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Accuracy of synovial fluid analysis compared to histology for the identification of calcium pyrophosphate crystals: an ancillary study of the OMERACT US Working Group - CPPD subgroup

S. Sirotti[1,2], M. Gutierrez[3], C. Pineda[3], D. Clavijo-Cornejo[3], T. Serban[4], A. Dumitru[5], A. Scanu[6], A. Adinolfi[7], C.A. Scirè[8], P. Sarzi Puttini[1,2], M.-A. D'Agostino[9], H.I. Keen[10], L. Terslev[11], A. Iagnocco[12], G. Filippou[2]

[1]Department of Clinical Sciences and Community Health, Università degli Studi di Milano, Italy; [2]Rheumatology Department, Luigi Sacco University Hospital, Milan, Italy; [3]Division of Musculoskeletal and Rheumatic Diseases, Instituto Nacional de Rehabilitacion, Mexico City, Mexico [4]Rheumatology Department, Ospedale La Colletta, ASL3 Genovese, Genoa, Italy; [5]Department of Pathology, "Carol Davila University of Medicine and Pharmacy", Bucharest, Romania; [6]Department of Medicine-DIMED, Rheumatology Unit, University of Padova, Italy; [7]Rheumatology Department, ASST Grande Ospedale Metropolitano Niguarda, Milano, Italy; [8]Società Italiana di Reumatologia, Epidemiology Research Unit, Milan, Italy; [9]Rheumatology Department, Università Cattolica del Sacro Cuore, Policlinico Universitario Agostino Gemelli IRCSS, Roma, Italy; [10]School of Medicine, The University of Western Australia, Murdoch, Perth, Australia; [11]Center for Rheumatology and Spine Diseases, Rigshospitalet, Copenhagen University, Copenhagen, Denmark; [12]Academic Rheumatology Centre, Department of Clinical and Biological Sciences, Università degli Studi di Torino, Italy

SUMMARY

The aim of this study was to evaluate the accuracy of synovial fluid analysis in the identification of calcium pyrophosphate dihydrate crystals compared to microscopic analysis of joint tissues as the reference standard.

This is an ancillary study of an international, multicentre cross-sectional study performed by the calcium pyrophosphate deposition disease (CPPD) subgroup of the OMERACT Ultrasound working group. Consecutive patients with knee osteoarthritis (OA) waiting for total knee replacement surgery were enrolled in the study from 2 participating centres in Mexico and Romania. During the surgical procedures, synovial fluid, menisci and hyaline cartilage were collected and analysed within 48 hours from surgery under transmitted light microscopy and compensated polarised light microscopy for the presence/absence of calcium pyrophosphate crystals. All slides were analysed by expert examiners on site, blinded to other findings. A dichotomic score (absence/presence) was used for scoring both synovial fluid and tissues. Microscopic analysis of knee tissues was considered the gold standard. Sensitivity, specificity, accuracy, positive and negative predictive values of synovial fluid analysis in the identification of calcium pyrophosphate crystals were calculated.

15 patients (53% female, mean age 68 yo ± 8.4) with OA of grade 3 or 4 according to Kellgren-Lawrence scoring were enrolled. 12 patients (80%) were positive for calcium pyrophosphate crystals at the synovial fluid analysis and 14 (93%) at the tissue microscopic analysis. The overall diagnostic accuracy of synovial fluid analysis compared with histology for CPPD was 87%, with a sensitivity of 86% and a specificity of 100%, the positive predictive value was 100% and the negative predictive value was 33%.

In conclusion synovial fluid analysis proved to be an accurate test for the identification of calcium pyrophosphate dihydrate crystals in patients with advanced OA.

***Key words**: Calcium pyrophosphate deposition disease, synovial fluid analysis, diagnosis, sensitivity and specificity, knee.*

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Corresponding author:
Silvia Sirotti
Rheumatology Department,
Luigi Sacco University Hospital
Via Giovanni Battista Grassi, 74
20157 Milan, Italy
E-mail: silvia.sirotti@unimi.it

INTRODUCTION

Calcium pyrophosphate deposition disease (CPPD) is an umbrella term used to describe all occurrences of calcium pyrophosphate (CPP) crystals in tissues (1). Even though its actual prevalence in the general population is not known, due to the

scarcity of epidemiological studies, CPPD seems to be the third most common inflammatory arthritis, after gout and rheumatoid arthritis (2). In patients over 50 years of age, who had conventional radiography of joints for any reason, the prevalence of CPPD ranges broadly between 3% (3) and 7%, and arguably CPPD is one of the most common reason for hospitalization due to musculoskeletal problems (4, 5). The disease increases with the aging of population (6) and may lead to acute and recurrent articular or peri-articular inflammation. The clinical presentation of CPPD is highly variable and CPP crystals can be found in joints and periarticular tissues of asymptomatic patients as well as in those presenting a range of signs and symptoms similar to other inflammatory arthritis (1), making a definite diagnosis challenging.

Historically, the milestone for diagnosing CPPD is the identification of crystals in synovial fluid (SF) aspirate using compensated polarised light microscopy. Synovial fluid analysis (SFA) in crystal-induced arthritis was first used in early 1960s by McCarty and colleagues (7) with the introduction of polarised light microscopy (8), capable of identifying monosodium urate (MSU) and CPP crystals. CPP crystals typically appear with a parallelepiped shape and are generally intracellular with absent or weak positive birefringence (1). Since then, the detection of CPP crystals in SF has always been considered the gold standard for CPPD diagnosis, firstly according to McCarty criteria (9) and, more recently, following the European Alliance of Associations for Rheumatology (EULAR) recommendations (1).

However, there have been very few published reports about its diagnostic accuracy (10, 11), and some studies raise concerns regarding the reliability of the test, with significant inter-readers error in the assessment of the identification of CPP crystals (12). Furthermore, arthrocentesis may not always be feasible in daily clinical practice, or may not be possible due to patient comorbidities or medications.

This is an ancillary study of the international multicentre study carried out by the Outcome Measures in Rheumatology (OMERACT) ultrasound working group for the validation of ultrasound in CPPD (13). In this study we aimed to evaluate the accuracy of SFA in the identification of CPP crystals compared to the microscopic analysis of joint tissues as reference standard.

PATIENTS AND METHODS

This ancillary study follows the design and methods of the previously published multicentre cross-sectional study conducted by the CPPD subgroup of the OMERACT Ultrasound working group. Patients were enrolled in two of the eleven participating centres in Mexico (*Instituto Nacional de Rehabilitacion,* Mexico City) and Romania (Carol Davila University, Bucharest). Inclusion and exclusion criteria were the same as those of the main study (13). Briefly, patients underwent total knee replacement (TKR) due to osteoarthritis (OA). During the surgical procedure, SF was collected, put in a sterile container and stored in a refrigerator at 4°C until it was examined within 48 hours from joint aspiration under optical light microscopy, a reasonable time for a reliable CPP crystals recognition (14). SF samples were analysed by expert examiners on site, blinded to clinical and histological findings. The slides were obtained by placing a small drop of SF on them and covering it with a coverslip. Crystal identification was carried out examining each slide under ordinary and compensated polarised light microscopy. CPP crystals were identified by their parallelepiped morphology and weak birefringence with positive elongation under compensated polarized light.

The anatomical specimens of the knee were collected, conserved and analysed according to methods of the main study (13). Patients were considered positive for CPPD, if at least one of the tissues examined was positive for CPP crystals at microscopic analysis. Tissues were analysed by expert pathologists on site, blinded to SFA results. A dichotomic score (absence/presence) was used for scoring both SF and tissues.

Microscopic analysis of the knee tissue was considered the reference standard for the final diagnosis of CPPD. Considering the pathogenetic mechanism of CPPD, where crystals are primarily formed in the hyaline cartilage and fibrocartilage of the joint and secondarily shed into the joint cavity, CPP crystals could be identified in the tissues before they were released into the SF.

Sensitivity, specificity, accuracy, positive and negative predictive values of SFA in the identification of CPP crystals were calculated with 95% confidence intervals (CI).

All participants signed an informed consent for participation in the study. This study was approved by the local ethics committee, and was reported according to the Standards for Reporting Diagnostic (STARD) accuracy studies guidelines (15).

RESULTS

We enrolled 15 patients with OA waiting for TKR, 8 patients from *Instituto Nacional de Rehabilitacion*, Mexico City, Mexico, and 7 patients from Carol Davila University, Bucharest, Romania. Eight of the fifteen participants were female (53%); mean age of the sample was 68±8.4 years; 6 patients had grade 3 OA, and 9 patients had grade 4 OA according to Kellgren-Lawrence scoring (16).

Table I - 2x2 table showing the relationship between synovial fluid analysis and tissue analysis (reference standard).

Test	CPPD+	CPPD-	Total
Positive	12	0	12
Negative	2	1	3
Total	14	1	15

The mean SF aspirated volume was 10.7 mL ± 10.5. The SF examination showed no crystals in 3 samples (20%), CPP crystals in 11 (73.5%), MSU crystals in 0 (0%), and both CPP and MSU crystals in 1 (6.5%).

Upon the microscopic analysis of the tissues (menisci and hyaline cartilage), 14 patients (93%) presented with at least one positive slide for CPP crystals. Among 12 SFA positive patients, all were positive for CPP crystals in either medial or lateral meniscus, and 11 were positive in both. Ten patients were positive at the hyaline cartilage, and they were also positive for at least one meniscus. Regarding the 3 SFA negative patients, only one had no crystals in the examined tissues, while the other 2 patients had CPP crystals in both menisci and hyaline cartilage.

The overall diagnostic accuracy of SFA for the identification of CPP crystals at knee level compared to tissues microscopic analysis as reference standard was of 87% with a sensitivity of 86% and specificity of 100%. The positive predictive value (PPV) of SFA was 100%, while the negative predictive value (NPV) was 33%.

Table I shows the relationship between SFA and the reference standard, whereas Table II summarizes the overall values of sensitivity, specificity, PPV, NPV and accuracy of SFA.

DISCUSSION AND CONCLUSIONS

In recent years, a growing body of evidence has focused on the use of imaging in CPPD, showing the potential of modern imaging techniques such as high resolution ultrasound or dual energy computed tomography (DECT) in the identification of CPP crystals in rheumatological clinical practice (17). Ultrasound is very sensitive and specific and has been tested for reli-

Table II - Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and diagnostic accuracy of synovial fluid (SF) analysis compared to the reference standard; in parentheses: 95% confidence intervals (CI).

	Sensitivity	Specificity	PPV	NPV	Accuracy
SF analysis	86% (57%-97%)	100% (75%-100%)	100% (75%-100%)	33% (13%-61%)	87%

ability and criterion validity with good results (13, 18, 19); while DECT has proven helpful in the diagnosis of gout, and emerging data suggest that it may be promising in calcium crystal recognition and differentiation as well (20, 21). Nonetheless, SFA via compensated polarized light microscopy is still considered the gold standard for the identification and diagnosis of CPPD-related arthropathies (1).

It is noticeable, however, that only very few studies were published on the diagnostic accuracy of SFA, in particular adopting histology as reference standard. Our data are consistent with the results of a recent work by Filippou et al. who reported an accuracy of 88% with a specificity of 100% and a sensitivity of 77% (10). Novel alternative methods for SFA, such as Raman spectroscopy and lens-free microscopy, seem to be more accurate in the detection of CPP crystals than polarized light microscopy (22, 23). However, these approaches are not widely available in the clinical practice. In this study SFA demonstrated a specificity of 100% and a sensitivity of 86%. Two patients with CPPD had negative SFA, even though CPP crystals were found in both menisci and hyaline cartilage of the joint. There is no unique explanation for these discrepancies. Swan et al. pointed out that there is a relative lack of reliability of SFA in detecting CPP crystals and that the recognition of CPP crystals was worse than for MSU (12). No shedding, small dimensions, variability in morphology, weak birefringence, and the relative scarcity of crystals in the synovial fluid could be some of the reasons (11). In addition, expertise in the interpretation of specimens and the quality of the microscope make SFA an operator-dependent technique even among trained professionals, even though accurate training could improve inter-reader agreement (24).

Predictive values for SFA in the diagnosis of CPPD were found to be 100% for PPV and 33% for NPV, which appears to be low. This result is partially inconsistent with previous studies that demonstrated again high PPV and higher NPV (100% and 78% respectively) than in the present study (10). This could be due to the small number of patients in this study and to the high prevalence of the disease that may influence negatively the results. It is worth mentioning that in case of non-life-threatening diseases with different phenotypes such as CPPD, a low NPV can be acceptable (25), but in the clinical practice it should be kept in mind that one negative SF test does not definitely rule out the diagnosis of CPPD, while a positive test is confirmatory.

This ancillary study presents some limitations. First, the number of patients included was small and the prevalence of CPPD was higher than previously reported, thus potentially reducing the external validity of our findings, and overestimating the PPV of the diagnostic test. Furthermore, the samples were exclusively obtained from symptomatic knee joints with advanced OA, thus increasing the risk of a selection bias. Despite these limitations, this international multicentre work has a high internal validity, being one of the few studies that used the microscopic analysis of tissues as reference standard.

In conclusion, SFA proved to be an accurate test for identifying CPPD in patients with advanced OA. However, due to the necessity of an arthrocentesis, which is not always feasible, and considering the availability of validated imaging techniques for the detection of CPPD such as ultrasound, we suggest that SFA as diagnostic test should be limited to those patients where imaging and clinical data are not definitely confirmatory of the disease. It is also important to remember that a negative SFA does not rule out CPPD.

Conflict of interest

The authors certify that there is no actual or potential conflict of interest in relation to this article.

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