) U/ml] were comparable to those in healthy subjects [1.59 (0.99–3.39) U/ml] and much lower than in gouty arthritis patients [4.55 (1.56–32.64) U/ml, $P < 0.001$] (Fig. 2C). We also monitored sUA and CRP, a parameter reflecting overall

FIG. 2 CA72-4 is elevated in patients with gouty arthritis



(**A**) Distribution of gout patients with different serum CA72-4 levels ($n = 833$). (**B**) Comparison of serum CA72-4 levels among gout patients [$n = 833$, including no flares within 1 year, $n = 65$; one flare within 1 year ($n = 691$); two or more flares within 1 year ($n = 77$)]; hyperuricaemia patients ($n = 120$); and healthy controls ($n = 541$). (**C**) Comparison of serum CA72-4 levels among gouty arthritis patients ($n = 833$), septic arthritis patients ($n = 43$), RA patients ($n = 532$), SpA patients ($n = 243$), OA patients ($n = 474$) and healthy controls ($n = 541$). (**D**) Serum CA72-4, CEA, CA19-9 and CA125 levels in gout patients ($n = 195$). Mann–Whitney *U* test was used for statistical analyses of CA72-4 levels. CEA: carcinoembryonic antigen.

inflammatory activity, and found that sUA levels were higher in gout and hyperuricaemia patients than in non-gouty arthritis patients (supplementary Fig. S1, available at *Rheumatology* online). The CRP level in gouty arthritis was lower than that in SA, comparable to that in RA and SpA, and higher than that in OA patients. The CRP level in OA patients was similar to that in hyperuricaemia and healthy subjects (supplementary Fig. S2, available at *Rheumatology* online).

Furthermore, serum CEA, CA 19-9 and CA125 levels in gouty arthritis patients were within the normal limits [CEA: 2.04 (1.32, 2.96) ng/ml; CA19-9: 8.81 (7.01, 14.04) U/ml; CA125: 10.67 (7.48, 14.47) U/ml], indicating that only CA72-4, but not other cancer glycoproteins, was highly expressed in gouty arthritis patients (Fig. 2D). Taken together, CA72-4 was highly associated with gouty arthritis, particularly in patients with recent flares.

CA72-4 as a predictor of flares

We prospectively followed the gout cohort. Of 833 gout patients, 722 completed 6 months of follow-up visits and were included in the statistical analyses (Fig. 1). Multiple linear regression analysis indicated that baseline CA72-4 and sUA, and the use of colchicine and glucocorticoids during the following 6 months, were independently correlated with future flares (Table 2). Logistic regression analysis identified baseline CA72-4 (per 50 U/ml, odds ratio = 2.81, 95% CI: 2.10, 3.76, $P < 0.001$), sUA (odds ratio = 1.12, 95% CI: 1.04, 1.21, $P = 0.003$) and colchicine use (odds ratio = 0.59, 95% CI: 0.42, 0.82, $P = 0.002$) as independent predictors for future flare risk (Fig. 3A). Kaplan–Meier survival curves showed a lower cumulative flare-free survival rate at the end of 6 months in patients with high CA72-4 than with low CA72-4 (72.3 *vs* 10.7%, $P < 0.05$) (Fig. 3B). We investigated factors associated with the cumulated flares during the 6-month

Table 1 Demographic and baseline clinical characteristics in gout patients ($n = 833$)

Characteristics	CA72-4 ≤6.9 U/ml	CA72-4 >6.9 U/ml	*P*-value
n	472	361	
Age, years	50.07 (14.34)	49.18 (14.86)	0.384
Male, *n* (%)	422 (89.41)	328 (90.86)	0.488
BMI, kg/m^2	27.47 (3.18)	27.40 (3.21)	0.831
Tophi, *n* (%)	88 (18.64)	65 (18.01)	0.814
Flare (within the last year), *n* (%)	287 (60.81)	256 (70.91)	0.002
Smoking[a], *n* (%)	131 (27.75)	116 (32.13)	0.170
Drinking[b], *n* (%)	290 (61.44)	206 (57.06)	0.202
Physical exercise[c], *n* (%)	126 (26.69)	114 (31.58)	0.123
Hypertension[d], *n* (%)	130 (27.54)	104 (28.81)	0.687
Cardiac disease[d], *n* (%)	20 (4.24)	16 (4.43)	0.891
Type 2 diabetes[d], *n* (%)	22 (4.66)	59 (16.34)	<0.001
Nephrolithiasis[d], *n* (%)	28 (5.93)	16 (4.43)	0.891
Colchicines[e], *n* (%)	208 (44.07)	165 (45.71)	0.637
NSAIDs[e], *n* (%)	128 (27.12)	99 (27.42)	0.922
Glucocorticoids[e], *n* (%)	70 (14.83)	29 (8.03)	0.001
ULT, *n* (%)	168 (35.59)	135 (37.40)	0.592
ALT, U/l	27 (18, 43)	28 (18, 42)	0.762
AST, U/l	20 (17, 27)	21 (17, 27)	0.948
Glucose, mmol/l	5.28 (0.81)	5.96 (1.46)	<0.001
TG, mmol/l	1.68 (1.19, 2.35)	1.74 (1.19, 2.51)	0.766
Cholesterol, mmol/l	4.51 (4.16, 5.31)	4.51 (4.16, 5.31)	0.465
Blood urea nitrogen, mmol/l	5.30 (4.00, 6.00)	5.30 (4.00, 6.00)	0.833
sCr, μmol/l	81.0 (71.0, 88.0)	81.0 (75.0, 90.5)	0.301
sUA, mg/dl	7.58 (2.18)	7.72 (2.26)	0.370
CRP, mg/l	5.61 (2.63, 25.11)	5.50 (3.12, 22.00)	0.301

Data are presented as *n* (%), mean (S.D.) or median (interquartile range). [a]At least 20 cigarette packs in a lifetime or at least one cigarette a day for at least 1 year. [b]Alcohol intake at least once a week for 6 months. [c]Mean cumulative exercise time per week of >30 min/day. [d]History of hypertension, cardiac disease, type 2 diabetes or nephrolithiasis. [e]Treatment within 2 weeks before gout flare and blood sample collection. ULT: urate-lowering treatment; ALT: alanine aminotransferase; AST: aspartate aminotransferase; TG: triglyceride; sCr: serum creatinine; sUA: serum uric acid.

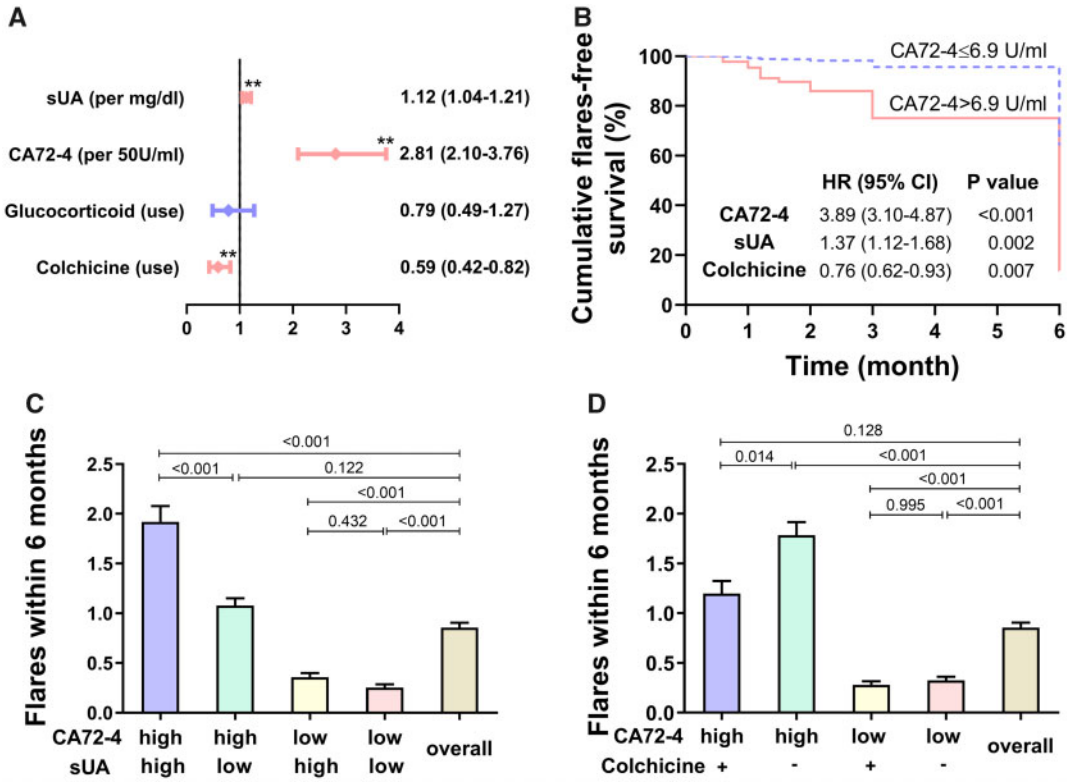
follow-up using a multivariable Cox proportional hazards model. Among nine biological variables potentially associated with flares, multivariable analysis identified high CA72-4 [>6.9 U/ml; hazard ratio (HR) = 3.889] as the strongest factor to predict flares, whereas sUA above the median value (HR = 1.373) and prophylactic colchicine (HR = 0.759) were weak factors associated and inversely associated with flares, respectively (Fig. 3B). CRP, tophi, triglyceride, BMI, NSAIDs and fasting glucose were not significantly associated with gout flares (supplementary Table S3, available at *Rheumatology* online).

In general, counting of flares based on CA72-4 level, baseline sUA level and prophylactic colchicine indicated that CA72-4 was the major determinant of flares (Fig. 3C and D). However, the probability of flares in patients with high sUA and high CA72-4 was higher than that in patients with high CA72-4 and low sUA (Fig. 3C). When CA72-4 was low, baseline sUA did not alter the flare frequency (Fig. 3C). Although the use of colchicine in patients with high CA72-4 reduced the flare frequency (Fig. 3D), colchicine did not alter the flare frequency when CA72-4 was low (Fig. 3D). The presence of tophi had no effect on gout flare frequency, whereas CA72-4 level was critical for the gout attack (supplementary Fig. S3, available at *Rheumatology* online). Receiver-operating characteristic curve analysis showed that CA72-4 had the highest area under the curve among CA72-4, sUA, CRP and tophi (0.82, 95% CI: 0.79, 0.85; 0.57, 95% CI: 0.52, 0.60; 0.51, 95% CI: 0.48, 0.55; 0.51, 95% CI: 0.47, 0.54, respectively, $P < 0.001$) (Fig. 4).

Discussion

CA72-4 is a mucin-like glycoprotein complex that can be identified by mAb B72.3, a murine antibody raised against a membrane-enriched fraction from a human breast carcinoma liver metastasis [6, 7]. Despite great efforts, CA72-4 has never been cloned [23, 24]. Evidence indicates that CA72-4 is not encoded by a single mucin gene; instead, the sialosyl-α(2–6)-*N*-acetylgalactosamine-α-serine structure carried by the unknown mucin core protein is the antigen [8, 9]. Clinically, CA72-4 has been found in a variety of human adenocarcinomas [10, 25]. However, accumulating evidence has shown that elevated CA72-4 is expressed in malignant diseases as well as non-cancer diseases, including

FIG. 3 CA72-4 level-based prediction of future flares in a cohort of gout patients



(**A**) Multiple logistic regression models were constructed for selecting baseline variables to assess independence, priority and confounding potentiality. (**B**) Kaplan–Meier analysis of the accumulated flare-free survival curves according to CA72-4 level (≤6.9 or >6.9 U/ml). (**C**) Number of flares in gout patients ($n = 722$) with distinct CA72-4 levels (≤6.9 or >6.9 U/ml) and sUA levels (<420 or ≥420 mmol/l). (**D**) Number of flares in gout patients ($n = 722$) with distinct CA72-4 levels (≤6.9 or >6.9 U/ml) and prophylactic colchicine use. ANOVA was used for statistical analyses of flares among the various groups. Double asterisk represents $P < 0.01$.

pancreatic and biliary diseases, type 2 diabetes and liver disorders, indicating that CA72-4 is not a unique product of cancer cells [26, 27]. Actually, glycosylation is the most diverse and common posttranslational modification of proteins. In association with systemic abnormalities like cancer and gout, cell membrane glycan structures often exhibit dramatic changes and incomplete synthesis followed by accumulation of precursor structures, which are proposed to be responsible for generating the sialosyl-α(2–6)-*N*-acetylgalactosamine-α-serine structures [28, 29]. It will be critical to understand the molecular mechanisms underlying the increased CA72-4 levels in patients suffering gout. Inflammation is the common feature for all these non-cancer diseases, prompting an investigation of CA72-4 as well as other glycoprotein cancer biomarkers in such disorders. The present study demonstrated that CA72-4, a known cancer biomarker, was aberrantly highly expressed in gout patients. Therefore, we explored its clinical utility in predicting future flares in a prospective cohort study. Our initial discovery confirmed that mean serum CA72-4 levels in gout patients were much higher than those in healthy subjects and hyperuricaemia patients. Assuming ≤6.9 U/ml as the reference normal range, ~40% of gout patients had aberrantly high CA72-4 levels, and a few patients exceeded the detection limit (300 U/ml). CA72-4 belongs to a glycoprotein family that includes CA19-9, CA125 and CEA, which have been approved for monitoring various gastrointestinal and gynaecological malignancies [30]. We found that only CA72-4, and not the three other common cancer biomarkers, was elevated in gout patients. Similar results were reported by another study on an autoinflammatory disease, FMF, in which patients also had elevated levels of CA72-4 but not the other three glycoproteins [31]. These data clearly indicate that elevated CA72-4 in this gout population was driven by gout itself rather than any cancer disorders.

Hyperuricaemia predisposes patients to gout, but is largely asymptomatic [32]. Gout flares are self-limiting and are followed by an intercritical phase. There are distinct clinical manifestations in terms of symptoms and parameters in laboratory tests among asymptomatic hyperuricaemia, acute gouty arthritis, intercritical-phase gout and non-gouty arthritis patients. sUA level does not predict flares and is not a useful biomarker for gout diagnosis. CRP is a common marker for inflammation

Table 2 Multiple linear regression analysis of correlations between variables and flare frequency in a prospective gout cohort ($n = 722$)

Variables	B	95% CI	*P*-value
Age, years	−0.002	−0.008, 0.004	0.511
BMI, kg/m^2	−0.008	−0.036, 0.021	0.588
Tophi present	0.018	−0.215, 0.252	0.876
CA72-4, U/ml	0.005	0.004, 0.006	<0.001
sUA, mg/dl	0.109	0.068, 0.150	<0.001
Glucose, mmol/l	−0.008	−0.105, 0.089	0.869
Type 2 diabetes[a]	0.187	−0.180, 0.554	0.316
TG, mmol/l	0.019	−0.052, 0.091	0.598
CRP, mg/l	−0.001	−0.004, 0.002	0.374
Colchicines[b]	−0.321	−0.503, −0.139	0.001
NSAIDs[b]	−0.213	−0.429, 0.002	0.053
Glucocorticoids[b]	−0.411	−0.687, -0.136	0.004

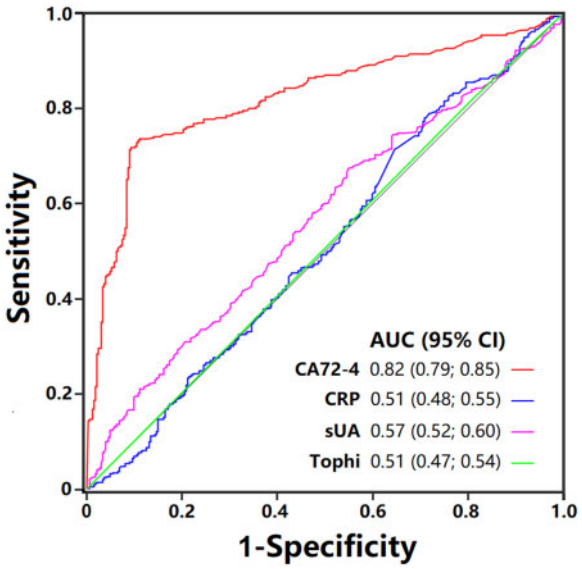
[a]History of type 2 diabetes. [b]Treatment during 6 months follow-up. sUA: serum uric acid; TG: triglyceride.

during gout flares. However, our study showed that CRP levels in gout patients were similar to those in RA and SpA patients, which diminished its value as a gout-specific marker. Generally, gouty arthritis lacks specific markers, unlike ACPA in RA and positive germ culture in SA [33], which allow them to be distinguished from other forms of arthritis. We examined other major arthritis forms: RA, SpA, OA and SA. Overall, CA72-4 levels were ranked from high to low in the order of gouty arthritis patients > hyperuricaemia patients = non-gouty arthritis patients = healthy subjects, indicating that high CA72-4 may be specific to gouty arthritis.

The selective expression of CA72-4 was also distinct from the CRP expression pattern in gouty arthritis and non-gouty arthritis patients, implying that CA72-4 was gouty arthritis-dependent, but independent of general inflammatory activity. However, CA72-4 levels were not high in hyperuricaemia patients, indicating that its level was related to a status prone to gouty activation rather than serum urate. Furthermore, patients with more frequent recent flares had much higher CA72-4 levels than patients with less frequent recent flares, indicating that CA72-4 may reflect disease stability (active or relatively stable) in gouty arthritis. Therefore, CA72-4 levels might predict gout flares.

A 6-month prospective cohort study of enrolled gout patients was conducted to determine the predictive value of CA72-4 for future gout flares. Among the clinical variables analysed, sUA above median value (HR = 1.373) was mildly associated with future gout flares, while prophylactic colchicine (HR = 0.759) was negatively associated with gout flares, consistent with previous studies [34, 35]. As expected, CA72-4 (HR = 3.889) was the strongest independent factor for predicting a flare in the prospective gout cohort. Logistic regression analysis confirmed that when CA72-4 increased by 50 U/ml, the flare risk increased 2.81-fold. Receiver-operating characteristic curve analysis

Fig. 4. Results of the receiver-operating characteristic curve analysis



The sensitivity and specificity of the four indexes are shown.

revealed that CA72-4 (area under the curve 0.82), but not sUA, CRP or presence of tophi (area under the curve 0.57–0.51), might predict gout flares. Thus, the prospective cohort study strongly supports the opinion that CA72-4 is a predictor of future gout flares.

Gout attack may be triggered by cold, physical exhaustion, trauma or severe changes in serum urate levels. However, there is a lack of clinically useful predictors or prognostic factors for gout flares. To the best of our knowledge, CA72-4 is the first serum-derived biomarker that specifically indicates gouty arthritis and predicts flares in the near future. Whether tophi is a flare-predictor is controversial [36]. Our data imply that tophi is not a convincing indicator for colchicine use, because tophi were not associated with flares in the prospective cohort, whereas CA72-4 level, but not presence of tophi, determined gout flare frequency. Current opinion suggests tophi may predict flares during the initiation of ULT because of the potential release of urate from tophi and consequent re-crystallization. This is coincident with the EULAR and ACR recommendations on gout treatment, in which prophylactic colchicine is recommended during the initiation of ULT for 6 months in gout patients with tophi, even after achieving the target sUA level [14, 19]. Our data suggest that prophylactic colchicine provides flare protection in patients with high CA72-4 levels, but may not be required in patients with low CA72-4 levels.

Although the findings of this pilot study are promising, there is no biological mechanistic explanation for the link between CA72-4 and gouty arthritis. Baseline data in the gout cohort showed that patients with low CA72-4 levels had more glucocorticoid use, fewer flares and

lower blood glucose levels than patients with high CA72-4 levels. Either glucocorticoids had an impact on CA72-4 expression or patients with high levels of CA72-4 had severe disease as manifested in the prospective cohort and were more often treated with glucocorticoids. However, the logistic regression model and Cox proportional hazards model confirmed that CA72-4, but not glucocorticoid use, was a predictor of gout flare. Based on these results, we hypothesize that glucocorticoid use lowers CA72-4 levels. Glucocorticoid is an anti-inflammatory drug with multiple targets including the NLRP3 inflammasome complex and subsequent IL-1β pathway, which is a major pathogenesis mechanism in gouty arthritis [37, 38]. The current results suggest a potential relationship between CA72-4 and the NLRP3 inflammasome. Several studies support this hypothesis. Serum CA72-4 levels were elevated in patients with FMF, a known NLRP3 inflammasome-related autoinflammatory disease, and were associated with the frequency of attacks [31]. Type 2 diabetes is considered to involve NLRP3-driven inflammation [39]. Shang *et al.* found that serum CA72-4 was related to hyperglycaemia as well as poor diabetes status [40]. Because CA72-4 is more likely a marker for immune pathway activation, it is reasonable to assume that glucocorticoid use will decrease CA72-4 levels. A prospective randomized trial is required to prove this.

One of the limitations of the present study was its observational nature. Because of the poor understanding of the mechanism for CA72-4 in gout, there is no way to identify a possible intervention with clear efficacy for the disease. Furthermore, CA72-4 can be influenced by many factors in addition to gouty arthritis; therefore, CA72-4 is unlikely to be well controlled in a randomized interventional study. Whether the findings of CA72-4 expression in the current study can be expanded to other races is uncertain, because all subjects in the current study were Chinese. Given the power of the sample size in the study, we could not observe a temporal relationship or exposure-based response for the predictive value of CA72-4.

In the current study, we observed the unexpected and specifically high expression of CA72-4 in gout patients with active status for flares. The present results have profound implications: (i) CA72-4 might be a useful routine test for gout management given its potential use as a marker reflecting gout stability, and thus indicating the chance of future flares; (ii) high CA72-4 levels indicate a relatively active status in gouty arthritis and prophylactic colchicine should be administered to these patients during the initiation of ULT; (iii) the biology of CA72-4 in gout warrants further investigation; and (iv) the current data do not provide evidence that conventional ULT reduces CA72-4 levels in gout patients. CA72-4 as a treatment target requires further investigation.

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Supplementary data

Supplementary data are available at *Rheumatology* online.

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