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ORIGINAL ARTICLE

Crystal identification of synovial fluid aspiration by polarized light microscopy. An online test suggesting that our traditional rheumatologic competence needs renewed attention and training

D. Berendsen[1] · **T. Neogi**[2] · **W. J. Taylor**[3] · **N. Dalbeth**[4] · **T. L. Jansen**[5,6]

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Abstract Testing a reading exercise for identification of several typical crystal such as the negatively birefringent needle-shaped crystals that are under polarized light microscopy is the gold standard for diagnosing gout. The objective of this study was to assess current performance of crystal identification by professionals involved in examining synovial fluid in routine care. Rheumatologists, trainees, lab technicians, and other physicians with an interest in crystal arthritis completed an online test. The test consisted of 30 images: 8 monosodium urate (MSU) crystals, 5 calcium pyrophosphate (CPP), 4 cholesterol, 2 depot methylprednisolone, 2 calcium oxalate, 2 rice bodies, 1 hydroxyapatite, 1 liquid lipid, 1 fibrin, 1 Charcot-Leyden, and 5 different artifacts. Of the 22 non-MSU slides, a subset of 8 was pre-designated that were thought to be clinically important to be identified as non-MSU. The primary outcome was defined as the correct identification of all eight MSU slides plus the identification of all eight pre-defined non-MSU slides as non-MSU. The online test was completed by 110 participants. The primary outcome was achieved by 39%. Correct identification of all MSU images was achieved by 81%, correct identification of all 8 pre-defined non-MSU, CPP images, and all 22 non-MSU images as non-MSU by 68, 68, and 23%, respectively. MSU crystals were well identified, but incorrect identification of non-MSU crystals occurred frequently. This study suggests that there is room for improvement regarding crystal identification of particularly CPP and other non-MSU crystals even in this highly motivated group.

Keywords Medical education · Microscopy · Monosodium urate crystalsc

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✉ D. Berendsen
d.berendsen@zgt.nl

T. Neogi
tneogi@bu.edu

W. J. Taylor
Will.Taylor@otago.ac.nz

N. Dalbeth
n.dalbeth@auckland.ac.nz

T. L. Jansen
tim.jansen@radboudumc.nl; tjansen@VieCuri.nl

[1] Department of Rheumatology, Ziekenhuisgroep Twente, Almelo, The Netherlands

[2] Clinical Epidemiology Research and Training Unit, Boston University School of Medicine, Boston, MA, USA

[3] Department of Medicine, University of Otago, POB 7343, Wellington, Wellington 6039, New Zealand

[4] Department of Medicine, University of Auckland, Auckland, New Zealand

[5] Department of Rheumatology, VieCuri MC, Venlo, The Netherlands

[6] Scientific IQ Healthcare RadboudUMC, Nijmegen, The Netherlands

Introduction

Different types of crystals can cause an acute inflammatory arthritis. The most common are monosodium urate (MSU) crystals causing gout and calcium pyrophosphate (CPP) crystals causing CPP-associated arthritis. These crystals are identified by clinicians or microscopists performing an examination of synovial fluid under polarized light microscopy, which is regarded as the gold standard for a diagnosis of gout and CPP-associated arthritis [1]. Obviously, correct crystal recognition depends on the competence of the clinician/

microscopist in addition to other factors such as the qualities of the microscope that are used, such as brightness of light and optical quality of lenses.

An accurate diagnosis of gout is critical to assure that patients are not inappropriately treated for gout with any of the potentially toxic urate lowering drugs [2]. In addition, exclusion of crystal presence can be critical for proper classification of other arthritic diseases such as rheumatoid arthritis (RA) before exposing those patients to effective, sometimes expensive, and also potentially toxic agents for those diseases [3, 4]. Proper recognition of crystals using polarized light microscopy requires training. The few evaluations of microscopic competency of practicing rheumatologists or of trainees in rheumatology that have been conducted support the need for further training and evaluation of competencies and that training results in higher consistency of findings [5–8].

As part of an effort to develop new classification criteria for gout, supported by ACR and EULAR [9, 10], potential participating investigators were required to demonstrate their individual competency in crystal recognition. Specifically, rheumatologists and others who wished to be involved in evaluation of synovial fluid aspirates participated in this online test for crystal recognition consisting of 30 images taken from microscopic examinations of synovial fluid. Only participants passing the online test and who then passed a test of examining actual synovial fluid samples were certified and thereby able to participate as a study investigator in a diagnostic study of gout. We here aim to report on the performance of the online test consisting of typical crystal pictures.

Methods

Participants and setting

Study participants included rheumatologists, laboratory technicians, rheumatology trainees (aspirant colleagues but not certified as such) and other physicians worldwide with an interest in crystal diagnosis or who wished to participate in a diagnostic study that was intended to inform the development of new gout classification criteria [9, 10]. Participants were invited from local rheumatology departments, from personal contacts of rheumatologists interested in gout, and from rheumatology centers who had expressed an interest in being part of a diagnostic study (in which crystal identification expertise was a pre-requisite for being involved in the study). Participants not only viewed and answered questions based on a series of standardized images of a similar adequate textbook quality (as judged by HRS, EP, WT, and TJ) online with the primary aim to ascertain their ability to identify MSU from non-MSU but also asked participants to specify the actual feature(s) presented in each slide. Participants could complete the test in approximately 30 min and were asked to do so, without seeking help from books on crystals. Once they completed each page, they were not able to return to change their first answer. Each page was available for only 1 min, then the next slide was presented.

Online crystal identification test

A compilation of 30 online images with characteristic crystals or artifacts was developed by 3 of the authors (TJ, EP, HRS). These images included 8 MSU (2 had micro-tophi) and 22 non-MSU slides. Among these non-MSU slides, there were five CPP slides, four cholesterol monohydrate, three depot methylprednisolone acetate, two calcium oxalate, two rice bodies, one hydroxyapatite, one liquid lipid, one fibrin, one Charcot-Leyden, and four slides with artifacts, such as broken glass and starch. Two slides presented a combination of crystals: one showed MSU plus cholesterol and another showed MSU plus CPP.

Of the 22 non-MSU slides, we specifically focused on a pre-defined subset of 8 slides that participants needed to be able to correctly identify as non-MSU (but not necessarily the precise feature) as part of the competency assessment. The slides that belonged to this subset of non-MSU were two slides with CPP, one slide with cholesterol, one slide with corticosteroid, one slide with calcium oxalate, one slide with a rice body, one slide with broken cover slip artifact, and one slide with fibrin.

Prior to the test, participants were asked about various characteristics that might be associated with the competence in crystal recognition: professional group, age category, total years of experience in their profession (not just with crystal identification), availability of a polarizing microscope in daily practice, how often diagnoses of gout based on microscopy, availability of ultrasound in daily practice, and number of gout patients treated each week (relevant only for clinician participants). In addition, participants were asked about subjective characteristics: rating of their self-perceived competency in identifying MSU and CPP crystals and their self-perceived competency for differentiating MSU from non-MSU crystals from 0 (very poor) to 10 (perfect).

For each slide, participants were asked two questions: (1) the presence of MSU (mutually exclusive options of "MSU only," "MSU with non-MSU," "non-MSU only") and (2) the presence of any of a list of non-MSU features (participants could choose as many features as they thought were present). For a slide to be marked as correctly identifying the specific feature(s), two approaches were used: (1) a stringent criteria which was that the actual crystal/artifact(s) that was present needed to be identified and no other feature(s) (that was not present) must have not been identified; and (2) a lenient criteria which was that the actual crystal/artifact(s) that was present needed to be identified whether or not other feature(s) (that was not present) was identified. The lenient criteria

meant that participants could potentially guess the answer by choosing more than one option, so we also report the distribution of the number of options chosen by participants for each slide.

Analysis

The primary outcome was defined as the correct identification of all eight MSU slides (as MSU) and correct identification of all eight pre-defined non-MSU slides (as non-MSU). Comparison of the primary outcome by categorical participant characteristics was assessed with chi-square tests. Continuous characteristics were described as medians with interquartile ranges and compared with an Independent Sample Mann-Whitney test. A secondary outcome was the performance regarding the correct specific crystal identification of the non-MSU slides. These data are presented as mean number of slides correctly identified (SD) with the Fisher's exact test used to compute p values.

Results

The test was completed by 110 participants. Of these, 57 were rheumatologists, 33 were laboratory technicians, 13 were rheumatology trainees, 6 were other physicians (GP or internist), and 1 unknown. The distribution of participants' characteristics by profession is shown in Table 1.

Forty-three participants (39%) achieved the primary outcome i.e., correct identification as MSU/non-MSU of 8 out of 8 MSU slides and of 8 out of 8 pre-selected non-MSU slides.

Accuracy regarding classification of the various crystal types is as follows (see Supplementary Table 1):

- MSU slides

All eight MSU slides were correctly identified as MSU by 81% of all participants. Seven out of eight MSU slides were correctly identified by another 15% of participants, see Table 2. No single MSU slide caused more problems than any other with correct identification as MSU by 94 to 98% of participants per slide. However, many participants incorrectly identified additional features that were not present in two slides or the wrong additional feature in two other slides, so by stringent scoring, correct identification occurred in 49 to 96% of the slides. Misclassification of MSU crystals as CPP crystals was made by 0.9 to 16% of participants (depending on the slide).

- Pre-selected non-MSU slides

All eight pre-selected non-MSU slides were correctly classified as non-MSU by 49% of participants, and another 36% correctly identified seven out of eight slides as non-MSU, see Table 2. However, the specific crystal was identified correctly in all 8 non-MSU slides by only 2 participants (1.8%) and in 7 of 8 slides by 13 (12%) of participants.

- Calcium pyrophosphate

All CPP slides were correctly identified by 71% of all participants as non-MSU, see Table 2. Only 8 (7%) did not identify any of the CPP slides correctly as CPP. Misclassification as MSU in CPP slides was made by 0.9 to 22% of participants (depending on the slide). Correct recognition as CPP was made by 33 to 76% of participants using stringent criteria (depending on the slide). Two or more options (i.e., guessing) were selected by 0.9 to 26.3% of participants (depending on the slide) and led to correct identification as CPP (lenient scoring) by 59 to 77% of participants.

- Lipids

All four slides with cholesterol monohydrate plates were identified correctly as non-MSU by 42% of all participants by stringent criteria. Non-identification of any cholesterol slides as cholesterol occurred in only 9% of participants. Correct recognition as a cholesterol crystal was made by 0 to 86% of participants using stringent criteria (depending on the slide). This led to correct identification as cholesterol by 54 to 90% of participants (lenient scoring). Liquid lipid crystals (single slide) were correctly identified by 54% of participants and easily identified as non-MSU (by 96% of participants).

- Calcium oxalate

The two oxalate slides were identified correctly as non-MSU by 97 to 99% of all participants and recognized correctly as oxalate by 78 to 83% (depending on the slide). Guessing occurred in only 0.9 to 3.5% participants, so the lenient scoring results were very similar at 78 to 86% of participants. Only 7.3% of participants did not recognize either oxalate slide as oxalate.

- Artifacts

Some artifact slides were more often misclassified as MSU than most other non-MSU slides, with 45 to 99% of participants correctly classifying these slides as non-MSU. Correct identification as an artifact was infrequent with only 16 to 50% correctly identified by stringent criteria and 20 to 56% by lenient criteria. Guessing (more than one option selected) occurred in 2.7 to 9.1% of participants.

Most of the 22 non-MSU crystals were easily recognized as non-MSU (median 98% of participants, range 45 to 100%), whereas the correct identification of the specific type of crystal

Table 1 Characteristics of participants

	Rheumatologist (n = 57) n (%)	Lab technician (n = 33) n (%)	Trainee (n = 13) n (%)	Other physician (n = 6)[a] n (%)
Age category				
25–35 years	6 (11)	13 (39)	8 (62)	4 (67)
36–45 years	28 (49)	7 (21)	4 (31)	1 (17)
46–55 years	17 (30)	9 (27)	1 (8)	0
56–70 years	6 (11)	4 (12)	0	1 (17)
Years of experience				
In training	0	1 (3)	8 (62)	3 (50)
<5 years	6 (11)	4 (13)	4 (31)	1 (17)
6–10 years	17 (30)	12 (38)	0	1 (17)
11–15 years	12 (21)	1 (3)	0	0
15–20 years	9 (16)	5 (16)	0	0
20–25 years	7 (12)	1 (3)	6 (8)	1 (17)
>26 years	6 (11)	8 (25)	0	0
Availability of polarized light microscope				
Yes, always	52 (91)	100	10 (77)	4 (67)
Sometimes	3 (5)	0	3 (23)	2 (33)
No, never	2 (4)	0	0	0
Diagnosis based on microscopy				
Nearly always (>95%)	16 (28)	NA	3 (23)	3 (50)
Mostly (>90%)	16 (28)	NA	4 (31)	3 (50)
Often (50–80%)	17 (30)	NA	5 (39)	0
Sometimes (<50%)	8 (14)	NA	1 (8)	0
Number of gout patients treated				
Commonly (>10/week)	25 (44)	NA	1 (8)	1 (17)
Often (2–9/week)	25 (44)	NA	4 (31)	1 (17)
Regularly (1/week)	6 (11)	NA	5 (39)	3 (50)
Sporadically (≤5/month)	1 (2)	NA	3 (23)	1 (17)

[a] n = 109, because one participant's characteristics were missing

was much less frequent (median 57.5% of participants, range 11 to 90%). The correct diagnosis of hydroxyapatite (18%), rice bodies (18 to 20%), depot methylprednisolone (37 to 69%), and artifacts (20 to 56%) was low, whereas correct diagnosis of CPP (59 to 77%), cholesterol slides (54 to 90%), and calcium oxalate was (78 to 86%) was higher; see supplementary Table 1.

The mean (SD) number of slides correctly identified with the specific crystal/artifact was 16.8 (5.1) by stringent scoring and 20.3 (5.3) by lenient scoring. Those who achieved the primary outcome generally had significantly better performance in identifying the specific slide findings, and this was generally apparent across the different crystal types whether or not stringent or lenient marking was used (Supplementary Table 2).

With respect to the objective test performance (achieving the primary outcome or not), there were no differences in participants' characteristics (Table 3). There was also no association between the objective test performance and self-perceived competence to identify MSU or CPP crystals (Table 4).

Discussion

This is the largest study to date quantifying the actual performance of crystal identification competencies of professionals involved in crystal diagnoses, i.e., rheumatologists, trainees, laboratory technicians, and other physicians, as assessed by viewing online images. While only 39% correctly identified all of the 16 pre-specified slides as MSU (n = 8) or non-MSU (n = 8), almost all participants correctly identified at least seven or eight out of eight MSU images. MSU is the best identified crystal in this online test and for this MSU identification the pattern recognition with microscopical pictures is highly reliable. A previous paper mentioned unreliability of polarized light microscopy for MSU and CPP because of false negative, false positive, and misclassification errors [11]. The rather good performance recognizing gout microscopically in our study is possibly due to the strong birefringent character with good visualization in modern microscopes of the often easily recognizable needles and possibly

Table 2 Correct crystal identification of MSU crystals as MSU and correct recognition of eight pre-selected non-MSU slides, all non-MSU slides, and CPP slides as non-MSU

	MSU (s = 8)		Pre-selected non-MSU (s = 8)		All non-MSU (s = 22)		CPP (s = 4)[a]	
Total participants (%) (n = 110)	8/8	81	8/8	49	22/22	23	4/4	71
	7/8	15	7/8	36	21/22	25	3/4	24
	≤6/8	5	≤6/8	12	≤20/2	53	≤2/4	5
Rheumatologists (%) (n = 57)	8/8	86	8/8	58	22/22	30	4/4	79
	7/8	11	7/8	33	21/22	23	3/4	18
	≤6/8	4	≤6/8	9	≤20/22	47	≤2/4	4
Lab technicians (%) (n = 33)	8/8	76	8/8	42	22/22	15	4/4	70
	7/8	18	7/8	39	21/22	33	3/4	30
	≤6/8	6	≤6/8	18	≤20/22	52	≤2/4	0
Trainees (%) (n = 13)	8/8	77	8/8	31	22/22	15	4/4	46
	7/8	15	7/8	39	21/22	8	3/4	39
	≤6/8	8	≤6/8	31	≤20/22	77	≤2/4	15
Other physicians (%) (n = 6)[b]	8/8	67	8/8	50	22/22	17	4/4	67
	7/8	33	7/8	33	21/22	33	3/4	17
	≤6/8	0	≤6/8	17	≤20/22	50	≤2/4	17
p value[c]	0.364		0.151		0.077		0.053	

All are percentages unless a quotient is given

MSU monosodium urate, *CPP* calcium pyrophosphate

[a] The slide that showed both MSU and CPP was counted as a MSU slide for this analysis

[b] The total number of participants of different professions here was 109, because 1 participant's characteristics were missing

[c] Fisher's exact test p value for difference between number of correct slides by professions (rheumatologists, laboratory technicians, trainees, other physicians)

partly due to day-to-day exposure to gout patients. On the other hand, MSU may still be somewhat over-diagnosed: artifacts and CPP were not infrequently misclassified as MSU, whereas only a few MSU slides were misclassified as CPP. Over-identification of MSU in acute, potentially gouty arthritis may create a risk of inappropriate initiation of possibly toxic urate lowering therapies in non-gout patients, as well as inappropriate initiation of urate lowering therapies in disorders that really need other therapies such as bacterial arthritides.

In addition to identification of MSU crystals, we report on CPP crystals, since this is the most important differential diagnosis of acute arthritis in which gout is clinically suspected. This online test suggests that about one in three will be able to identify all (i.e., 100%) of presented CPP crystals and one in 20 will not identify any typical picture of CPP at all. These data suggest that CPP, even if present, will not always be identified by its rod-like or rhomboid shape and faint positive or even absent birefringence under polarized light microscopy. The findings based only on crystal identification on slides viewed online suggest that for the sake of daily practice rheumatology, a diagnosis such as CPP-associated arthritis can be improved in two out of three colleagues. Still, problems were addressed earlier and not fully tackled by EULAR and ACR training sessions. [11, 12] Almost similar conclusions can be drawn for cholesterol, correctly identified by one in 2.5 (42%) of participants; but this problem in cholesterol synovitis is of less concern due to its less common occurrence and lower clinical significance. Calcium oxalate slides were correctly read by 70% of test takers, which may be considered quite adequate.

Considering that microscopic examination of synovial fluid is the gold standard, we acknowledge that this online test with pathognomonic slides is not the perfect test for measuring the real-life competence of a clinician handling synovial fluid analysis with his/her own microscope. In this online test, we did not test actual handling of specimens nor the skill of handling with one's own microscope, and one did not get additional time nor pictures which can be done by doing the polarized light microscopy oneself. The online test reveals the theoretical knowledge, whereas real-time microscopy depends on recognition of specific crystals and confounders PLUS the quality of the microscope and handling of the fluid. If there is uncertainty about the presence or type of crystals, one also has the opportunity to look at additional fields of view when visualizing under polarized microscopy directly. From Table 3, we can deduce that less availability of the microscope is associated with a lower percentage achieving the primary outcome of correct identification possibly via lower awareness of the difference in phenotypes of the different crystals.

Previously, optical microscopy appeared to be equivalent to virtual microscopy in a teaching situation [13], which may

Table 3 Characteristics of participants and test performance

Characteristics of participants (n)[a]	Achieved primary outcome (N, %)	Number of correctly identified slides (mean, SD)	
		Stringent criteria (maximum 30)	Lenient criteria (maximum 32)[b]
Profession			
Rheumatologist (57)	26 (46)	18.1 (5.2)*	22.8 (4.8)*
Laboratory technician (33)	11 (33)	16.6 (3.2)	20.5 (3.4)
Trainees (13)	4 (31)	14.4 (6.4)	18.6 (7.5)
Other physicians (6)	2 (33)	14.5 (3.5)	18.8 (4.0)
Years of experience			
In training (12)	3 (25)	13.6 (6.1)	17.0 (7.2)
<5 years (15)	6 (40)	16.9 (4.9)	21.6 (4.5)
6–10 (30)	15 (50)	16.9 (4.3)	21.4 (4.4)
11–15 (13)	5 (38)	19.1 (5.8)	23.5 (5.3)
15–20 (14)	2 (14)	18.0 (3.7)	21.8 (4.0)
20–25 (10)	7 (70)	17.8 (6.4)	23.1 (4.2)
>26 (14)	5 (36)	16.9 (3.4)	21.7 (4.4)
Availability of a polarizing microscope			
Yes, always (99)	40 (40)	17.2 (4.9)	21.5 (5.1)
Sometimes (8)	3 (38)	15.1 (3.5)	21.1 (3.1)
No, never (2)	0 (0)	12.5 (9.2)	17.5 (9.2)
Diagnosis based on microscopy			
Nearly always >95% (42)	17 (41)	17.4 (4.8)	21.5 (5.4)
Mostly >90% (26)	9 (35)	17.8 (4.8)	21.9 (5.0)
Often 50–80% (23)	12 (52)	16.5 (5.3)	21.3 (5.3)
Sometimes <50% (11)	2 (18)	14.1 (5.3)	20.2 (4.6)
Number of gout patients treated			
Commonly (>10/week) (29)	13 (45)	17.5 (4.6)	
Often (2–9/week) (33)	13 (39)	18.6 (4.6)	
Regularly (1/week) (14)	5 (36)	15.6 (6.1)	
Sporadically (≤5/month) (9)	4 (44)	14.0 (6.9)	

[a] Several characteristics of participants were missing

[b] Two slides had two features, so these were scored separately

*$p < 0.05$

plea for testing microscopical recognition of crystals via an online slide show as we did. Currently, this is not the case in rheumatology training in most countries. Our findings may not be generalizable given the fact that most of the participants were specifically interested in gout; thus, these results are likely to be better than might otherwise be obtained in a more general representative sample of rheumatologists, lab technicians, trainees, and other physicians.

Table 4 Self-competence ratings based upon achievement of the primary outcome

Area of self-perceived competence	Self-competence rating (median, IQR)		P value
	Those achieving the primary outcome (n = 43)	Those not achieving the primary outcome (n = 67)	
MSU identification	9 (9–10)	9 (8–10)	0.30
Differentiating MSU from non-MSU	9 (8–10)	9 (7–10)	0.90
CPP identification	8 (8–10)	9 (7–10)	0.86
Differentiating MSU from non-MSU	9 (8–10)	9 (7–10)	0.33
Differentiating MSU from CPP	9 (8–10)	8.5 (6–10)	0.16

Modern techniques with online training and testing systems may be useful components of tests for improving diagnostic performance or pattern recognition in crystals. Both the ACR and the EULAR have sponsored training courses at their annual meetings. The results of this pilot study suggest that there is a necessity for renewed attention to this topic, a necessity for training of polarized light microscopy, and possibly preparation of specimens with crystals, and still, these data show we have a challenge for improvement of pattern recognition at microscopy, perhaps, at least in part, online for improved theoretical knowledge of the patterns one may encounter and certainly for giving more attention to the microscope competence of rheumatologist trainees, rheumatologists, and laboratory technicians.

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Compliance with ethical standards

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