

Synovial fluid uric acid level aids diagnosis of gout

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Abstract. Examination of urate crystal in synovial fluid (SF) remains the gold standard for diagnosis of gout, but is not universally available. SF uric acid (UA) level may be measured by the uricase method with an automated analyzer. The present study aimed to evaluate the utility of SF to serum UA ratio (SSR) for diagnosis of gout. A cross-sectional study was conducted at the National Center for Rheumatic Diseases, Nepal. Patients presenting with acute (<1 day) joint pain and/or swelling were included. Aspiration was performed in all patients and fluid was subjected to testing for urate level, pH and cell counts and microscopy. Serum samples were also assessed for urate levels, and the SSR was calculated for each patient. A receiver operating characteristic curve was plotted to determine the cutoff value for indicating diagnosis of gout. The difference in SSR between gout and non-gout effusion was evaluated by one-way analysis of variance. A total of 181 patients were included of which 77 had gout. The remaining cases included osteoarthritis, pseudogout, rheumatoid arthritis and ankylosing spondylitis. SSR was significantly higher in gout patients than in any other group ($P<0.05$). An SSR of ≥ 1.01 had the highest sensitivity and specificity at 89.6 and 66.3%, respectively, for identifying gout effusion. The present results indicated that SSR may be used as an aid for gout diagnosis when polarizing microscopy is not available.

Introduction

Gout is among the most common inflammatory arthritides, characterized by deposition of monosodium urate (MSU) crystals in and around the joint and soft tissue (1-3). Persistent hyperuricemia is an important factor in the development of gout, but not all hyperuricemic patients develop gouty arthritis (4). The risk of supersaturation and crystal formation increases with increasing urate concentration. Alteration in

various factors including pH and temperature also aggravates crystal formation and deposition in joints such as the first metatarsophalangeal (MTP) joint, a normally lower-temperature joint (5). The release of these preformed crystals initiates an intense inflammatory response in the synovium causing acute gouty arthritis (6).

Worldwide prevalence of gout ranges from 0.1 to 10%, with an incidence of 0.3 to 6 cases per 1,000 person-years (7). However, Asian countries including India (8), Bangladesh (9) and Pakistan (10) have been reported as having low gout prevalence, with prevalence rates below 0.5% according to data published by the Community Oriented Programme for Control of Rheumatic Diseases (7).

Gout typically presents in middle-aged men as severe pain and/or swelling with redness around joints, which may last from hours to days. Acute gouty arthritis usually has monoarticular presentation with a predisposition to develop in joints of lower extremities, particularly the first MTP joint. Other joints including the ankle and wrist may also be affected. Oligo- or polyarticular presentation may also occur, albeit rarely, typically in conditions such as chronic untreated gout or in postmenopausal women (11).

The gold standard test for the diagnosis of gout remains to be identification of MSU crystals in the synovial fluid (SF) by polarizing microscopy (12-14), which has previously been reported to have a sensitivity of at least 84.4% (15) and specificity of 97.2% (16). This technique requires specialized equipment and training, and is not available freely in countries with limited resources. Although hyperuricemia is a feature of gout, serum uric acid (UA) level may be within normal range during an acute attack (11). Such normouricemia at the time of an acute attack may be attributed to release of interleukin 6 (IL-6) during the acute inflammatory process (17). UA levels may be efficiently measured in the SF by colorimetric and enzymatic methods. The enzymatic method of UA measurement results from oxidation of UA by the uricase enzyme (18). The present study used the latter method with the aim of evaluating the utility of SF to serum UA ratio (SSR) for diagnosis of gout.

Materials and methods

Study design. The current study followed a cross-sectional design and was conducted at the rheumatology outpatient clinic of the National Center for Rheumatic Diseases (NCRD) from June 2013 to September 2016. Informed written consent

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was obtained from all patients prior to enrollment in the study or performing aspiration of joint fluid. The study protocol was approved by the ethical review board of the NCRD.

Study population. The study population comprised all patients presenting with joint pain at the NCRD. The sample size was calculated using the worldwide prevalence of gout of 1.7% (7), a 95% confidence interval and an 80% power of the study. Though the calculated sample size was 106, 181 patients were included in the study as the patient pool was adequate. The inclusion criteria were as follows: Patients with pain and swelling in one or multiple joints of acute onset (≤ 1 day). The exclusion criteria were as follows: i) Patients with chronic joint pain (>14 days); ii) diagnosed cases of rheumatoid arthritis (RA), spondyloarthritis, or gout on treatment with disease-modifying anti-rheumatic drugs or urate lowering therapy; iii) those not willing to give consent for the study protocol. Gout was diagnosed on the basis of American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) 2015 classification criteria (19). RA was diagnosed on the basis of the ACR/EULAR 2010 criteria (20). Spondyloarthritis was diagnosed using the Assessment of Spondyloarthritis International Society classification criteria (21), and ankylosing spondylitis (AS) on the basis of modified New York criteria (22). Osteoarthritis (OA) was diagnosed on the basis of X-ray findings of reduced medial joint space in the absence of chondrocalcinosis with or without apatite crystals in the SF. Pseudogout was diagnosed if there was detection of chondrocalcinosis by X-ray or calcium pyrophosphate dihydrate crystals on SF examination.

Sample collection and analysis. At least 1 ml of SF was aspirated from any swollen joint, which included the first MTP, ankle, knee, wrist and metacarpophalangeal joints. SF aspiration was performed with a heparinized syringe in all patients and the fluid was sent for assessment of UA level, crystallization, pH, cell counts and gram staining at the NCRD. Additionally, serum samples were assessed for UA levels. Serum and SF UA levels were measured by the enzymatic uricase method using an automated analyzer (Erba XL 200; Erba Diagnostics, Mannheim, Germany). The sample collection and microcrystal examination were performed by a standard method as in a previous study by Montagna *et al* (23). The analyses of SF cell count and gram staining and culture were performed as described by Brannan and Jerrard (24). However, crystal count was not determined. The SSR was calculated for each patient.

Statistical analysis. Statistical tests were performed using SPSS version 17.0 (SPSS, Inc., Chicago, IL, USA). Data were expressed as the mean \pm standard deviation unless otherwise stated. The difference in SSR ratio between gout and non-gout effusions was calculated using one-way analysis of variance followed by Bonferroni's post hoc tests for multiple comparisons. A receiver operating characteristic (ROC) curve was plotted to calculate the cutoff value of SSR ratio for the diagnosis of gout. Internal validation was performed using bootstrapping. $P < 0.05$ was considered to indicate a statistically significant difference.

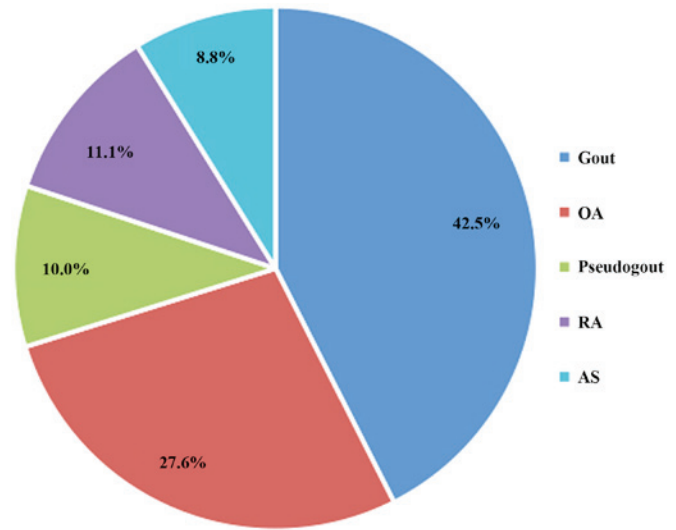


Figure 1. Frequencies of diagnoses. OA, osteoarthritis; RA, rheumatoid arthritis; AS, ankylosing spondylitis.

Results

Clinicodemographic profile. A total of 181 patients who presented with acute joint pain and/or swelling were included. The mean age of patients was 51.5 ± 9.6 years, and 79% were males. The overall male: female ratio was 3.7:1. Gout was diagnosed in 77 (42.5%) of patients. The frequencies of other diagnoses are presented in Fig. 1.

The differences in age, gender, and SF and serum urate levels of patients with each diagnosis are presented in Table I.

SSR. The SF UA level was significantly higher in patients with gout when compared with that in patients with pseudogout ($P = 0.001$) or AS ($P < 0.001$; Table I). SSR was significantly higher in gout patients than in patients with OA ($P < 0.001$), pseudogout ($P = 0.013$), RA ($P < 0.001$) or AS ($P < 0.001$; Table II). When comparing SSR between groups excluding gout, no significant differences were determined (Table II). Serum UA level did not differ significantly between the groups except between RA and pseudogout ($P = 0.015$; Table I).

A ROC curve was plotted for SSR (Fig. 2). An SSR of ≥ 1.01 was associated with the highest sensitivity and specificity, at 89.6 and 66.3%, respectively, for diagnosis of gout. The area under the curve was 0.82. The higher the value of SSR above 1.01, the greater the specificity for diagnosis of gout.

Discussion

SF may be considered as a dialysate of blood plasma (25). As small molecules including urate pass freely through the double barrier of the synovial membrane into SF, the concentration of most non-electrolytes and micromolecules in SF is similar to that of plasma (26,27) except for the few macromolecules that are secreted by the synovial membrane and surrounding tissues (28). Most non-electrolytes diffuse freely between serum and SF, while electrolytes follow the Gibbs-Donnan equilibrium. UA is filtered from plasma to SF, and the normal SF urate level is equal to or slightly lower than the serum levels (29). There are different hypotheses

Table I. Comparison of urate levels between groups.

Variable	Diagnosis					P-value
	Gout (n=77)	OA (n=50)	Pseudogout (n=18)	RA (n=20)	AS (n=16)	
Age, years	49.0±10.0	54.6±9.8	50.9±8.6	53.0±10.2	51.9±3.8	
Serum UA	6.2±0.8	6.3±0.7	5.8±0.8 ^a	6.7±0.5 ^a	6.0±0.9	0.019
Synovial UA	6.6±0.8b ^c	6.1±0.9	5.7±0.9 ^b	6.3±0.9	5.5±0.9 ^c	<0.001
Synovial fluid to serum UA ratio	1.1±0.1	1.0±0.1	1.0±0.1	0.9±0.1	0.9±0.1	<0.001
SF pH	7.4±0.0	7.4±0.1	7.4±0.0	7.4±0.0	7.4±0.0	0.800
SF total cell count/cumm, median (range)	9,300 (1,000-18,000)	3,000 (800-10,000)	10,200 (2,000-22,000)	8,500 (3,000-30,000)	6,700 (2,700-16,000)	0.720
SF gram stain	All negative	All negative	All negative	All negative	All negative	-

Values are presented as the mean ± standard deviation unless otherwise stated. Values with alike superscript letters were significantly different (determined by Bonferroni's post hoc tests); ^aP=0.015, ^bP=0.001, ^cP<0.001. UA, uric acid; OA, osteoarthritis; RA, rheumatoid arthritis; AS, ankylosing spondylitis.

Table II. Comparison of SSR between different study groups.

Diagnosis (SSR)	P-value
Gout (1.06±0.07)	
OA (0.96±0.08)	<0.001
Pseudogout (0.98±0.09)	0.013
RA (0.94±0.11)	<0.001
AS (0.92±0.14)	<0.001
OA (0.96±0.08)	
Pseudogout (0.98±0.09)	1.000
RA (0.94±0.11)	1.000
AS (0.92±0.14)	1.000

SSR, synovial fluid to serum uric acid ratio; OA, osteoarthritis; RA, rheumatoid arthritis; AS, ankylosing spondylitis.

regarding the distribution of urate in serum and SF. According to Beutler *et al* (30), the concentration of UA in SF is identical to that in the blood. However, previous study has demonstrated that SF urate levels were higher than serum levels in patients with acute gouty attack (31). During the acute attack of gout, approximately 40% of patients may be normouricemic which may be attributed to various factors including long-term excessive alcohol intake, diuretic therapy, obesity, release of IL-6 and use of urate lowering therapy, among others (32). Thus, the current study selected the ratio of SF urate to serum urate as a measure to verify higher UA concentration in the SF of patients with gout.

In the present study, the percentage of patients with gout was higher in comparison with those of other inflammatory and non-inflammatory arthritides. This may be because of the criteria of acute onset joint pain and/or swelling. This may also explain the higher percentage of male patients. It was identified that an SSR of ≥1.01 was significantly associated with diagnosis of gout determined through MSU crystal examination

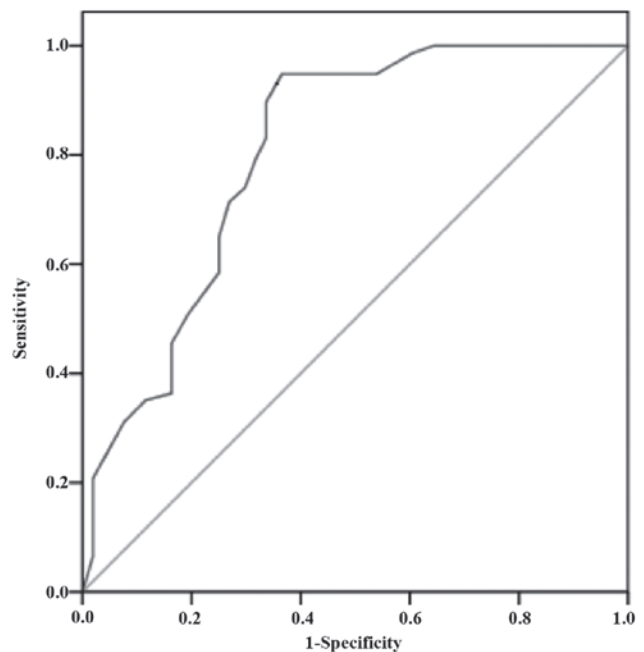


Figure 2. ROC curve for the diagnostic value of synovial fluid to serum uric acid ratio. Diagonal segments are produced by ties. The curved line represents the ROC curve; the straight line represents the intercept at 45°. A synovial fluid to serum uric acid ratio of 1.01 was associated with the highest sensitivity and specificity at 89.6 and 66.3%, respectively. ROC, receiver operating characteristic.

by polarizing microscopy. Values <1.01 were associated with non-gout effusions including those of pseudogout or OA cases. These findings are consistent with the hypothesis that a higher level of UA in SF may in part mediate gout onset. However, there are multiple factors that may be involved in gout onset, including pH (MSU crystal solubility increases with increasing pH to a maximum at pH 7.7 at 37°C), osmolality, and serum-SF gradients of calcium, phosphorus, albumin and globulin (32).

A study in 1965 observed a similar finding of higher SF urate level in patients with gout when compared with those with knee swelling due to other causes (31). However, their data was limited to 27 patients and lacked calculation of the predictive ratio for diagnosis of gout. The present study elaborated on this by obtaining more samples, and determined the predictive value of SSR to be ≥ 1.01 with 89.6% sensitivity.

Consistent with the findings of the current study, Wangkaew *et al* (33) demonstrated that SF urate and SSR were significantly different in patients with gout compared within other types of arthritis. However, in their study, the serum UA levels were also higher in gout patients. By contrast the present data indicated that serum UA as an independent marker may not be reliable for identification of gouty arthritis.

Beutler *et al* (30) reported that SF urate levels tended to reflect serum levels in patients with gout and non-inflammatory arthropathies but not with inflammatory joint disorders. The results of their study did not explain the higher level of SFUA in non-inflammatory arthritis, and contradict the present data. This may be due to the small sample size in their study and selection differences of the study population. Nonetheless, the results of the current study may aid to clarify the relationship between synovial and serum UA levels in gout and non-gout populations in a larger group of patients. The results also indicate a cutoff value for SSR in predicting gout as a cause of joint pain and swelling when the gold standard test for diagnosis is not available. Notably, the higher the SSR value, the more specific it was indicated to be for identifying gout-associated effusion. An external validation of the data with larger groups is now required for confirmation of these findings.

In conclusion, though identification of MSU crystals by polarized microscopy is the gold standard method for the diagnosis of gout, some laboratories and diagnostic centers lack this facility. It also requires a qualified and experienced technician. In this context, measurement of SF urate level may be used as an alternative method, as it is inexpensive, does not require special training or equipment and may be performed in regions where technologically advanced laboratories and equipment may not be available. It may also be effective in differentiating gout effusions from others when there is diagnostic uncertainty. Thus, measurement of SF urate level may be used as an alternative test for diagnosis of gout when polarized microscopy is not available.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

BV conceived the study and was responsible for data analysis and drafting of the manuscript. MB was responsible for data collection and reviewing the literature. SN was responsible for data analysis and drafting of the manuscript. All authors read and approved the final version.

Ethics approval and consent to participate

The study protocol was approved by the ethical review board of the NCRD. Informed written consent was obtained from all participants prior to enrollment.

Consent for publication

Consent for publication of relevant data was obtained from patients following anonymization of personal information.

Competing interests

The authors declare that they have no competing interests.

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