

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

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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-1 β dependent innate inflammatory processes^[3]. However, the inflammasome complex and IL-1 β 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

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4 performed a 6-month prospective observational cohort study of the gout patients to test and verify the
5 hypothesis that CA72-4 is a predictor of future flares. During the 6-month follow-up, gout flares,
6 CA72-4 levels, and other gout-related clinical variables were observed at 1, 3, and 6 months. Other
7 glycoprotein cancer markers including serum carcinoembryonic antigen (CEA), CA125, and CA19-9,
8 were also examined in patients with gouty arthritis. The study was approved by the Ethics Committee
9 of the Affiliated Hospital of Qingdao University and all study participants provided written informed
10 consent.
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17 **Study population**

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19 Between June 2016 and May 2018, patients with gout or asymptomatic hyperuricaemia who visited
20 the dedicated Gout Clinic at the Affiliated Hospital of Qingdao University were consecutively
21 recruited, as were patients with RA, SpA, OA, or SA who visited the Department of Rheumatology.
22 The diagnoses were confirmed by senior gout experts and rheumatologists. Gout patients were
23 diagnosed according to the 2015 American College of Rheumatology/European League Against
24 Rheumatism (ACR/EULAR) gout classification criteria^[14]. Asymptomatic hyperuricaemia was
25 defined as fasting sUA >7 mg/dl detected at least twice on 2 separate days in patients on a normal
26 purine diet (defined as avoiding alcohol and purine-rich food such as seafood, red meat, especially
27 organ meat, but not purine-rich leafy-green vegetables) without previous or current arthritis. RA
28 patients included recent-onset and long-term patients who met the 2010 ACR/EULAR classification
29 criteria^[15]. SpA was diagnosed according to the Assessment of Spondyloarthritis International Society
30 classification criteria^[16]. Diagnoses of OA involving the hands and knees were established in
31 accordance with two classification standards^[17, 18]. SA was confirmed by synovial fluid germ culture
32 or based on clinical assessments. Any patients manifesting sophisticated arthritis or with uncertain
33 diagnosis according to the above criteria would not be recruited. Healthy controls, age and gender-
34 matched to gout patients, were recruited from the Health Examination Center at the hospital during the
35 same period, and defined as persons with normal sUA, benign joint conditions, and no diagnosis of
36 cancer or arthritis. All subjects were aged ≥ 18 years but were <80 years (**Supplementary Table S1,**
37 **available at *Rheumatology* online).**
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56 Informed consent and permission to use spare serum samples were obtained from all participants. All
57 subjects had their serum CA72-4, sUA, and C-reactive protein (CRP) levels measured. Those with
58 elevated CA72-4 were transferred to gastroenterologists and screened for neoplastic diseases. The
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4 exclusion criteria included: (a) presence or history of malignant disease; (b) pregnant or lactating
5 women; (c) chronic renal failure (estimated glomerular filtration rate (eGFR) <15 ml/min/1.73 m²);
6 and (d) hepatic dysfunction (alanine aminotransferase/total bilirubin greater than or equal to twice the
7 upper limit of normal range).
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11 Overall, 120 hyperuricaemia patients, 833 gout patients, 532 RA patients, 243 SpA patients, 474 OA
12 patients, 43 SA patients, and 541 healthy controls were enrolled. Patients who refused to follow the
13 procedure, withdraw their consent, or failed to meet the inclusion criteria for any other reason were
14 excluded.
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19 **Follow-up procedures**

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21 The gout cohort of 833 patients was followed for an additional 6 months after enrolment as a
22 prospective study to verify whether CA72-4 is a predictor of gout flares. Patients were followed at
23 baseline (time of enrolment), and at 1, 3, and 6 months after enrolment (**Figure 1**). All patients were
24 treated with a standard protocol according to the 2012 ACR guidelines^[19, 20]. Patients with a gout flare
25 were treated with colchicine and/or nonsteroidal anti-inflammatory drugs (NSAIDs) and sodium
26 bicarbonate. Patients in the intercritical phase or beyond 2 weeks after the flare were started on urate-
27 lowering therapy (ULT) and prophylactic low-dose colchicine for 3 months. Patients who had no flares
28 after 3 months of ULT ceased prophylactic colchicine. All patients were required to keep diaries of
29 flares and medication compliance. Gout flares, CA72-4 levels, and other gout-related clinical variables,
30 including medical history, presence or absence of tophi, body mass index (BMI), sUA, CRP,
31 transaminase, fasting glucose, and eGFR, were collected at every visit. For the gout patients, serum
32 CEA, CA125, and CA19-9 were also screened at baseline.
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44 **Outcome measurements**

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46 The serum samples were spare samples in the hospital laboratory reserved for biochemical purposes.
47 All samples were stored at -80°C for future use. The levels of CA72-4, CEA, CA125, and CA19-9
48 were measured by an electrochemiluminescence method using Elecsys kits (CA72-4/11776258 122,
49 CEA/11731629 322, CA125/11776223 190, CA19-9/11776193 122, Roche Diagnostic GmbH,
50 Germany). According to the kit instructions, the normal range of CA72-4, CEA, CA125, and CA
51 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
52 biochemical parameters were detected by enzyme colorimetric methods. CRP was detected by a latex-
53 enhanced turbidimetric assay using reagents and instrumentation provided by Goldsite (Shenzhen,
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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 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 (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

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4 low CA72-4 (72.3% vs 10.7%, $P<0.05$) (**Figure 3B**). We investigated factors associated with the
5 cumulated flares during the 6-months' follow-up using a multivariable Cox proportional hazards model.
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7 Among nine biological variables potentially associated with flares, multivariable analysis identified
8 high CA72-4 (>6.9 U/ml; HR=3.889) as the strongest factor to predict flares whereas sUA above the
9 median value (HR=1.373) and prophylactic colchicine (HR=0.759) were weak factors associated and
10 inversely associated with flares, respectively (**Figure 3B**). CRP, tophi, triglyceride, BMI, NSAIDs,
11 and fasting glucose were not significantly associated with gout flares (**Supplementary Table S3**,
12 **available at *Rheumatology* online**).

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19 In general, counting of flares based on CA72-4 level, baseline sUA level, and prophylactic colchicine
20 indicated that CA72-4 was the major determinant of flares (**Figure 3C, 3D**). However, the probability
21 of flares in patients with high sUA and high CA72-4 was higher than that in patients with high CA72-4
22 and low sUA (**Figure 3C**). When CA72-4 was low, baseline sUA did not alter the flare frequency
23 (**Figure 3C**). Although the use of colchicine in patients with high CA72-4 reduced the flare frequency
24 (**Figure 3D**), colchicine did not alter the flare frequency when CA72-4 was low (**Figure 3D**). The
25 presence of tophi had no effect on gout flare frequency, whereas CA72-4 level was critical for the gout
26 attack (**Supplementary Figure S3, available at *Rheumatology* online**). Receiver operating
27 characteristic (ROC) curve analysis showed that CA72-4 had the highest AUC among CA72-4, sUA,
28 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,
29 95%CI (0.47–0.54), respectively, $P<0.001$) (**Figure 4**).

40 Discussion

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42 CA72-4 is a mucin-like glycoprotein complex that can be identified by monoclonal antibody B72.3, a
43 murine antibody raised against a membrane-enriched fraction from a human breast carcinoma liver
44 metastasis^[6, 7]. Despite all the efforts, CA72-4 has never been cloned^[23, 24]. Evidences indicate that
45 CA72-4 is not encoded by a single mucin gene, instead, the sialosyl- α (2-6)-N-acetylgalactosamine- α -
46 serine structures carried by the unknown mucin core protein is the antigen^[8, 9]. Clinically, CA72-4 has
47 been found in a variety of human adenocarcinomas^[10, 25]. However, accumulating evidence has shown
48 that elevated CA72-4 is expressed in malignant diseases as well as non-cancer diseases including
49 pancreatic and biliary diseases, type 2 diabetes and liver disorders, indicating that CA72-4 is not a
50 unique product of cancer cells^[26, 27]. Actually, glycosylation is the most diverse and common
51 posttranslational modification of proteins. In association with systemic abnormalities like cancer and
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4 gout, cell membrane glycan structures often exhibit dramatic changes and incomplete synthesis
5 followed by accumulation of precursor structures are proposed to be responsible for generating the
6 sialosyl- α (2-6)-N-acetylgalactosamine- α -serine structures^[28, 29]. It will be critical to understand the
7 molecular mechanisms underlying the increased CA72-4 levels in patients suffering gout.
8 Inflammation is the common feature for all these non-cancer diseases, prompting an investigation of
9 CA72-4 as well as other glycoprotein cancer biomarkers in such disorders. The present study
10 demonstrated that CA72-4, a known cancer biomarker, was aberrantly highly expressed in gout
11 patients. Therefore, we explored its clinical utility in predicting future flares in a prospective cohort
12 study. Our initial discovery confirmed that mean serum CA72-4 levels in gout patients were much
13 higher than those in healthy subjects and hyperuricaemia patients. Assuming ≤ 6.9 U/ml as the
14 reference normal range, approximately 40% of gout patients had aberrantly high CA72-4 levels, and a
15 few patients exceeded the detection limit (300 U/ml). CA72-4 belongs to a glycoprotein family that
16 includes CA19-9, CA125, and CEA, which have been approved for monitoring various gastrointestinal
17 and gynaecological malignancies^[30]. We found that only CA72-4, and not the three other common
18 cancer biomarkers, was elevated in gout patients. Similar results were reported by another study on an
19 autoinflammatory disease, familial Mediterranean fever, in which patients also had elevated levels of
20 CA72-4, but not the other three glycoproteins^[31]. These data clearly indicate that elevated CA72-4 in
21 this gout population was driven by gout itself rather than any cancer disorders.

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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.

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4 The selective expression of CA72-4 was also distinct from the CRP expression pattern in gouty arthritis
5 and non-gouty arthritis patients, implying that CA72-4 was gouty arthritis-dependent but independent
6 from general inflammatory activity. However, CA72-4 levels were not high in hyperuricaemia patients,
7 indicating that its level was related to a status prone to gouty activation rather than serum urate.
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9 Furthermore, patients with more frequent recent flares had much higher CA72-4 levels than patients
10 with less frequent recent flares, indicating CA72-4 may reflect disease stability (active or relatively
11 stable) in gouty arthritis. Therefore, CA72-4 levels might predict gout flares.
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17 A 6-month prospective cohort study of enrolled gout patients was conducted to determine the
18 predictive value of CA72-4 for future gout flares. Among the clinical variables analysed, sUA above
19 median value (HR=1.373) was mildly associated with future gout flares, while prophylactic colchicine
20 (HR=0.759) was negatively associated with gout flares, consistent with previous studies^[34, 35]. As
21 expected, CA72-4 (HR=3.889) was the strongest independent factor for predicting a flare in the
22 prospective gout cohort. Logistic regression analysis confirmed that when CA72-4 increased per 50
23 U/ml, the flare risk increased 2.81-fold. ROC curve analysis revealed that CA72-4 (AUC 0.82), but
24 not sUA, CRP and presence of tophi (AUC 0.57–0.51), might predict gout flares. Thus, the prospective
25 cohort study strongly supports the opinion that CA72-4 is a predictor of future gout flares.
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35 Gout attack may be triggered by cold, physical exhaustion, trauma or severe changes in serum urate
36 levels. However, there is a lack of clinically useful predictors or prognostic factors for gout flares. To
37 the best of our knowledge, CA72-4 is the first serum-derived biomarker that specifically indicates
38 gouty arthritis and predicts flares in the near future. Whether tophi is a flare-predictor is
39 controversial^[36]. Our data imply that tophi is not a convincing indicator for colchicine use, because
40 tophi were not associated with flares in the prospective cohort, whereas CA72-4 level, but not presence
41 of tophi, determined gout flare frequency. Current opinion suggests tophi may predict flares during the
42 initiation of ULT because of the potential release of urate from tophi and consequent re-crystallization.
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Although the findings of this pilot study are promising, there is no biological mechanistic explanation

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4 for the link between CA72-4 and gouty arthritis. Baseline data in the gout cohort showed that patients
5 with low CA72-4 levels had more glucocorticoid use, fewer flares, and lower blood glucose levels than
6 patients with high CA72-4 levels. Either glucocorticoids had an impact on CA72-4 expression or
7 patients with high levels of CA72-4 had severe disease as manifested in the prospective cohort and
8 were more often treated with glucocorticoids. However, the logistic regression model and COX
9 proportional hazards model confirmed that CA72-4, but not glucocorticoid use, was a predictor of gout
10 flare. Based on these results, we hypothesize that glucocorticoid use lowers CA72-4 levels.
11 Glucocorticoid is an anti-inflammatory drug with multiple targets including the NLRP3 inflammasome
12 complex and subsequent IL-1 β pathway, which is a major pathogenesis mechanism in gouty arthritis^{[37,}
13 ^{38]}. The current results suggest a potential relationship between CA72-4 and the NLRP3 inflammasome.
14 Several studies support this hypothesis. Serum CA72-4 levels were elevated in patients with familial
15 Mediterranean fever, a known NLRP3 inflammasome-related autoinflammatory disease, and were
16 associated with the frequency of attacks^[31]. Type 2 diabetes is considered to involve NLRP3-driven
17 inflammation^[39]. Shang et al., found that serum CA72-4 was related to hyperglycaemia as well as poor
18 diabetes status^[40]. Because CA72-4 is more likely a marker for immune pathway activation, it is
19 reasonable to assume that glucocorticoid use will decrease CA72-4 levels. A prospective randomized
20 trial is required to prove this.

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

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52 In the current study, we observed the unexpected and specifically high expression of CA72-4 in gout
53 patients with active status for flares. The present results have profound implications: 1) CA72-4 might
54 be a useful routine test for gout management given its potential use as a marker reflecting gout stability,
55 and thus indicating the chance of future flares; 2) high CA72-4 levels indicate a relatively active status
56 in gouty arthritis and prophylactic colchicine should be administered to these patients during the
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4 initiation of ULT; 3) the biology of CA72-4 in gout warrants further investigation; and 4) the current
5 data do not provide evidence that conventional ULT reduces CA72-4 levels in gout patients. CA72-4
6 as a treatment target requires further investigation.
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12 together with X.W., X.B., and M.S., designed, obtained, and analysed the majority of the data. M.S.
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References

1. Hainer BL, Matheson E, Wilkes RT: **Diagnosis, treatment, and prevention of gout.** *Am Fam Physician* 2014, **90**(12):831-836.
2. McCarty DJ: **Gout without hyperuricemia.** *JAMA* 1994, **271**(4):302-303.
3. Goldberg EL, Asher JL, Molony RD *et al*: **beta-Hydroxybutyrate Deactivates Neutrophil NLRP3 Inflammasome to Relieve Gout Flares.** *Cell reports* 2017, **18**(9):2077-2087.
4. Mangan MSJ, Olhava EJ, Roush WR *et al*: **Targeting the NLRP3 inflammasome in inflammatory diseases.** *Nat Rev Drug Discov* 2018, **17**(8):588-606.
5. Zhang Y, Chen C, Choi H *et al*: **Purine-rich foods intake and recurrent gout attacks.** *Ann Rheum Dis* 2012, **71**(9):1448-1453.
6. Colcher D, Hand PH, Nuti M *et al*: **A spectrum of monoclonal antibodies reactive with human mammary tumor cells.** *Proceedings of the National Academy of Sciences of the United States of America* 1981, **78**(5):3199-3203.
7. Nuti M, Teramoto YA, Mariani-Costantini R *et al*: **A monoclonal antibody (B72.3) defines patterns of distribution of a novel tumor-associated antigen in human mammary carcinoma cell populations.** *Int J Cancer* 1982, **29**(5):539-545.
8. Johnson VG, Schlom J, Paterson AJ *et al*: **Analysis of a human tumor-associated glycoprotein (TAG-72) identified by monoclonal antibody B72.3.** *Cancer research* 1986, **46**(2):850-857.
9. Thor A, Ohuchi N, Szpak CA *et al*: **Distribution of oncofetal antigen tumor-associated glycoprotein-72 defined by monoclonal antibody B72.3.** *Cancer research* 1986, **46**(6):3118-3124.
10. Mariampillai AI, Cruz JPD, Suh J *et al*: **Cancer Antigen 72-4 for the Monitoring of Advanced Tumors of the Gastrointestinal Tract, Lung, Breast and Ovaries.** *Anticancer Res* 2017, **37**(7):3649-3656.
11. Schmiegel W: **Tumor markers in pancreatic cancer--current concepts.** *Hepatogastroenterology* 1989, **36**(6):446-449.
12. Zhao B, Zhang MM, Xie J *et al*: **An abnormal elevation of serum CA72-4 due to taking colchicine.** *Clin Chem Lab Med* 2018, **56**(1):E13-E15.
13. Zhang YR, Zhang M, Bai XS *et al*: **Increased serum CA724 levels in patients suffering gout vs cancers.** *Prog Mol Biol Transl* 2019, **162**:177-186.
14. Neogi T, Jansen TLTA, Dalbeth N *et al*: **2015 Gout classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative.** *Ann Rheum Dis* 2015, **74**(10):1789-1798.
15. Aletaha D, Neogi T, Silman AJ *et al*: **2010 Rheumatoid Arthritis Classification Criteria An American College of Rheumatology/European League Against Rheumatism Collaborative Initiative.** *Arthritis Rheum-Us* 2010, **62**(9):2569-2581.
16. Sieper J, Rudwaleit M, Baraliakos X *et al*: **The Assessment of SpondyloArthritis international Society (ASAS) handbook: a guide to assess spondyloarthritis.** *Ann Rheum Dis* 2009, **68**:1-44.
17. Altman R, Alarcon G, Appelrouth D *et al*: **The American College of Rheumatology criteria for the classification and reporting of osteoarthritis of the hand.** *Arthritis Rheum* 1990, **33**(11):1601-1610.
18. Altman R, Asch E, Bloch D *et al*: **Development of criteria for the classification and reporting of osteoarthritis. Classification of osteoarthritis of the knee. Diagnostic and Therapeutic Criteria Committee of the American Rheumatism Association.** *Arthritis Rheum* 1986, **29**(8):1039-1049.
19. Khanna D, Khanna PP, Fitzgerald JD *et al*: **2012 American College of Rheumatology guidelines for management of gout. Part 2: therapy and antiinflammatory prophylaxis of acute gouty arthritis.** *Arthritis Care Res (Hoboken)* 2012, **64**(10):1447-1461.
20. Khanna D, Fitzgerald JD, Khanna PP *et al*: **2012 American College of Rheumatology guidelines for management**

- 1
2
3
4 **of gout. Part 1: systematic nonpharmacologic and pharmacologic therapeutic approaches to hyperuricemia.**
5 *Arthritis Care Res (Hoboken)* 2012, **64**(10):1431-1446.
- 6 21. Dalbeth N, Merriman TR, Stamp LK: **Gout.** *Lancet* 2016, **388**(10055):2039-2052.
- 7 22. Proudman C, Lester SE, Gonzalez-Chica DA *et al*: **Gout, flares, and allopurinol use: a population-based study.**
8 *Arthritis Res Ther* 2019, **21**(1):132.
- 9 23. Ho JJ, Kim YS: **Serological pancreatic tumor markers and the MUC1 apomucin.** *Pancreas* 1994, **9**(6):674-691.
- 10 24. Katari RS, Fernsten PD, Schlom J: **Characterization of the shed form of the human tumor-associated**
11 **glycoprotein (TAG-72) from serous effusions of patients with different types of carcinomas.** *Cancer research*
12 1990, **50**(16):4885-4890.
- 13 25. Liang Y, Wang W, Fang C *et al*: **Clinical significance and diagnostic value of serum CEA, CA19-9 and CA72-4 in**
14 **patients with gastric cancer.** *Oncotarget* 2016, **7**(31):49565-49573.
- 15 26. Demirci H, Erdamar H, Karakoc A *et al*: **CA 72-4 levels in patients with type 2 diabetes mellitus.** *Int J Clin Pract*
16 2010, **64**(1):34-38.
- 17 27. Carpelan-Holmstrom M, Louhimo J, Stenman UH *et al*: **CEA, CA 19-9 and CA 72-4 improve the diagnostic**
18 **accuracy in gastrointestinal cancers.** *Anticancer Res* 2002, **22**(4):2311-2316.
- 19 28. Hu M, Lan Y, Lu A *et al*: **Glycan-based biomarkers for diagnosis of cancers and other diseases: Past, present,**
20 **and future.** *Prog Mol Biol Transl Sci* 2019, **162**:1-24.
- 21 29. Fu C, Zhao H, Wang Y *et al*: **Tumor-associated antigens: Tn antigen, sTn antigen, and T antigen.** *HLA* 2016,
22 **88**(6):275-286.
- 23 30. Stowell SR, Ju T, Cummings RD: **Protein glycosylation in cancer.** *Annu Rev Pathol* 2015, **10**:473-510.
- 24 31. Balaban YH, Simsek H, Yilmaz R *et al*: **Tumor markers in familial Mediterranean fever and their correlation with**
25 **the frequency of attacks.** *Clin Exp Rheumatol* 2008, **26**(4 Suppl 50):S114-116.
- 26 32. Tausche AK, Jansen TL, Schroder HE *et al*: **Gout--current diagnosis and treatment.** *Dtsch Arztebl Int* 2009,
27 **106**(34-35):549-555.
- 28 33. Van Hoovels L, Jacobs J, Vander Cruyssen B *et al*: **Performance characteristics of rheumatoid factor and anti-**
29 **cyclic citrullinated peptide antibody assays may impact ACR/EULAR classification of rheumatoid arthritis.** *Ann*
30 *Rheum Dis* 2018, **77**(5):667-677.
- 31 34. Borstad GC, Bryant LR, Abel MP *et al*: **Colchicine for prophylaxis of acute flares when initiating allopurinol for**
32 **chronic gouty arthritis.** *J Rheumatol* 2004, **31**(12):2429-2432.
- 33 35. Neogi T: **Clinical practice. Gout.** *N Engl J Med* 2011, **364**(5):443-452.
- 34 36. Abhishek A, Valdes AM, Zhang W *et al*: **Association of Serum Uric Acid and Disease Duration With Frequent**
35 **Gout Attacks: A Case-Control Study.** *Arthritis Care Res (Hoboken)* 2016, **68**(10):1573-1577.
- 36 37. Dalbeth N, Choi HK, Joosten LAB *et al*: **Gout.** *Nat Rev Dis Primers* 2019, **5**(1):69.
- 37 38. Frank MG, Hershman SA, Weber MD *et al*: **Chronic exposure to exogenous glucocorticoids primes microglia to**
38 **pro-inflammatory stimuli and induces NLRP3 mRNA in the hippocampus.** *Psychoneuroendocrinology* 2014,
39 **40**:191-200.
- 40 39. Masters SL, Dunne A, Subramanian SL *et al*: **Activation of the NLRP3 inflammasome by islet amyloid**
41 **polypeptide provides a mechanism for enhanced IL-1beta in type 2 diabetes.** *Nat Immunol* 2010, **11**(10):897-
42 904.
- 43 40. Shang X, Song C, Du X *et al*: **The serum levels of tumor marker CA19-9, CEA, CA72-4, and NSE in type 2 diabetes**
44 **without malignancy and the relations to the metabolic control.** *Saudi Med J* 2017, **38**(2):204-208.
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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.

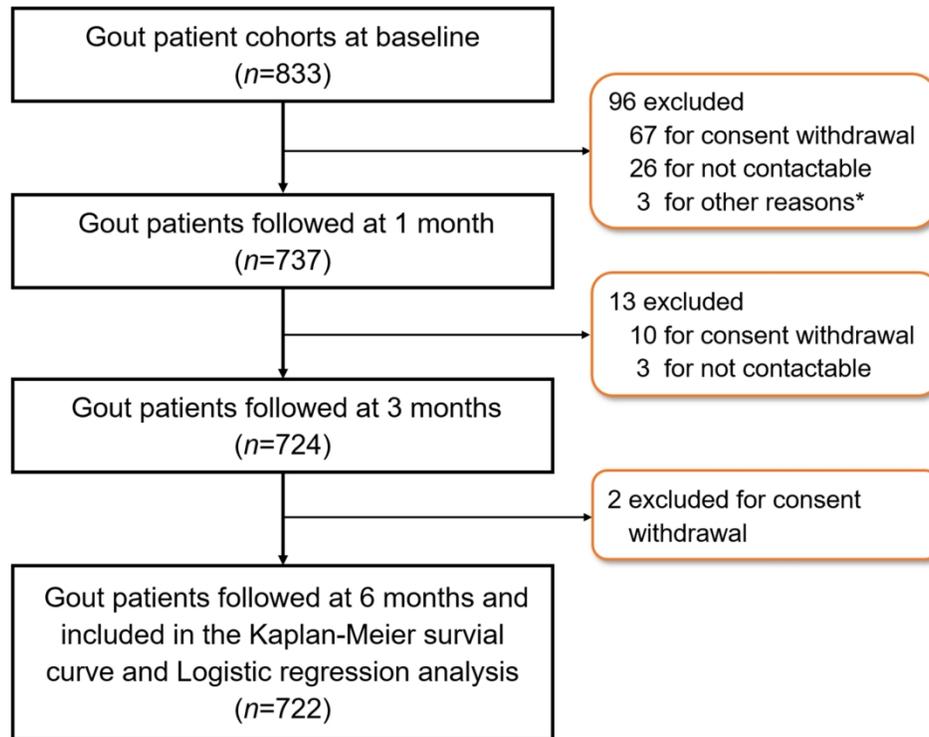


Figure 1. Overall study design. Asterisk (*) represents unable to commit time, poor health condition, or subjected to surgery.

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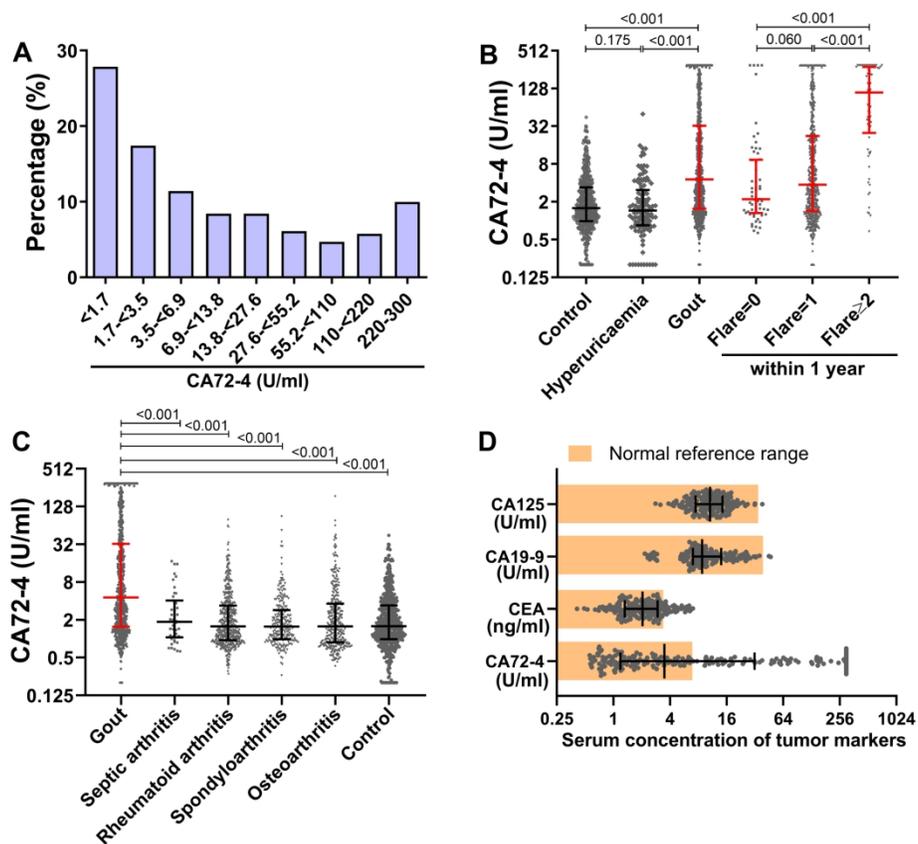


Figure 2. CA72-4 is elevated in patients with gouty arthritis. A. Distribution of gout patients with different serum CA72-4 levels (n=833). B. Comparison of serum CA72-4 levels among gout patients (n=833). Including no flares within 1 year, n=65; 1 flare within 1 year (n=691); ≥ 2 flares within 1 year (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.

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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/m²	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;

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

5 ^aAt least 20 cigarette packs in a lifetime or at least one cigarette a day for at least 1 year.

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7 ^bAlcohol intake at least once a week for 6 months.

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9 ^cMean cumulative exercise time per week of more than 30 min/day.

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11 ^dHistory of hypertension, cardiac disease, type 2 diabetes, or nephrolithiasis.

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13 ^eTreatment within 2 weeks before gout flare and blood sample collection.
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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
^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

^aHistory of type 2 diabetes.

^bTreatment 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.

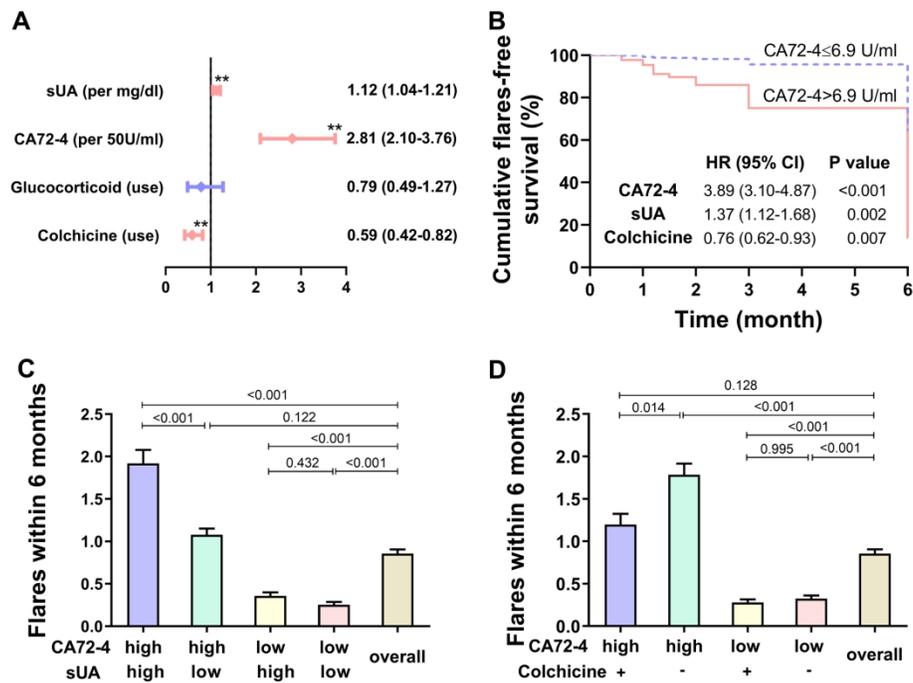


Figure 3. CA72-4 level-based prediction of future flares in a cohort of gout patients. A. Multiple logistic regression models were constructed for selected baseline variables to assess independence, priority, and confounding potentiality. B. Kaplan–Meier analysis of the accumulated flare-free survival 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 $P < 0.01$.

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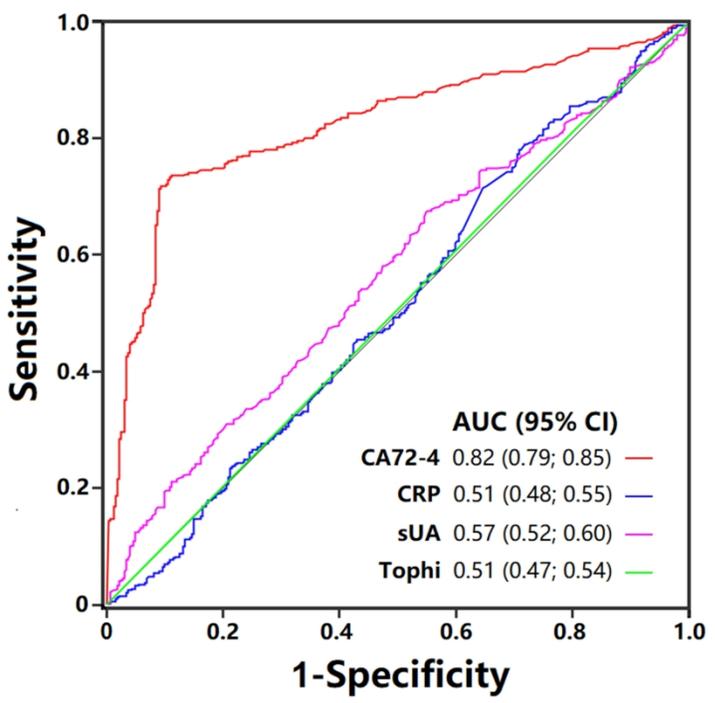


Figure 4. Receiver-operating characteristic curve showing the capacity of CA72-4 (red), CRP (blue), sUA (pink), or tophi (green) to predict gout flares. The sensitivity and specificity of the four indexes are shown.

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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)

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Glucocorticoid, n (%)	99 (11.88)	0	0	172 (32.33)	3 (1.23)	0	0
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sUA: serum uric acid; ALT: alanine aminotransferase; AST: aspartate aminotransferase; TG: triglyceride; sCr: serum creatinine; CRP: C-reactive protein.

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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

^aHistory of type 2 diabetes

^bTreatment 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. ^aTreatment during 6 months follow-up. The cut-off values were defined as: CA72-4: \leq or $>$ normal upper limit (6.9 U/ml); CRP: \leq or $>$ normal upper limit (5 mg/l); triglyceride: \leq or $>$ median level (1.69 mmol/l); BMI: \leq or $>$ 24 kg/m²; glucose: \leq or $>$ normal upper limit (7.0 mmol/l); and sUA: \leq 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.

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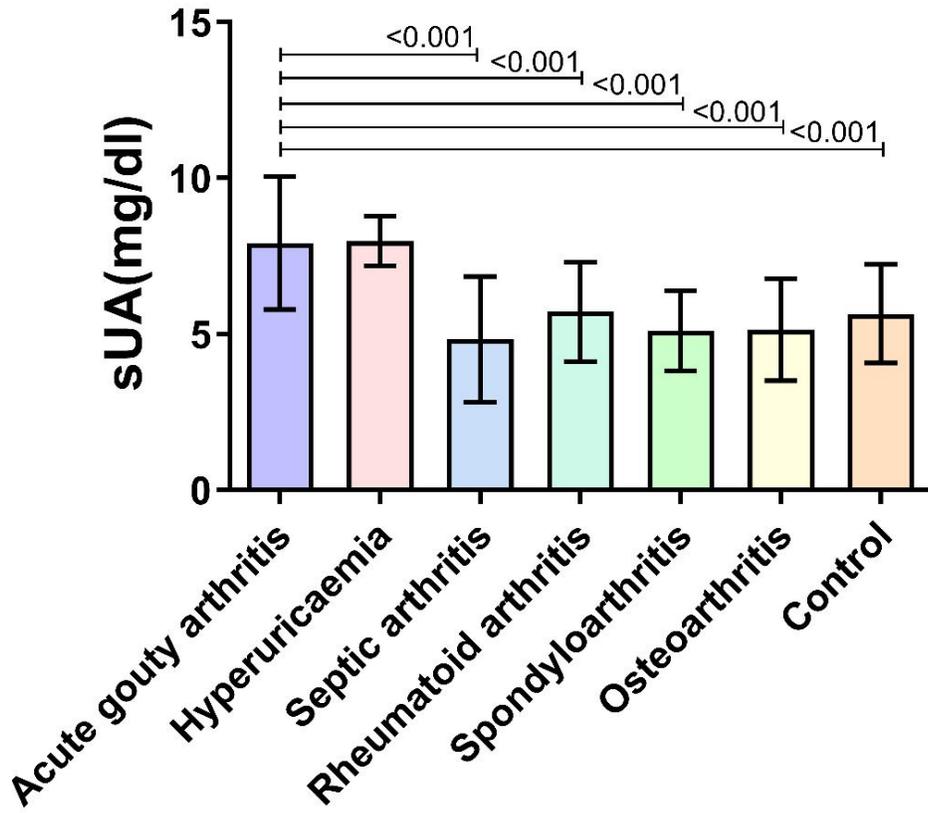
ANOVA was performed for statistical analysis of flares among the various groups.

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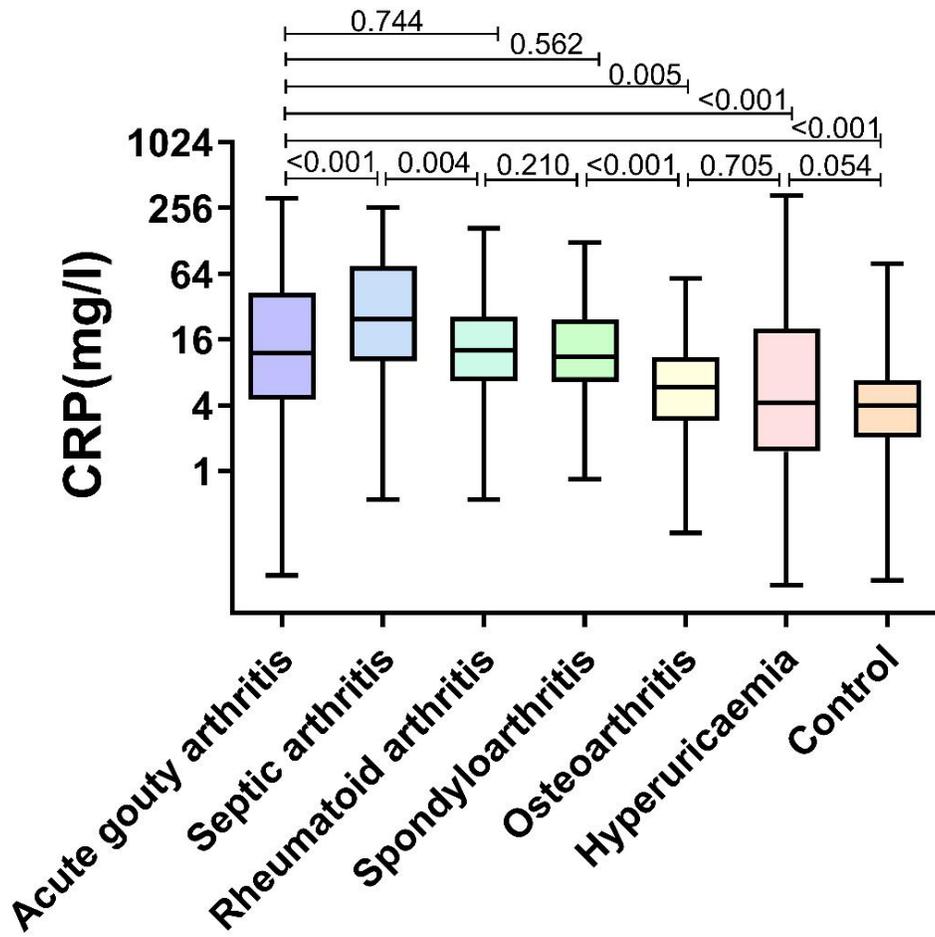
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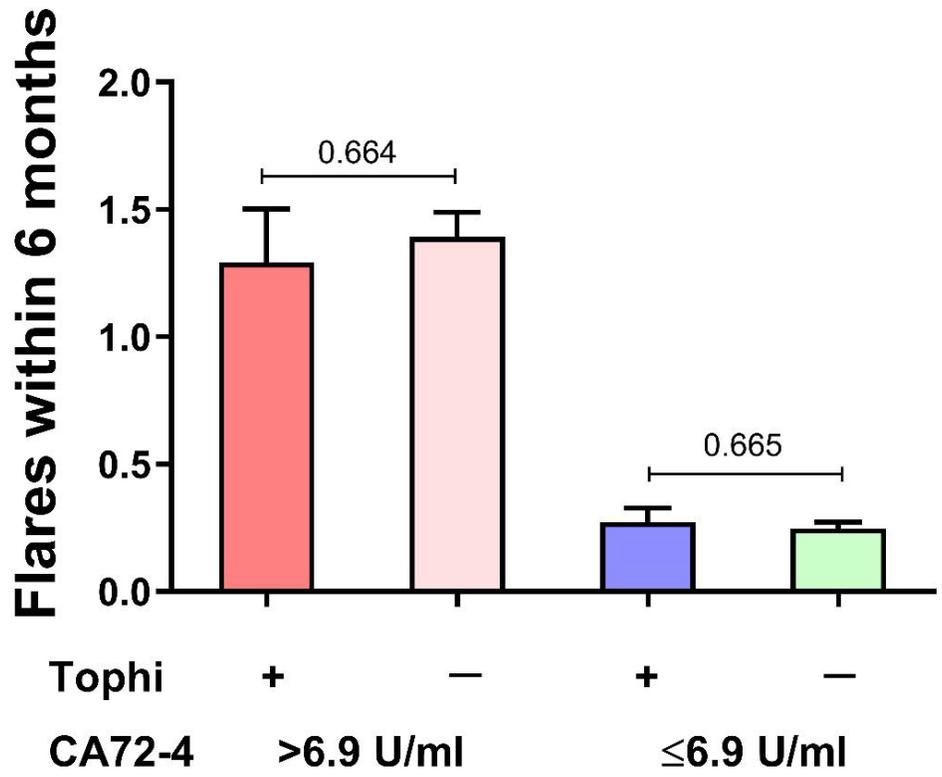


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