A method for counting monosodium urate crystals in synovial fluid

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SUMMARY

This study was aimed to standardize the technique for counting monosodium urate (MSU) crystals in the synovial fluid (SF) of patients with gout. A total of 52 SF specimens were examined under a polarized light microscope. The amount of SF ranged between 0.1 and 45 mL (median 3 mL). MSU crystals were counted in four areas with the same size at 400x magnification. Cytological examination of the same specimens was also performed. Median leukocyte count was 400 cells/mm^3 (range 50-14,000 cells/mm^3), with a median percentage of polymorphonuclear leukocytes of 9% (range 0%-98%). Median crystal count was 179.5 (range 3-1600). Inter-reader and intra-reader agreement in crystal counting were good with a weighed k of 0.89 [95% confidence interval (CI) 0.85-0.94] and 0.89 (95% CI 0.84-0.93), respectively. Our data indicate that the SF MSU crystal count is a feasible and highly reliable technique.

Key words: *Gout, Monosodium urate crystals, Synovial fluid, Polarized microscopy.*

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INTRODUCTION

The prevalence of gout, the most common form of arthritis, is increasing (1). The mainstay of diagnosis is the identification of monosodium urate (MSU) crystals in the synovial fluid (SF) and tophi of affected patients, since MSU crystals play a role in the development of the joint lesions. The identification of these crystals by compensated polarized light microscopy is a fast procedure providing a conclusive diagnosis (2). MSU crystals cause inflammation and damage when present within the joint and soft tissues, whereas the concentration of serum uric acid (SUA) can be very high without causing any tissue damage. Although clinical classification criteria for the diagnosis of gout are already available (3) and new ones are being developed (4), microscopic detection of MSU crystals is necessary for the clinical evaluation of the patient. It should also be noted that although the presence of MSU crystals in the SF is considered indicative of joint inflammation, they can often be aspirated from asymptomatic joints (5, 6).

In addition to its diagnostic value, SF examination can also be used to evaluate the efficacy of therapy, since the common goal of treatment protocols is to reduce MSU concentration in the tissues rather than simply reducing SUA. In the clinical practice, changes in SUA are often measured as a convenient surrogate for tissue MSU deposition, because the dissolution of MSU crystals depends on the reduction of SUA levels and is associated with a decrease in inflammatory episodes (7). Therefore, counting MSU crystals in the SF may be useful to assess the efficacy of the hypouricemic treatment. This paper describes a reproducible, objective method to standardize MSU crystal counting.

MATERIALS AND METHODS

Patients

Forty-four consecutive patients affected by gout, which was diagnosed according to the American College of Rheumatology (ACR) preliminary criteria (8), were enrolled in this study. Six were examined at

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different time points during the course of the disease and a total of 52 SF specimens were collected. SF aspiration was performed under aseptic conditions following the relevant Italian recommendations (9). Written, informed consent for SF aspiration was obtained from all patients. Before the procedure, the patients were asked if the aspirated joint had been symptomatic in the previous week. The study protocol was not submitted to the local ethical committee because SF aspiration and analysis is part of the standard procedure in patients with suspected gout arthritis.

Sample preparation

Immediately after the arthrocentesis, the SF specimen was divided into two test tubes, one containing ethylenediaminetetraacetic acid for cytological evaluation, including leukocyte count and differential analysis; the second tube was used for microcrystal detection. Fresh SF decays rapidly and should be examined promptly (10). In our laboratory SF is examined without centrifugation, usually within 4 h from aspiration and in any case no later than 24 h, provided it is refrigerated at +4°C (11).

Cytological evaluation

SF leukocyte count provides information about the severity of joint inflammation (10). It was performed using the Burker chamber, after adding Trypan blue to the SF with a 1:2 dilution ratio. Differential counting was performed using ready to use, pre-stained slides (Testsimplets, Waldeck GmbH & Co, Münster, Germany). The stain contains two dyes, cresyl violet acetate and new methylene blue. The percentage of polymorphonuclear leukocytes (PMN) was determined.

Microcrystal detection and identification

Slide examination was performed by two observers (PM, RB) who had passed a two-stage MSU-identification certification procedure, which consisted of a web-based crystal detection test followed by the examination of 5 to 8 vials of SF with different types of crystals, such as calcium pyrophosphate dihydrate crystals, MSU, and apatite (basic calcium phosphate), or depot methylpredisolone crystals, as part of the development of the new classification criteria for gout (4). In order to become certified, the operator had to state correctly whether each SF sample contained MSU crystals or not.

In our procedure, 20 μL of SF were pipetted onto a microscope slide, then a coverslip was overlaid, and the unstained specimen was examined under a compensated polarized light microscope with a 400x magnification. This type of microscope is currently considered the standard instrument for crystal identification in SF because it makes it possible to distinguish crystals depending on their birefringence (12). Initially, the SF specimen was examined without polarization to adjust the focus on the field. Subsequently the samples were examined under polarized light, which highlights the crystals for easy recognition. Whenever crystals of any kind were detected, a first-order red compensator, added to the microscope, was used to identify MSU crystals by their negative birefringence. In practice, detection and identification of crystals are straightforward, almost simultaneous procedures. If crystals were not identified on the slide, but a microcrystalline arthropathy was still suspected, a second slide was prepared and analyzed.

Microcrystal count

After MSU crystals identification, the slide was divided into four areas with the same size separated by a cross mark. The examination was performed at a magnification of 400x, which makes crystal identification both easy and timely. Crystals were counted with a continuous view of the fluid, rather than a fixed number of fields. In each area, the crystals were counted up to a maximum of 400 (maximum crystal count per slide was 1600).

Reliability studies

The consistency of the crystal count was investigated by evaluating its inter-reader reproducibility. The observers evaluated the same slide preparation in 27 SF specimens. Intra-reader agreement was assessed

by repeating the crystal count in 21 SF specimens on another microscope slide. These were examined 24 h after aspiration to test also the effect of 24 h preservation at +4°C on the crystal count.

Statistics

Data were expressed as means and medians, where appropriate. The Kruskal-Wallis test for nonparametric figures was used to compare medians. Correlations were calculated by Spearman's test. Agreement between observers was tested by kappa statistics and intraclass correlation coefficient. All statistical calculations were performed using Medcalc software (Beerse, Belgium).

RESULTS

Of the 44 patients considered, 40 (90.9%) were men. Mean age was 65.2±11.8 years. SF was taken from the knee (48 patients), elbow (1 patient), 1st metatarsophalangeal (MTP) joint (2 patients), and ankle (1 patient). The aspirated amount of SF ranged between 0.1 and 45 mL (median 3 mL). Median leukocyte count was 400 cells/mm^3 (range 50-14,000 cells/mm^3) with PMNs averaging 9% (range 0%-98%). Median crystal count was 179.5 (range 3-1600). Inter-reader concordance for crystal count was good, with a weighed k of 0.89 [95% confidence interval (CI) 0.85-0.94] for the first examination and 0.86 (95% CI 0.80-0.92) for the second. Intra-reader concordance was also good with a weighed k of 0.89 (95% CI 0.84-0.93) for the first observer, and 0.85 (95% CI 0.78-0.93) for the second one. The intraclass correlation coefficient for the four readings was 0.998 (95% CI 0.996-0.999). Maximum time needed for the count was 30 min.

The number of crystals in the SF did not correlate with the amount of SF (P=0.15), nor with leukocyte count (P=0.52), percentage of PMN (P=0.69), gender (P=0.46), or age (P=0.89). Patients with recent-onset symptoms of inflammation (<1 week) in the target joint had a higher crystal count than asymptomatic ones [460.5 (range 3-1600) *vs* 48.5 (range 4-1600), P=0.03].

DISCUSSION AND CONCLUSIONS

In our experience, MSU crystal count in the SF is a feasible and reliable technique with high intra- and inter-reader agreement. It can be easily performed by any laboratory operator with experience in the detection of crystals in the SF. It involves, however, a significant increase in the examination time. Our results were obtained by readers with experience in the evaluation of MSU crystals, who had undergone specific training and certification. Our hypothesis, which we intend to investigate in further studies, is that the crystal count correlates with the clinical course of patients with gout and can be useful to assess the local effects of urate-lowering treatment. A somewhat similar technique has been already used to study the time necessary for crystal clearance after an effective treatment of hyperuricemia (7). In that study, however, the count was discontinued whenever 10 crystals per field were found. This is a semiquantitative procedure in which, for statistical calculations, SF samples with more than 10 crystals per field were considered as having 11 crystals. In our study, the crystals were counted up to a maximum of 400 per quadrant, with a maximum count for slide of 1600 crystals. It was felt unnecessary to count more than 400 crystals, because of the excessive time needed for the procedure. In addition, such a high count was seen in less than 20% of the patients.

Crystal counting does not depend on the amount of aspirated SF. This finding confirms an observation by Pascual et al., who found no difference between SF aspirated from the knee or 1st MTP joint in the number of microscope fields that had to be examined to find crystals (13). Similarly, the number of crystals was not associated with that of leukocytes. This is not surprising because nearly half of the samples were from non-inflamed joints. Although a count greater than 50,000 cells/mm^3 was considered the cut-off for the diagnosis of septic arthritis, gout, acute pseudogout and acute spondyloarthropathy may occasionally

show an effusion within the septic range, especially if smaller joints are affected (14, 15). On the other hand, infective arthritis may show SF cell counts below 50,000 cells/mm^3.

The percentage of PMN leukocytes in SF broadly relates to the amount of local inflammatory reaction. However, this has not been tested fully and its diagnostic value in individual cases is minimal (12). The SF of patients with gout includes mainly PMN leukocytes (more than 90%), low amounts of lymphocytes and monocytes, rare eosinophils, basophils and synovial cells (10). No correlation was found in our study between the number of crystals and the percentage of PMN.

In addition, our study confirms that preserving the SF for 24 h does not affect MSU crystals identification, and adds the information that also crystal count is unaffected.

Finally, the SF from joints with recent active inflammatory reaction had more crystals than those drawn from joints that were not inflamed. This finding corroborates the view that the intra-articular concentration of urates is important in inflammation. Further clinical studies are necessary to evaluate if the SF count of MSU crystals can be a useful biomarker in gout, especially for the evaluation of the effect of urate lowering therapies.

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