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ORIGINAL ARTICLE

The Persistency and Visibility of Synovial Fluid Crystals After at Least 10 Days in Refrigerator by Light and Polarized Microscopy

Saeedeh Shenavandeh[1]

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Abstract

Introduction Synovial fluid (SF) analysis is one of the most important tests used in approach to arthritis and is necessary for rheumatology fellowship training. It depends on the operator's experience and can be affected by handling, processing, temperature, type of preservative, and time from aspiration to analysis. Therefore, we aimed to reevaluate the SF with positive results for crystals, after at least 10 days for the persistency and visibility of the crystals.

Method For 1 year, we reevaluated crystal positive synovial fluid samples after at least 10 days under light and polarized microscopy. The samples were sent in tubes without any preservative. After the first day of diagnosis of a crystal arthropathy, all the samples were kept in 4 °C refrigerator in a syringe without any preservative and then reevaluated.

Results 14 calcium pyrophosphate (CPP); 12 monosodium urate (MSU), 1 of which was combined CPP and MSU; and 1 post-methylprednisolone (Depomedrol) injection, steroid crystal arthropathy were found and reevaluated. In all reevaluated samples [between 10–24 (median:14 days)], the crystals were detectable again.

Conclusion Our results suggests that SF CPP, MSU and methylprednisolone crystals at 4 °C without preservation could be detectable after 10–24 days (median: 14, 15.5, and 10 days, respectively) under light and polarized microscopy. It seems that the samples evaluated in emergency settings without enough time and those sent from other centers or gathering samples for trainees can be kept to detect crystals at least 10 days for all and till 22 days (for CCP and MSU) after sampling.

Keywords Crystal · CPP · MSU · Synovial fluid analysis · Polarized microscopy

Introduction

Synovial fluid (SF) analysis is one of the most important tests in approach to arthritis. It determines the degree of inflammation, finding the pathologic crystals and microorganisms [1]. Therefore, the capability of SF analysis and diagnosis of the crystals under polarized microscopy is a requirement in rheumatology fellowship training [2, 3].

Synovial fluid analysis is operator dependent, and its interpretation depends on the experience and presence of specially trained personnel/trainees [4]. Moreover, it can be affected by its handling, i.e., processing method, temperature, type of preservative, and time from aspiration to analysis. SF specimen handling methods remain controversial. The optimal recommendation is to examine the SF promptly, ideally immediately after aspiration, that is usually not applicable. In emergency room settings, where the focus is on infection evaluation, crystals sometimes can be missed due to restricted time for evaluation, and it is important to re-examine the SF for crystalline when an infection is excluded, maybe after 3–5 days. Sometime because of crowded clinics or lack of experienced clinicians or training or proper equipment in rural clinics and its time-consuming process of searching for crystals, the sample needs to be evaluated in equipped clinics. Also, we sometimes need to keep the samples for the best time when trainees are all available to learn the method of its interpretation in university clinics [5].

Therefore, we made an attempt to reevaluate the samples of SF with positive results for crystals after 10–14 days under polarized microscopy to find the time of the persistency of crystals when they were kept in refrigerator without any preservation.

✉ Saeedeh Shenavandeh
shenavande@sums.ac.ir

[1] Division of Rheumatology, Department of Internal Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

Discussion

We evaluated the persistency and visibility of crystals in SF samples of patients with crystal induced arthritis after keeping the samples in 4 °C refrigerator in a syringe without any preservation materials to find the accuracy of these samples for re-evaluation and finding crystals if there were no time or experienced person or microscopy for exact diagnosis on the first day of evaluation or keeping samples for teaching purposes in our clinics.

Our results suggest that SF MSU, CPPD, and steroid crystals can be defined after 10–22 (median:14) days (for CPP:14 (10–24), for MSU:15.5 (10–22) and for methylprednisolone crystals after 10 days) when the samples are stored at 4 °C without EDTA or sodium heparin as an anticoagulant or any other preservation. Several studies have looked at SF handling for CPP analysis in SF with different results. These studies have not been systematically evaluated. Only one recent systematic review article evaluated the effect of SF handling, time, temperature, and preservation effect on CPP and MSU crystals. It showed that most articles mentioned that MSU crystals were mostly unaffected by storage time until 72 h. Two studies looked at storage over several weeks. One study found 100% stability for up to 12 months on air-dried slides; in another study that used preservative-free vials at 24 weeks, MSU was detected in 87, 69, and 89% in the 20, 4, and 20 °C groups, respectively. For CPP, one study demonstrated no loss in CPP detection at 2 and 4 weeks, and in sum it was shown that, unlike MSU crystals, CPP crystals do vanish with time and the best time for their detection was within 24 h. However, they can be fairly detectable for up to 72 h, especially if they are refrigerated. Some articles showed that they could be detected for weeks, but their quantities can be declined. Among different sample preservatives, it was shown that there was no significant difference between heparin, ethylenediaminetetraacetic acid (EDTA) versus no preservative on MSU detection. For the CPP, the results were the same. In sum, it showed that both CPP and MSU crystals remained stable independent of the preservative for 24 h and up to 72 h, so it can be stored in a syringe or EDTA tubes. As to the temperature, both crystals remain very stable at room temperature for the clinically relevant duration of 72 h. There was a small drop in the percentage of detections in 4 °C when the preservation was the heparin. Therefore, it was recommended that for analysis beyond 24 h (usually 48–72 h), SF should be refrigerated ideally in an EDTA tube as this preserves the white blood cells and if there was enough sample and CPP crystals suspected, some SF might also be placed in a heparin tube. Also, it is recommended that if there is high clinical suspicion for crystal arthropathies and initial sample is negative, it would be better to re-examine refrigerated SF after 24 h [5]. It was known that very low temperature (4 °C) had a slowing effect on the dissolution of MSU crystals and that this effect continued over several weeks. Kerolus et al. worked on 7 synovial fluids with MSU crystals and showed that they did not disappear over the first few days, but over 1–8 weeks they did decrease in number; also, compared with the aliquots of the same specimens kept at room temperature, refrigeration seemed to protect MSU crystals from dissolution. They also showed on five samples with CPP crystals that they could disappear with time and were difficult to recognize by the next day in 3 of 5 samples and all crystals were dissolved within 8 weeks; however, some samples still had a few crystals after 3 months of storage. Refrigeration does not seem to protect CPPD crystals from dissolution after a long time in their study [6].

We did not find any article about the detection of steroid crystals in joint fluids, too, as those samples were mostly excluded from studies. Our experience on steroid crystal showed that it persisted after 10 days intracellularly and was detectable easily.

There are also laboratory recommendations that if MSU and CPP crystal analysis are to be delayed or used for educational purposes, the sample might be stored at room temperature (with or without sodium heparin or EDTA) up to 72 h and if for more hours, at 4 °C refrigerator till 8 weeks [7].

A study assessed the re-evaluation of negative samples after 24 h and identified an additional 3 and 2% of cases of gout and CPPD, respectively; it was found that in some patients the number, size, and/or birefringence (visibility) of crystals might increase over time in vitro and in vivo [8]. In our evaluation, we did not check the number of crystals or time consumption to find the crystals compared to the first evaluation, and it was our further plan of research although all of them were detectable after at least 10 days.

Conclusion

Our results suggest that SF MSU, CPP can be defined after 10–24 (median:14) days and for the steroid crystals after at least 10 days, when the samples are stored at 4 °C without EDTA or sodium heparin as an anticoagulant. Therefore, it seems that the negative or suspected samples that were evaluated in emergency settings without enough time for complete evaluation, or gathering samples for trainees and samples from other centers with no available equipment for crystal analysis can be re-evaluated and detectable for crystals till at least 2–6 weeks after sampling.

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Data availability All data and materials in this article are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors have declared that they have no conflicts of interest.

Ethics approval and consent to participate The ethics committee of Shiraz University of Medical Sciences approved this study (Ethical code: IR.SUMS.MED.REC.1401.330). Patients' information was anonymized before data analysis and confidentiality of patient information was guaranteed and protected.

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