

Apatite crystal identification in dried smears and synovial fluid pellets with alizarin red staining

Sirs,

Monosodium urate (MSU) and calcium pyrophosphate (CPPD) crystals can often be easily identified in a wet drop preparation of synovial fluid with compensated polarized light microscopy (1-3), and identification of apatite or basic calcium phosphate, can be facilitated by using an alizarin red S stain in a single wet drop preparation of synovial fluid (4). Wet drop examination requires prompt examination once the drop is prepared and is not a permanent preparation that can later be reviewed. MSU and CPPD crystal identification in Gram and Wright stained dried smears have been described (5, 6). Use of dried smears has not been used for alizarin red staining to identify apatite and has not yet been reported. The use of dried smears with alizarin red permits the detection of apatite crystals and quantification of the number before and after spinning the fluid.

Alizarin red S stain 2.0gm (Fisher Scientific, Malvern, PA) was prepared as previously described (4). Forty-five samples of fresh synovial fluid were examined over four months. Twenty-five fluids that had some evidence of positive alizarin red stain for calcium crystals were included. Each specimen was examined as a single wet drop mixed with alizarin and a dried smear of a single drop, onto which a drop of alizarin was added and allowed to dry. Following this, the unused fluid was centrifuged in a Beckman Microfuge™ 11 and a pellet was obtained. This was re-suspended in approximately 0.1 cc fluid. This suspended pellet was then prepared as a wet drop and as a dried smear and stained as described above. Evaluation was performed by two observers trained in crystal identification in synovial fluid (HC, GC), who recorded the presence and number of apatite crystal clumps in a wet drop preparation per high power field (HPF) as described elsewhere (7). Quantification of suspected apatite crystals was as follows: 0 = no alizarin positive clumps, 1+ = 1 clump/HPF, 2+ = 2-3 clumps/HPF, 3+ = > 4-10 clumps/HPF and 4+ = > 10 crystal clumps/HPF. Results were evaluated using a Chi-square statistical test.

Apatite crystals clumps were easily identified in wet drop preparations with alizarin red S as well as in dried smears with alizarin. A large number of crystal clumps were within aggregates of fibrils has typically been seen in osteoarthritis (Fig. 1). The presence and quality of crystals observed was similar comparing both methods. The number of crystals and cells observed increased after spinning the fluid sample for a pellet in the wet drop preparation ($p < 0.02$), as well as in the dried smears ($p < 0.01$).

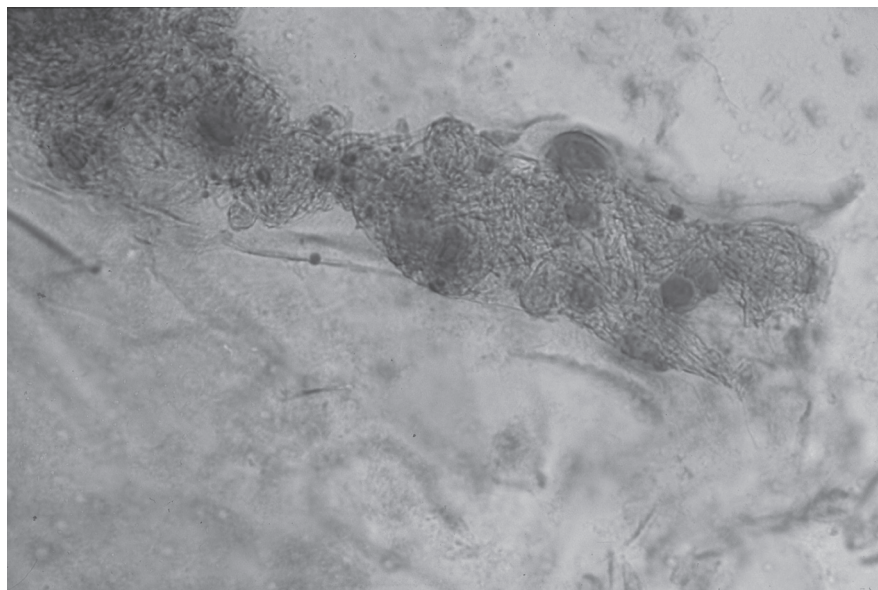


Fig. 1. Alizarin positive clumps among fibrils in dried smear of pellet from patient 10 with knee osteoarthritis. Note also occasional homogeneous pale red artifacts. 400X

The quality of smears decreased once the fluid examination was delayed and artifacts drop oil alike may appear. Six patients were negative on the initial wet drop but positive on the pellet. Only one patient was positive on the initial wet drop and negative on the initial smear.

Mc Carty *et al.* (8) by x-ray diffraction and later Schumacher *et al.* (9), described the presence of apatite crystals in joint fluids with otherwise unexplained arthritis and in patients with osteoarthritis (OA) (3). Apatite crystal presence in OA correlates well with the presence of destructive arthritis (10). Clinical recognition and identification may be important to explain some attacks of arthritis and perhaps to suggest that more apatite clumps correlate well with more advanced and destructive knee OA.

We have now shown that smears can be prepared from the synovial fluid and centrifuging to make a pellet helps to identify a larger number of crystals than before spinning the sample. Dried smears may present artifacts seen as orange stained spots but apatite crystals are easily distinguishable. All dried smears are good enough to detect apatite crystals for at least 24-48 hours, and some crystals have still been visible for over 6 weeks.

Alizarin red S stains on dried smears allow further evaluation of synovial fluid samples for more than one clinician, thus, samples can be kept for a short period for further studies and observations.

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