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Quality control of synovial fluid crystal identification

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Abstract

***Objective*—To establish a quality assessment programme for the diagnosis of crystal arthropathies by synovial fluid (SF) microscopy.**

***Methods*—Three or four cytocentrifuge slides prepared from suitable patient SF specimens were distributed to 25–47 predominantly Finnish clinical laboratories once a year. Sodium urate crystals were included in every survey.**

***Results*—Returns for the years 1989–1996 were reviewed. Laboratories that participated in > four surveys made on an average one error a year (range 0.25–2). The error rate for specimens containing abundant crystals was acceptable but it increased considerably for specimens showing few crystals per microscope field. No laboratory characteristic predictive of successful performance was found.**

***Conclusion*—Errors in quality assessment results for crystal identification were much more frequent than in the fields of, for example, clinical chemistry or microbiology. Despite efforts to provide educational feedback, no improvement was seen during the study period. Because of the dearth of data from other parts of the world it is not known for certain whether this study has merely pinpointed a local problem or if the same trend applies elsewhere.**

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Synovial fluid (SF) analysis is something of an orphan in the clinical laboratory. The annual number of specimens is in most cases modest[1] and reference values for normal SF are scarce. Dedicated specialist SF laboratorians (haematologists) are exceedingly rare; conversely, many rheumatologists are not actively engaged in laboratory procedures. Yet SF analysis can be crucially important. The rheumatic diseases can be pragmatically divided into three categories: bacterial arthritis, which can be cured; gout, for which efficient and effective treatment is available; and other rheumatic diseases, at best symptomatically controlled. The first two,

Table 1 Analytes and responses to the query: "Does the specimen contain sodium urate and/or calcium phosphate chrystals?"

Year	*Number of participants*	*Specimens*	*Correct results for MSU/ CPPD (%)*	*False positive for MSU/ CPPD (%)*	*False negative (%)*	*Labs with no errors (%)*	*Mean number of errors*
1989	25	MSU abundant	100	NA	0	80	0.32
		Cholesterol	80	20			
		Triamcinolene hexacetonide	88	12			
		Glove powder	100	0			
1990	36	MSU abundant	86	NA	14	17	1.0
		MSU moderate	53	NA	47		
		MSU rare	33	NA	67		
1991	47	MSU moderate, extracellular	70	NA	30	25	1.1
		MSU moderate, intracellular	60	NA	40		
		Cholesterol	63	37			
1992	35	MSU moderate	89	5	5	43	0.57
		MSU rare	67	3	31		
		CPPD abundant	63	3	34		
1993	40	MSU moderate	76	17	7	38	1.0
		MSU rare	76	7	17		
		Betamethasone acetate	53	47			
1994	45	MSU moderate	94	6	0	38	0.44
		MSU rare	84	8	8		
		Sodium oxalate	66	34			
1995	41	MSU abundant, intracellular chrystal fragments	57	5	38	39	0.61
		MSU moderate	89	3	11		
		MSU rare	89	5	5		
1996	47	MSU moderate	54	4	40	13	1.6
		MSU rare	26	7	67		
		MSU abundant, triamcinolone hexacetonide abundant	64	30	7		

MSU = monosodium urate, CPPD = calcium pyrophosphate dihydrate, NA = not applicable: the CPPD alternative was not included in 1989–1991.

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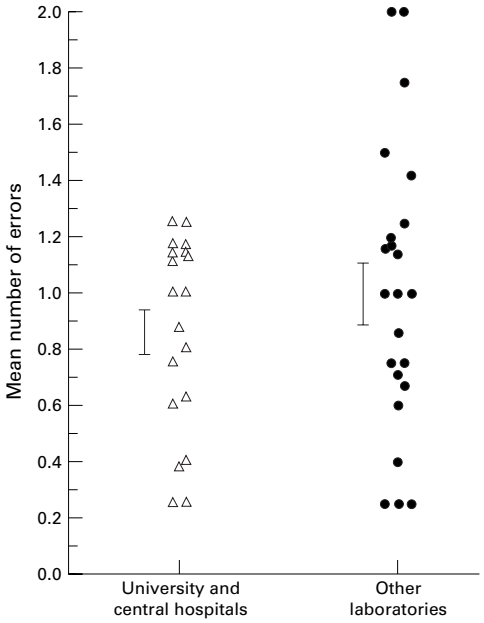

Figure 1 Mean annual number of errors for 19 university and central hospital laboratories and 23 other clinical laboratories that participated in >4 SF crystal identification surveys. Bars = standard error.

and pyrophosphate arthropathy besides, can be diagnosed with certainty only by SF analysis. But whereas simulated bacterial arthritis specimens of SF specimens routinely figure in microbiology external quality assessment programmes a need for similar assessment of SF crystal examination has not to date been generally recognised. Here we present the disconcerting results of eight annual SF crystal identification surveys in Finland.

Methods

For specimen preparation we used a procedure modified after our preliminary study.[2] Cytocentrifuge (Shandon Ltd, UK) slides were prepared from crystal containing, suitably diluted SFs. We designed special microscope slides, covered with a dark hydrophobic film of paint except for a single 6 mm 0 circular well of bare glass surface aligned to the hole in the cytocentrifuge specimen funnel (Danbrit Co, Helsinki, Finland). A similar slide has since become available from the cytocentrifuge manufacturer. All positive specimens contained at least one crystal per microscope field, 0.78 mm^3 at × 250 magnification. Sets of three coded slides (four in 1989) were sent to the participating laboratories together with an instruction sheet and a reply form. Early on, we enclosed a questionnaire concerning the number of specimens annually examined, microscope equipment, internal controls, and procedures for examining specimens and validating results. Participants wishing to keep a permanent specimen for future reference were advised to examine the slides as such and to refrain from using immersion oil. For better optical quality and a closer illusion of a conventional SF "wet prep", participants were instructed to place a drop of 10 µl serum in the well, to place a coverslip on top and apply gentle pressure (for example, with the eraser end of a pencil) in the centre, and, to prevent solubilisation of crystals, to examine the slide immediately.

Results

Table 1 shows the number of participants, analytes, and survey results. Only the identification of MSU and CPPD were taken into account in the result evaluation; however some participants did correctly identify various other birefringent substances. Emphasis was heavily on MSU identification. Apart from the greater clinical importance of diagnosing gout than diagnosing pyrophosphate arthropathy, SF specimens with a high enough CPPD concentration to be suitable for the preparation of survey samples were rare in our patient material. Except for the first year, 1989, the mean number of errors stayed roughly similar, with no trend towards improvement.

Figure 1 summarises the distribution of errors between individual laboratories over time. While the most error prone were smaller laboratories, the mean number of errors is similar for large hospitals and other laboratories. Together the two sets of figures form a continuum, not a dichotomy of competents and incompetents. Neither did the questionnaire responses, often incomplete, reveal any meaningful correlations between laboratory practices and survey results.

Discussion

Crystal identification is only one facet of SF analysis.[3] It is qualitative in nature, and that way more akin to clinical microbiology or haematology than to clinical chemistry. But while the number of crystals is diagnostically irrelevant, it is evident that false negative results increase when crystals are scarce (table 1). The same trend has been noted in other crystal analysis studies on identical liquid SF specimens, performed by different preselected laboratories, or by different observers.[1 4–6] We preferred dry cytocentrifuge specimens, partly for logistic reasons, partly because of the prevailing uncertainty concerning dissolution and loss of birefringence of true SF crystals and the appearance of artefactual ones in stored SF specimens.[7–10] Cytocentrifugation is also a convenient technique for making stained reference preparations that show up SF cells as well as crystals. Moreover we have found it useful for crystal detection in dilute specimens such as dry joint wash fluid, because the method places all crystals in the same optical plane. Though cytocentrifuge specimens may have been unfamiliar to the participants early on, it seems unlikely that the specimen format would have affected the results adversely in the long run.

We were unable to link survey results to laboratory practices through the questionnaire responses. While the lack of correlation to laboratory type and annual number of specimens could be real, microscope properties are not adequately disclosed this way. A vexing aspect of our results is the lack of improvement over time. Procedures for SF crystal detection by polarisation light microscopy have not

changed since they were first described,[11] whereas microscope illumination and optics have steadily improved. The technique is covered in detail in the standard rheumatology textbooks, and two special atlases on the subject have been published, with splendid illustrations of both the relevant crystals and other birefringent materials that SF may contain.[3 12] But as the books may not be readily available to all Finnish laboratorians, we have tried to cover the subject by lectures, articles in the national quality control journal, and by video, apparently in vain. Very limited experience suggests that the effective though difficult to implement method for improvement is site visits, as differences in microscope construction can be the cause of confusion.

It could be argued that our unsatisfactory results are a local problem that is unrelated to the situation elsewhere, and which could be corrected by referring all tests to a few expert laboratories. Though centralisation probably would reduce errors, there are practical problems with handling and mailing the not uncommon specimens that consist of a few µl of aspirate in a syringe; perhaps also reluctance to refer, and thus delay, an examination that demands no special equipment unavailable on site. While we are not aware of any other long term quality assessment studies, SF crystal surveys in New Hampshire and Vermont,[1] in Bristol,[5] and in Sydney[13] all produced far from perfect returns. In conclusion, the prevalent complacence regarding SF crystal identification seems to be based on the theoretical simplicity of the test method rather than on the available data, which show that both false positives and false negatives may occur, with potentially serious medical consequences.

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