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| Journal: | *Rheumatology* |
| Manuscript ID | RHE-19-1392.R2 |
| Manuscript Type: | Original Article |
| Date Submitted by the Author: | 24-Nov-2019 |
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| Keywords<br>Please select a minimum FIVE keywords from the list provided. These keywords will be used to select reviewers for this manuscript. The keywords in the main text of your paper do not need to match these words.: | Crystal arthropathies < RHEUMATIC DISEASES, Biochemistry < BASIC & CLINICAL SCIENCES, Inflammation < BASIC & CLINICAL SCIENCES, Biomarkers < DIAGNOSTIC METHODS, Primary care rheumatology < HEALTH SERVICES AND PRACTICE |

# 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 comprehensive 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 (osteoarthritis [OA], rheumatoid arthritis [RA], spondyloarthritis [SpA], septic arthritis [SA]), 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 (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 (SA, 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 (≥2 times in the last year) had higher CA72-4 levels than patients with fewer flares (<2 times 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 biomarker to predict future flares.


**Keywords:** CA72-4, biomarker, gout, flare

**Rheumatology key messages**

- CA72-4 was specific upregulated in patients with gouty arthritis, but not with other major arthritis.
- 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 (MSU) crystal deposition in joints[1]. Hyperuricaemia is the prelude to gout. Only 10% of patients with hyperuricaemia develop gout, and high serum uric acid (sUA) level is not always associated with gout flares[2]. In gouty arthritis, MSU crystals induce 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 and other non-gout diseases, such as familial Mediterranean fever, type 2 diabetes mellitus, and Alzheimer 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 tumor 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 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 (rheumatoid arthritis [RA], spondyloarthritis [SpA], osteoarthritis [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 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) gout classification criteria[14]. Asymptomatic hyperuricaemia was defined as fasting sUA >7 mg/dl detected at least twice on 2 separate days in patients on a normal purine diet (defined as avoiding alcohol and purine-rich food such as seafood, 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 ≥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 C-reactive protein (CRP) levels measured. Those with elevated CA72-4 were transferred to gastroenterologists and screened for neoplastic diseases. The

exclusion criteria included: (a) presence or history of malignant disease; (b) pregnant or lactating women; (c) chronic renal failure (estimated glomerular filtration rate (eGFR) <15 ml/min/1.73 $m^2$); and (d) 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, withdraw 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 (**Figure 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 nonsteroidal anti-inflammatory drugs (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 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, body mass index (BMI), sUA, CRP, transaminase, fasting glucose, and eGFR, 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 Diagnostic GmbH, Germany).). According to the kit instructions, the normal range 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 (standard deviation), or median (interquartile range), frequency or percent as appropriate. Comparisons of baseline demographic and clinical measures between different groups were undertaken using $\chi^2$ 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 (ROC) 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 (**Figure 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% gout patients' CA72-4 levels exceeded the upper limit of the normal reference range (>6.9 U/ml) (**Figure 2A, 2B**). Interestingly, patients who experienced only one flare in the last year before enrolment had similar CA72-4 levels with patients who did not have any flare, while patients with more frequent flares (≥2 per year)[21, 22] had higher serum CA72-4 levels than patients who experienced fewer flares (0 or 1 per year) (**Figure 2B**). CA72-4 levels in asymptomatic hyperuricaemia patients and healthy subjects were within the normal reference range (**Figure 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 mmol/L *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 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) (**Figure 2C**). We also monitored sUA and CRP, a parameter reflecting overall inflammatory activity, and found that sUA levels were higher in gout and hyperuricaemia patients than in non-gouty arthritis patients (**Supplementary Figure S1, available at *Rheumatology* online**). 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 with that in hyperuricaemia and healthy subjects (**Supplementary Figure S2, available at *Rheumatology* online**).

Furthermore, serum CEA, CA 19-9, and CA 125 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 (**Figure 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 (**Figure 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, OR=2.81, 95%CI (2.10–3.76), *P*<0.001), sUA (OR=1.12, 95%CI (1.04–1.21), *P*=0.003) and colchicine use (OR=0.59, 95%CI (0.42–0.82), *P*=0.002) as independent predictors for future flare risk (**Figure 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) (**Figure 3B**). We investigated factors associated with the cumulated flares during the 6-months' 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; HR=3.889) as the strongest factor to predict flares where as sUA above the median value (HR=1.373) and prophylactic colchicine (HR=0.759) were weak factors associated and inversely associated with flares, respectively (**Figure 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 (**Figure 3C, 3D**). 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 (**Figure 3C**). When CA72-4 was low, baseline sUA did not alter the flare frequency (**Figure 3C**). Although the use of colchicine in patients with high CA72-4 reduced the flare frequency (**Figure 3D**), colchicine did not alter the flare frequency when CA72-4 was low (**Figure 3D**). The presence of tophi had no effect on gout flare frequency, whereas CA72-4 level was critical for the gout attack (**Supplementary Figure S3, available at *Rheumatology* online**). Receiver operating characteristic (ROC) curve analysis showed that CA72-4 had the highest AUC 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) (**Figure 4**).

## Discussion

CA72-4 is a mucin-like glycoprotein complex that can be identified by monoclonal antibody B72.3, a murine antibody raised against a membrane-enriched fraction from a human breast carcinoma liver metastasis[6, 7]. Despite all the efforts, CA72-4 has never been cloned[23, 24]. Evidences indicate that CA72-4 is not encoded by a single mucin gene, instead, the sialosyl-α(2-6)-N-acetylgalactosamine-α-serine structures 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 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 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, approximately 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, familial Mediterranean fever, 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 during gout flares. However, our study showed that CRP levels in gout patients were similar to that in RA and SpA patients, which diminished its value as a gout-specific marker. Generally, gouty arthritis lacks specific markers, unlike anti-citrullinated peptide antibodies in RA and positive germ culture in septic arthritis[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 from 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 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 per 50 U/ml, the flare risk increased 2.81-fold. ROC curve analysis revealed that CA72-4 (AUC 0.82), but not sUA, CRP and presence of tophi (AUC 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 familial Mediterranean fever, 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: 1) 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; 2) 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; 3) the biology of CA72-4 in gout warrants further investigation; and 4) 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.

**Acknowledgements:** The authors thank the investigators in the trials. C.L. conceived the study and, together with X.W., X.B., and M.S., designed, obtained, and analysed the majority of the data. M.S. and X.B. wrote, and C.L. and Y H. edited, the manuscript. F.W. provided statistical support. Y.H., R.L., L.C., C.W., M.W., X.L., and H.L. conducted the clinical work.

*Funding*: This work was supported by research project grants from the National Key Research and Development Program (#2016YFC0903400), National Natural Science Foundation of China (#81520108007, #81770869), and Shandong Province Key Research and Development Program (#2018CXGC1207).

*Disclosure statement:* The authors have declared no conflicts of interest.

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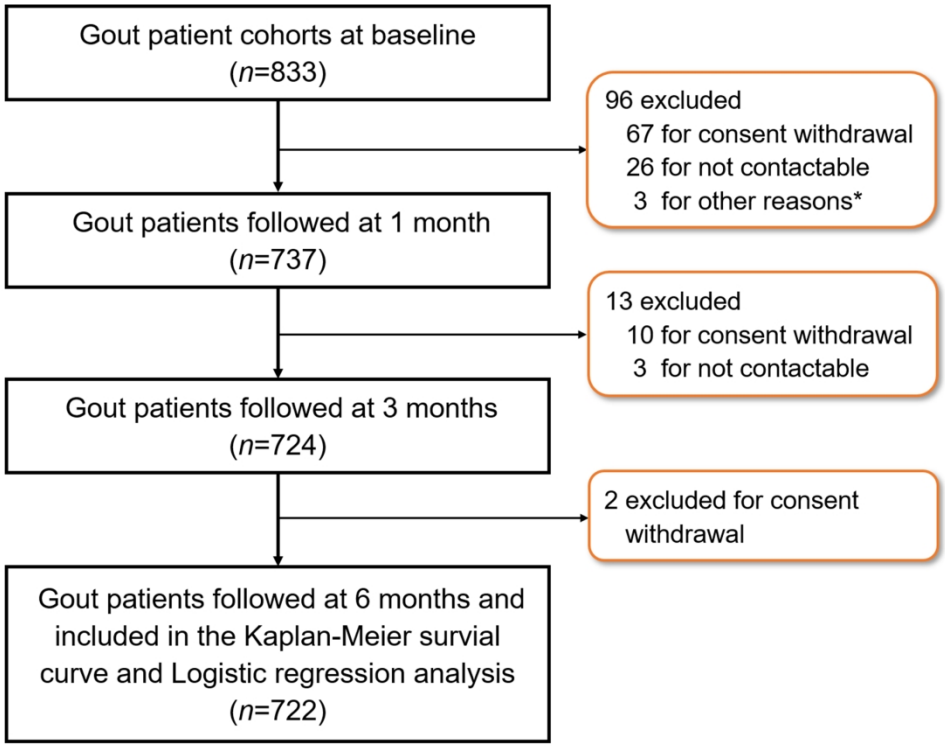
## FIGURE LEGENDS

**Figure 1. Overall study design.** Asterisk (*) represents unable to commit time, poor health condition, or subjected to surgery.

**Figure 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); 1 flare within 1 year (*n*=691); ≥2 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), rheumatoid arthritis patients (*n*=532), spondyloarthritis patients (*n*=243), osteoarthritis 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.

**Figure 3. CA72-4 level-based prediction of future flares in a cohort of gout patients. A.** Multiple logistic regression models were constructed for selected 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 $P<0.01$.

**Figure 4. Results of the receiver-operating characteristic curve analysis.** The sensitivity and specificity of the four indexes are shown.

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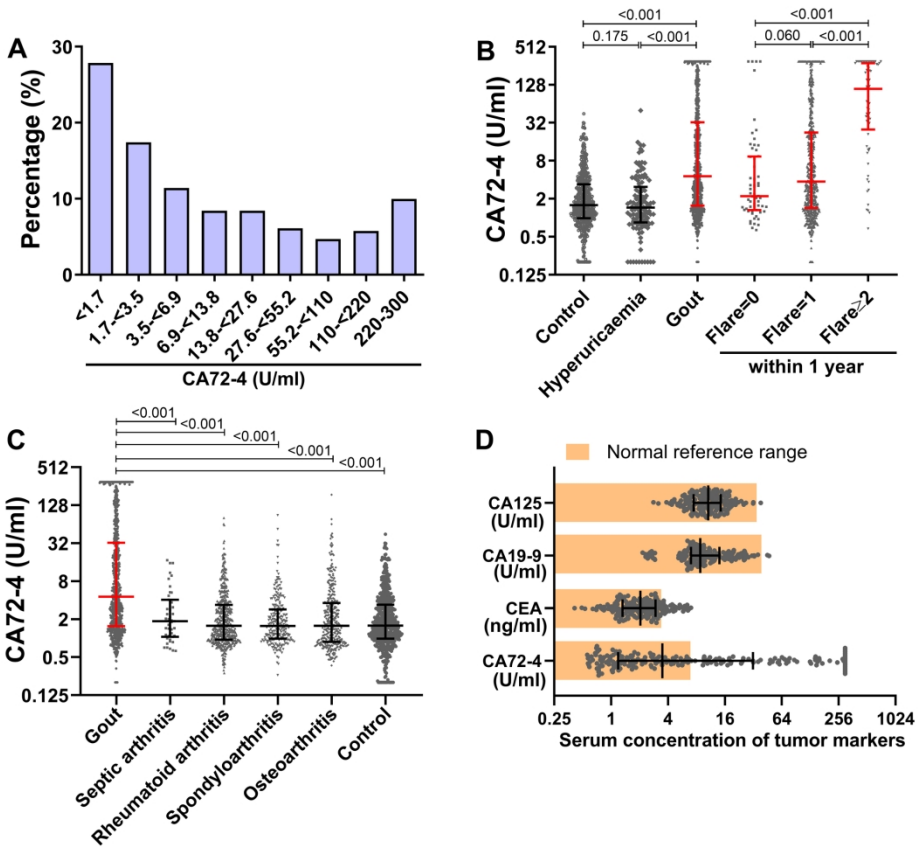


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**Table 1. Demographic and baseline clinical characteristics in gout patients (n=833).**

| 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 |
| **[a]Smoking, n (%)** | 131 (27.75) | 116 (32.13) | 0.170 |
| **[b]Drinking, n (%)** | 290 (61.44) | 206 (57.06) | 0.202 |
| **[c]Physical exercise, n (%)** | 126 (26.69) | 114 (31.58) | 0.123 |
| **[d]Hypertension, n (%)** | 130 (27.54) | 104 (28.81) | 0.687 |
| **[d]Cardiac disease, n (%)** | 20 (4.24) | 16 (4.43) | 0.891 |
| **[d]Type 2 diabetes, n (%)** | 22 (4.66) | 59 (16.34) | <0.001 |
| **[d]Nephrolithiasis, n (%)** | 28 (5.93) | 16 (4.43) | 0.891 |
| **[e]Colchicines, n (%)** | 208 (44.07) | 165 (45.71) | 0.637 |
| **[e]NSAIDs, n (%)** | 128 (27.12) | 99 (27.42) | 0.922 |
| **[e]Glucocorticoids, 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 |

BMI: body mass index; NSAIDs: non-steroidal anti-inflammatory drugs; ULT: urate-lowering treatment; ALT: alanine aminotransferase; AST: aspartate aminotransferase;

TG: triglyceride; sCr: serum creatinine; sUA: serum uric acid; CRP: C-reactive protein.

[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 more than 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.

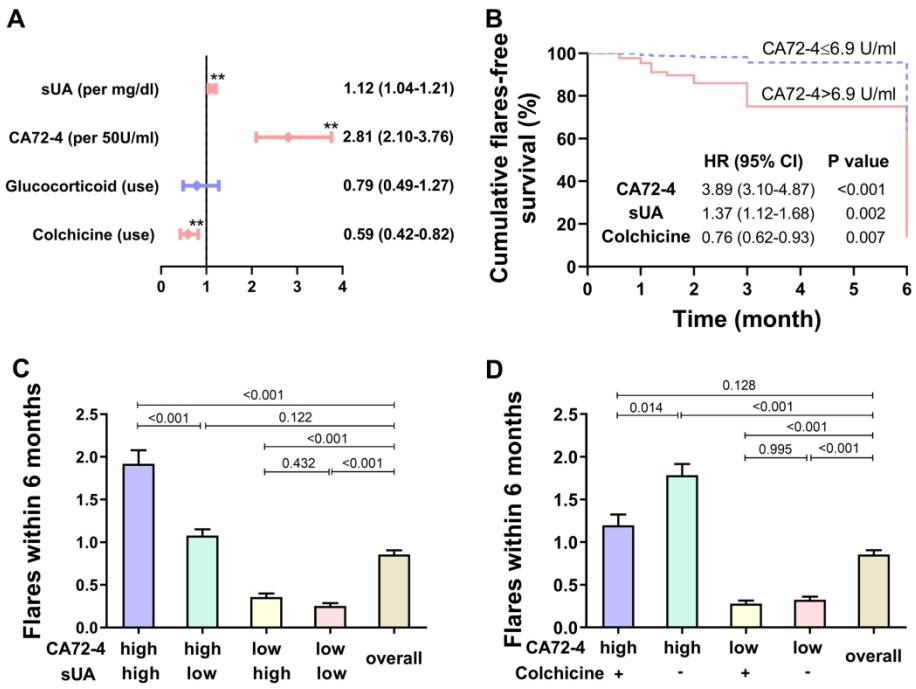
**Table 2. Multiple linear regression analysis of correlations between variables and flare frequency in a prospective gout cohort (*n*=722)**

| Variables | B | 95% CI | *P*-value |
|---|---|---|---|
| **Age, years** | -0.002 | -0.008, 0.004 | 0.511 |
| **BMI, kg/m²** | -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 |
| **[a]Type 2 diabetes** | 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 |
| **[b]Colchicines** | -0.321 | -0.503, -0.139 | 0.001 |
| **[b]NSAIDs** | -0.213 | -0.429, 0.002 | 0.053 |
| **[b]Glucocorticoids** | -0.411 | -0.687, -0.136 | 0.004 |

[a]History of type 2 diabetes.

[b]Treatment during 6 months follow-up.

BMI: body mass index; sUA: serum uric acid; TG: triglyceride; CRP: C-reactive protein; NSAIDs: non-steroidal anti-inflammatory drugs.

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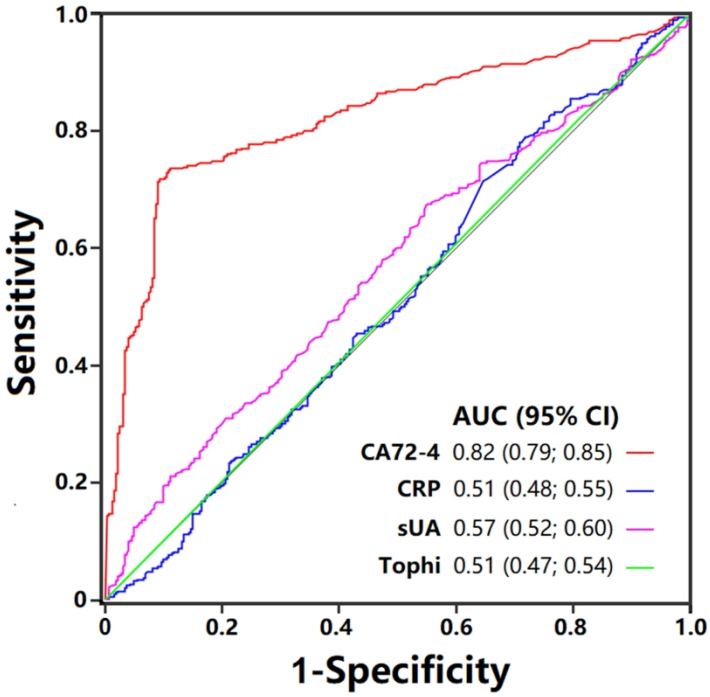


Figure 4. Receiver-operating characteristic curve showing the capacity of CA72-4 (red), CRP (blue), sUA (pink), or tophi (green) to predict gout flares. The sensitivity and specificity of the four indexes are shown.

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## SUPPLEMENTARY MATERIAL

**Supplementary Table S1. Demographic data and baseline clinical characteristics among participants with different forms of arthritis or healthy controls**

| | Gout (*n*=833) | Hyperuricaemia (*n*=120) | Septic arthritis (*n*=43) | Rheumatoid arthritis (*n*=532) | Spondyloarthritis (*n*=243) | Osteoarthritis (*n*=474) | Control (*n*=541) |
|---|---|---|---|---|---|---|---|
| Male, n (%) | 750 (90.04) | 116 (96.67) | 22 (51.16) | 140 (26.32) | 172 (70.78) | 156 (42.98) | 479 (88.54) |
| Age, years | 50 (37-62) | 42 (35-51) | 51 (34-73) | 64 (56-71) | 44 (33-53) | 63 (55-72) | 52 (43-59) |
| sUA, mg/dl | 7.64 (2.22) | 7.98 (0.79) | 4.83 (2.02) | 5.71 (1.59) | 5.11 (1.28) | 5.16 (1.78) | 5.65 (1.58) |
| ALT, U/l | 27.00 (17.00-40.00) | 25.00 (17.00-38.00) | 32.00 (22.00-54.00) | 28.00 (19.00-43.00) | 28.00 (18.00-44.00) | 28.00 (19.00-47.00) | 16.00 (12.00-23.00) |
| AST, U/l | 20.00 (17.00-26.00) | 21.00 (18.00-27.00) | 22.00 (19.00-28.00) | 21.00 (17.00-27.00) | 22.00 (18.00-28.00) | 21.00 (18.00-28.00) | 18.00 (15.00-23.00) |
| Glucose, mmol/l | 5.32 (4.85-5.89) | 5.47 (5.05-5.99) | 5.50 (5.05-6.00) | 5.67 (5.25-6.12) | 5.54 (5.19-6.04) | 5.63 (5.27-6.18) | 4.41 (4.05-4.78) |
| TG, mmol/l | 1.70 (1.09-2.37) | 1.58 (1.08-2.58) | 2.06 (1.48-2.71) | 1.87 (1.31-2.56) | 1.94 (1.38-2.95) | 1.85 (1.29-2.74) | 0.99 (0.73-1.34) |
| sCr, μmol/l | 81.00 (67.00-88.00) | 79.00 (70.00-85.00) | 78.00 (67.00-89.00) | 81.00 (72.00-90.00) | 83.00 (74.00-97.00) | 81.00 (73.00-91.00) | 62.00 (51.00-74.00) |
| CRP, mg/l | 5.51 (2.79-23.22) | 4.20 (1.51-19.98) | 24.53 (10.05-74.85) | 12.67 (6.69-25.70) | 11.13 (6.56-24.50) | 5.91 (2.89-11.02) | 3.96 (2.03-6.79) |
| CA72-4, U/ml | 4.55 (1.56-32.64) | 1.45 (0.84-3.09) | 1.38 (0.99-2.66) | 1.58 (0.95-3.37) | 1.56 (0.98-2.85) | 1.56 (0.88-3.50) | 1.59 (0.99-3.39) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Glucocorticoid, n (%) | 99 (11.88) | 0 | 0 | 172 (32.33) | 3 (1.23) | 0 | 0 |

sUA: serum uric acid; ALT: alanine aminotransferase; AST: aspartate aminotransferase; TG: triglyceride; sCr: serum creatinine; CRP: C-reactive protein.

**Supplementary Table 2. Multiple linear regression analysis of correlations between variables and CA72-4 level in gout patients (*n*=833)**

| Variables | B | 95% CI | *P*-value |
|---|---|---|---|
| Age, years | 0.155 | -0.295, 0.605 | 0.499 |
| BMI, kg/m$^2$ | -0.387 | -2.395, 1.621 | 0.706 |
| Tophi present | -7.735 | -24.096, 8.627 | 0.354 |
| Flare frequencies | 28.676 | 14.982, 42.371 | <0.001 |
| sUA, mg/dl | -0.734 | -3.651, 2.183 | 0.622 |
| Glucose, mmol/l | 15.878 | 8.688, 23.089 | <0.001 |
| [a]Type 2 diabetes | -6.715 | -36.403, 22.972 | 0.657 |
| [b]Colchicine | 3.580 | -9.422, 16.582 | 0.589 |
| [b]NSAIDs | -2.852 | -17.668, 11.964 | 0.706 |
| [b]Glucocorticoid | -16.758 | -36.608, 3.093 | 0.098 |

[a]History of type 2 diabetes

[b]Treatment within 1 month before enrollment.

BMI: body mass index; sUA: serum uric acid; NSAIDs: non-steroidal anti-inflammatory drugs.

**Supplementary Table 3. Multivariable Cox proportional regression analysis of explanatory variables for flare frequency during 6 months follow-up (*n*=722)**

| Variables | P value | HR | 95% CI |
|---|---|---|---|
| CA72-4, U/ml | 0.000 | 3.889 | 3.104-4.874 |
| CRP, mg/l | 0.680 | 0.958 | 0.782-1.174 |
| Tophi present | 0.765 | 1.040 | 0.804-1.347 |
| TG, mmol/l | 0.946 | 0.993 | 0.815-1.210 |
| BMI, kg/m$^2$ | 0.962 | 0.996 | 0.859-1.155 |
| [a]NSAIDs | 0.158 | 0.839 | 0.658-1.071 |
| [a]Glucocorticoid | 0.519 | 0.900 | 0.655-1.238 |
| Glucose, mmol/l | 0.733 | 1.060 | 0.757-1.185 |
| [a]Colchicine | 0.007 | 0.759 | 0.621-0.927 |
| sUA, mg/dl | 0.002 | 1.373 | 1.124-1.677 |

All variables were categorical data. [a]Treatment during 6 months follow-up. The cut-off values were defined as: CA72-4: ≤ or > normal upper limit (6.9 U/ml); CRP: ≤ or > normal upper limit (5 mg/l); triglyceride: ≤ or > median level (1.69 mmol/l); BMI: ≤ or > 24 kg/m$^2$; glucose: ≤ or > normal upper limit (7.0 mmol/l); and sUA: ≤ or > median level (7.64mg/dl). CRP: C-reactive protein; TG: triglyceride; BMI: body mass index; NSAIDs: non-steroidal anti-inflammatory drugs; sUA: serum uric acid.

**Supplementary Table 4. Spearman rank correlation coefficients between CA72-4 level and other characteristics of gout patients (r (*P*))**

| | CA72-4 | CRP | sUA | Tophi |
|---|---|---|---|---|
| **CRP** | -0.036 (0.328) | - | | |
| **sUA** | 0.028 (0.452) | 0.106 (0.004) | - | |
| **Tophi** | -0.047 (0.211) | 0.060 (0.107) | 0.034 (0.365) | - |
| **Age** | -0.046 (0.215) | 0.144 (0.001) | -0.054 (0.145) | 0.022 (0.561) |

CRP: C-reactive protein; sUA: serum uric acid.

**Supplementary figure legends**

**Supplementary Figure S1. Comparison of sUA levels among acute gouty arthritis patients (*n*=833).**

Hyperuricaemia patients (*n*=120), SA patients (*n*=43), RA patients (*n*=532), SpA patients (*n*=243), OA patients (*n*=474), and healthy controls (*n*=541). ANOVA was performed for statistical analysis.

sUA: serum uric acid; SA: septic arthritis; RA: rheumatoid arthritis; SpA: spondyloarthritis; OA: osteoarthritis.

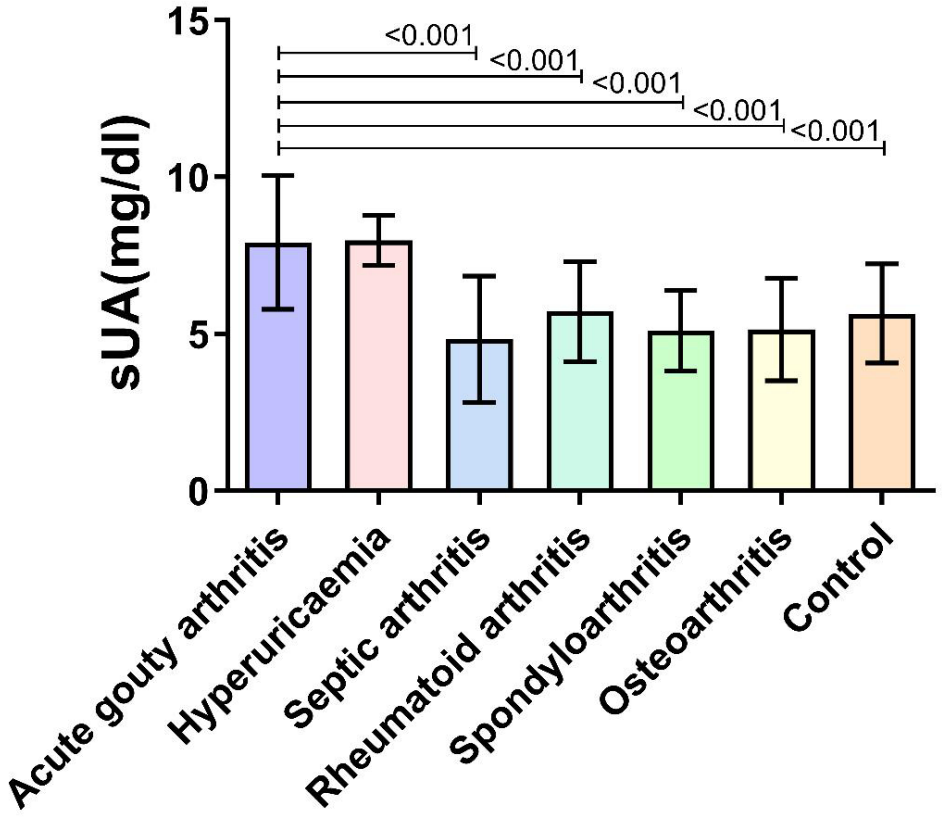
**Supplementary Figure S2. Comparison of serum CRP levels among acute gouty arthritis patients (*n*=833),**

SA patients (*n*=43), RA patients (*n*=532), SpA patients (*n*=243), OA patients (*n*=474), hyperuricaemia patients (*n*=120) and healthy controls (*n*=541). The Mann–Whitney *U*-test was performed for statistical analysis.

CRP: C-reactive protein; SA: septic arthritis; RA: rheumatoid arthritis; SpA: spondyloarthritis; OA: osteoarthritis.

**Supplementary Figure S3. Comparisons of flare frequencies between gout patients (n=722).** The mean flare frequency within 6 months in the indicated groups is shown.

ANOVA was performed for statistical analysis of flares among the various groups.

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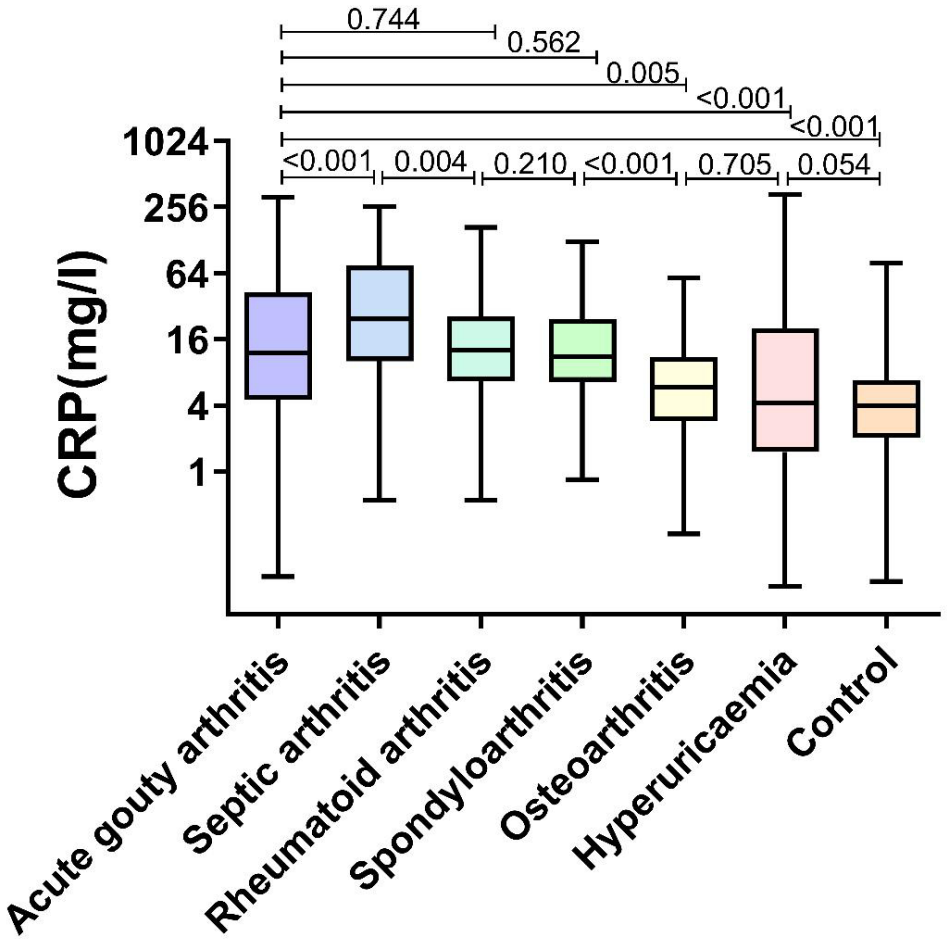


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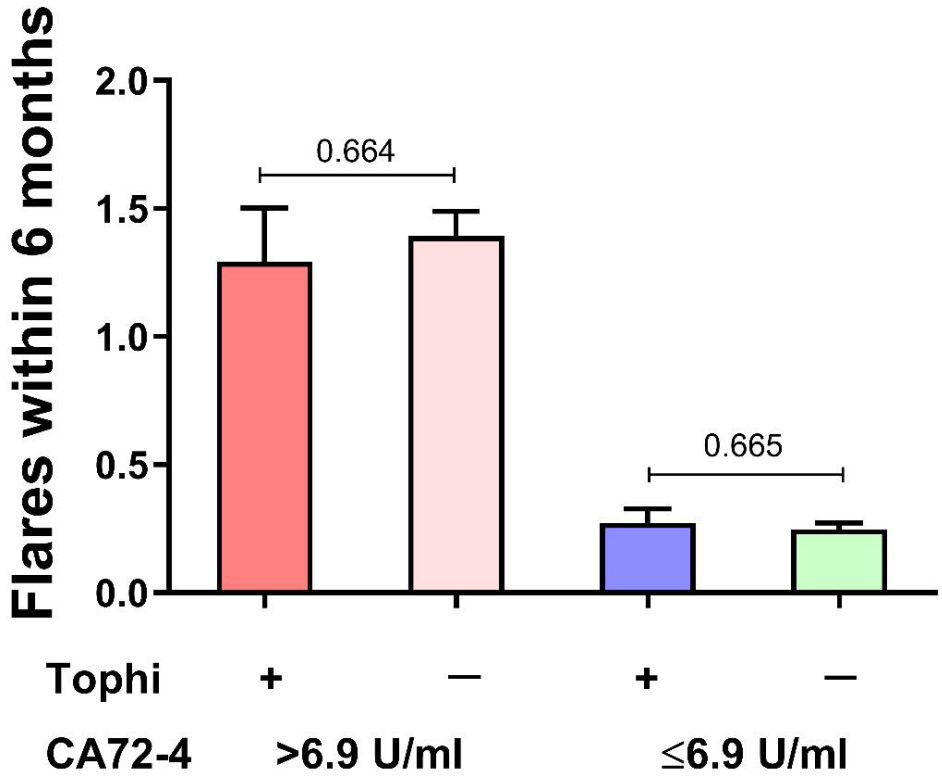


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