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The Clinical Significance of Cytoplasmic Inclusions(CPI) in Synovial Fluid Examination

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The clinical significance of cytoplasmic inclusions(CPI) in synovial fluid(SF) examination was evaluated. We examined SF specimens collected from major rheumatology clinics in the Philadelphia area during the period of January to December 1995. Among 759 patients in the initial study group, 419 cases with established diagnoses and full synovial analyses were included. Their diagnoses and SF analysis results including leukocyte counts, differential counts and wet preparations were collected and analysed. Ninety seven of the 419 SF specimens were found to have CPI. CPI were found in SF from almost all rheumatic diseases. They were most likely to be found in inflammatory arthropathy including rheumatoid arthritis(RA, 46%), juvenile rheumatoid arthritis(JRA, 78%) and psoriatic arthritis(55%). On the contrary, CPI were least common in crystal-induced arthropathy among the inflammatory arthropathy. CPI were found 8 out of 98 gout cases(8%) and 2 among 53 calcium pyrophosphate dihydrate(CPPD) deposition disease(4%). In noninflammatory arthropathy, CPI were found in only 6 cases(6%) out of the 103 osteoarthritis(OA). In RA cases with noninflammatory SF, 4 of the 20 SF(20%) had CPI while only 6% of OA SF had CPI. OA SF with CPI were all noninflammatory SF. In summary, CPI were a common finding on SF examination. CPI were more likely to be found in inflammatory arthropathy than noninflammatory. Among inflammatory arthropathy, CPI can favor non-crystal arthropathy than crystal arthropathy. Awareness of the presence of CPI is suggested as an addendum to routine SF analysis. Renewed investigation of the several types of CPI may add further to the understanding of joint disease.

Key Words : *Cytoplasmic inclusions, Synovial fluid*

INTRODUCTION

Cells with small round cytoplasmic inclusions(CPI) visible on wet preparations have been described in rheumatoid arthritis(RA) synovial fluid(SF) specimens and termed RA cells or ragocytes(Delbarre et al., 1964; Hollander et al., 1965). The demonstration that some such intracellular inclusions contain immunoglobulin and rheumatoid factor(RF) has played an important part in the development of modern concepts of the pathophysiology of RA(Hasselbacher, 1979). However, since simple morphologic demonstration of CPI was found to

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be nonspecific and found in diseases other than RA (Brandt et al., 1968), not much attention was paid to this subject. This study was done to reevaluate the clinical significance of demonstration of CPI in SF examination.

MATERIALS AND METHODS

SF Specimens : SF specimens, which had been collected by physicians in several clinics and delivered the same day to our laboratory during January to December 1995, were evaluated. The great majority of specimens were from the Hospital of the University of Pennsylvania, Medical College of Pennsylvania and Hahnemann University Medical Center, Childrens Hospital of Philadelphia and Philadelphia Veterans Affairs Medical Center. Specimens studied were from patients who had a definite clinical diagnosis at the time of arthrocentesis without regard to the presence or absence of other specific criteria. Excluded from this study were specimens from patients with unestablished or unclassified diagnoses and with localized periarticular diseases. Among 759 patients in our initial study group, 488 SF specimens with established diagnoses were included retrospectively ; full synovial fluid examinations were done in 419 of these cases.

SF Examination : All SF specimens were examined manually for white blood cell(WBC) counts(with 0.3% saline as a diluent). A wet preparation was immediately examined under polarised light and Wright's stained smears were done for differential counts. CPI were recorded as positive or negative. CPI were defined under light microscopy(LM) as small round 0.5-2.0 μm nonbirefringent structures in the cytoplasm, not in the nucleus. To be considered positive they had to be visible in virtually every field and in several cells per high power field. Numbers or percentages of cells with CPI or the quantification of CPI were not recorded.

RESULTS

The most common diagnosis in 419 cases with full SF examination was osteoarthritis(OA), followed by gout and rheumatoid arthritis(RA). Ninety seven among 419 cases were found to have CPI. CPI had various sizes and were usually round in shape(Fig. 1). Under EM, CPI appeared round in shape and either contained granular protein-like material or lipid(Fig. 2). CPI were a common finding in SF examination. They were a nonspecific finding and, in addition to that, they were found in almost all rheumatic diseases. They were more likely to be found in inflammatory arthropathy, and among inflammatory arthropathy, CPI were more likely to be found in non-crystal arthropathy. As shown in Table 1, CPI were commonly found in JRA(78%), psoriatic arthritis(55%), RA(46%), systemic lupus erythematosus(43%) and reactive arthritis(33%). On the contrary, they were less commonly found in gout(8%), calcium pyrophosphate dihydrate(CPPD) deposition disease(4%) and OA(6%). CPI were also found in other rheumatic diseases including ankylosing spondylitis(100%), dermatomyositis(100 %), Reiter's syndrome(33%), Lyme arthritis(25%) and polymyalgia rheumatica(25%), although the number of evaluations was small in these categories. Comparison

Table 1. Incidence of CPI

Diagnosis	No. of cases	No. of CPI (+)	Incidence (%)
Inflammatory arthropathy			
Non-crystal arthropathy			
Rheumatoid arthritis	94	43	46
Juvenile RA	23	18	78
Psoriatic arthritis	11	6	55
Reactive arthritis	9	3	33
SLE	7	3	43
Crystal arthropathy			
Gout	98	8	8
CPPD	53	2	4
Non-inflammatory arthropathy			
osteoarthritis	103	6	6

CPPD : calcium pyrophosphate dihydrate
SLE : systemic lupus erythematosus
Among 419 cases, the 21 cases with small numbers of evaluation were not shown.

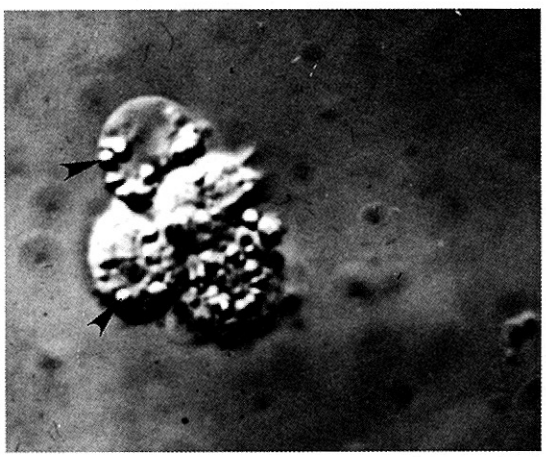

Fig. 1. Cytoplasmic inclusions(arrowheads) enhanced by phase contrast microscopy. ×600.

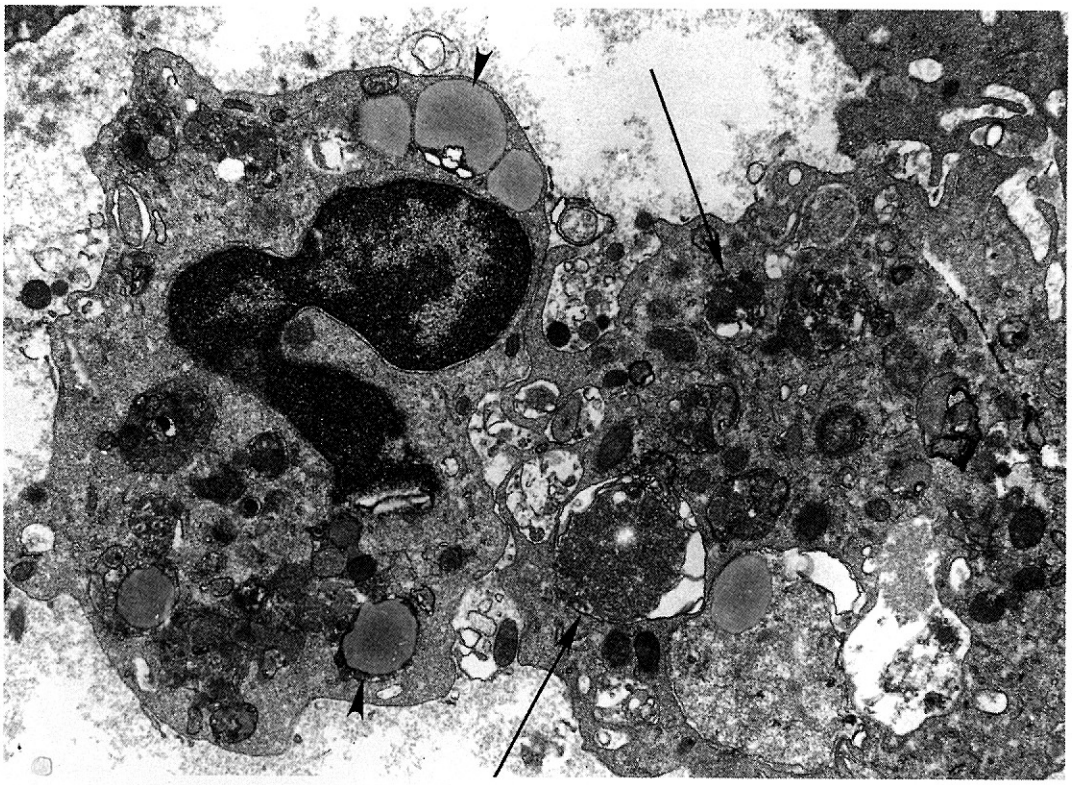

Fig. 2. A macrophage under electron microscopy showing multiple cytoplasmic inclusions(arrows) and lipid bodies(arrowheads). ×6300.

Table 2. Comparison of incidence of CPI between inflammatory and noninflammatory SF

Diagnosis	Incidence(%)	
	Inflammatory	Noninflammatory
Inflammatory arthropathy		
Non-crystal arthropathy		
Rheumatoid arthritis	39/74 (53)	4/20 (20)
Juvenile RA	18/21 (86)	0/2 (0)
Psoriatic arthritis	6/8 (75)	0/3 (0)
Reactive arthritis	3/6 (50)	0/3 (0)
SLE	3/6 (50)	0/1 (0)
Crystal arthropathy		
Gout	5/54 (9)	3/44 (7)
CPPD	2/15 (13)	0/38 (0)
Non-inflammatory arthropathy		
osteoarthritis	0/3 (0)	6/100 (6)

of incidence of CPI between inflammatory and non-inflammatory SF is shown in Table 2. CPI were more likely to be found in inflammatory SF in non-crystal inflammatory arthropathy. There were 20 RA cases with noninflammatory SF as defined by SF with WBC less than 2000/mm^3; 4 of these cases had CPI found(20%), while only 6% of OA SF had CPI. OA cases with CPI were all noninflammatory SF.

Differential counts were done in 79 of the 97 specimens with CPI. Polymorphonuclear leukocytes(PMNL) were the predominant cell in 67 SF specimens, and mononuclear cells in 11 SF specimens. However, in percentages, CPI were found in 39.6 percent of SF with PMNL predominant and in 34.3 percents of SF with mononuclears predominant among the total 419 SF specimens.

DISCUSSION

Ever since cells with CPI in RA SF specimens were termed as RA cells by Hollander et al.(1965) or ragocytes by Delbarre et al.(1964), many studies have been done to evaluate CPI and their significance in RA. The inclusions were thought to represent phagocytosed precipitates of rheumatoid factor(Broderick et al., 1976),

or immune complexes from the SF(Zvaifler, 1973; Stiehl, 1977). Although the simple morphologic demonstration of CPI was found to be nonspecific and CPI were found in diseases other than RA(Brandt et al., 1968), they were reportedly more common in RA(Gatter and Richmond, 1975 ; Broderick et al., 1976 ; Stiehl, 1977). The demonstration of intracellular inclusions containing immunoglobulin and rheumatoid factor(RF) has played an important part in the development of modern concepts of the pathophysiology of RA(Hasselbacher, 1979). The full details of their nature remain unknown. Using ultrastructural immunocytochemistry, intracellular inclusions of IgG, IgM and C_3 were observed in vacuoles of greater than 75% of PMNLs and mononuclear cells (Cherian and Schumacher, 1983).

None of previous studies defined exactly what was meant by CPI at a light microscopic level. CPI were defined in our study under light microscopy(LM) as small round 0.5-2.0 μm non-birefringent structures in the cytoplasm, not in the nucleus. They had to be visible in virtually every field and in several cells per high power field in the SF specimens. Various kinds of intracytoplasmic structures create this appearance so that CPI also include intracytoplasmic lipid droplets which appear identical under LM. Crystals and the larger inclusions typical of Reiter's cells were excluded.

CPI were more commonly found in RA with inflammatory SF than OA with noninflammatory SF, and this was not surprising. According to the study by Davis et al.(1988), positive correlations in the serial specimens between proportions of PMNLs and cells with CPI were found when serial RA SF specimens were analysed cytologically. However, in our study, even with noninflammatory SF, CPI were also more commonly found in RA than in OA. Thus it seems true that CPI are more likely to be related with inflammatory disease processes even when inflammation is not intense. In OA, most of them showed noninflammatory SF as expected, and only six cases had CPI(6%). It may be possible to say that positive correlations are present between cells and CPI in a certain disease entities. However, CPI were present in OA without regard to the cell numbers, and interestingly they were all found with noninflammatory SF. Whether these 6 cases represent a different subset of OA will require further study(Schumacher, 1995). Although CPI have been most commonly discussed as characteristics of PMNL, they were found in mononuclear cells including synovial monocytes and macrophages in our study. We have seen CPI in a similar proportion in PMNL and monuclear cell populations and their possible role in chronic inflammatory cells remains conjectural at this moment.

CPI were found far less often in SF specimens with crystal arthropathies comparing to those with other non-crystal arthropathies. CPI were found 8 out of 98 gout cases(8%) and 2 among 53 CPPD cases(4%). This confirms the previous report of Gatter and Richmond(1975) in which they noted CPI were found more commonly in RA than in crystal arthropathy; our results expand the observation in that almost all inflammatory arthropathy had more CPI than those with crystal arthropathy, although the number of evaluations was small in some disease categories. Thus it seems true that CPI found in SF examination can favor non-crystal arthropathy than crystal arthropathy among inflammatory arthropathy.

CPI are polymorphic. They have various sizes and are usually round in shape(Fig. 1), but some also can be seen as vaguely square or short rod-like structures that could be confused by the unwary with crystals such as CPPD. Although we elected to call CPI only as positive or negative this is obviously an oversimplification. Some fluids have rare CPI and the meaning of these is not clear. Other SF specimens may contain similar inclusions which are too small to be identified by conventional LM or too few in number to meet our criteria. As noted above, CPI should be differentiated from CPPD crystals, apatite clumps, small calcium oxalate crystals, cell debris and lipid liquid crystals under polarized LM. Without an excellent microscope it may be especially difficult to differentiate CPI from intracellular CPPD crystals because they have also a variable morphology, size and birefringence. Although there were only 2 cases of CPI present with CPPD crystals in our study, we believe that intracellular CPPD crystals can confuse less experienced examiners. Actually among the SF specimens which were sent to us for intracellular CPPD crystal confirmation and also were included in this study, we noted that large numbers of CPI were occasionally confused with CPPD crystals by less experienced examiners(Clayburne et al., 1995). In those cases, electron microscopy(EM) was helpful to differentiate CPI from CPPD crystals. Under EM, CPI appeared round in shape and either contained granular protein-like material or lipid(Fig. 2). Further attention seems warranted in examining SF specimens when CPI versus CPPD crystals are being considered. CPI are most common in RA but CPPD can occasionally occur in RA(Moskowitz et al., 1965 ; Good and Rapp, 1967 ; Resnick et al., 1981 ; Zyskowski et al., 1983 ; Doherty et al., 1984 ; Brasseur et al., 1987) and gout(Ho and

DeNuccio, 1993). Awareness of the presence of CPI seems to be needed to increase the diagnostic value of the SF analysis especially in inflammatory SF specimens and/or in a condition which an intracellular CPPD crystal is suspicious.

CPI were found in SF specimens with almost all rheumatic disease categories including OA and it is interesting because they have completely different etiopathogenic mechanisms and diverse clinical manifestations, and that seems to justify further research work, such as finding and comparing the possible differences in CPI present in SF cells with all different disease entities by using ultrastructural immunocytochemistry. Recently there are increasing numbers of papers about so called lipid bodies, which we believe are the same intracytoplasmic lipid droplets and indistinguishable from other CPI that we have seen in SF specimens so far under LM. Lipid bodies in human leukocytes function as intracellular sites for eicosanoid formation and they become more prominent in number and size when these leukocytes are engaged in inflammatory responses(Weller et al., 1989, 1991 ; Weller and Dvorak, 1994). Increased numbers of lipid bodies were recognized in cells recovered from inflammatory reactions, such as pyogenic joint effusions(Coimbra and Lopes-Vaz, 1971). How often the lipid bodies, rather than phagocytized immunoglobulin, explain the light microscopic appearance of CPI can only be determined by further study.

In addition, the significance of what were termed gray globular structures(GGS) of Zucker-Franklin (Zucker-Franklin, 1966), which were first described in RA SF WBC by EM with only the larger of these detected as CPI with the conventional microscope, needs to be reevaluated. SF cells with other rheumatic diseases had similar structures although to a lesser extent. The GGS may have some relation with lipid bodies. Although Zucker-Franklin inferred they were carbohydrate or protein rather than lipid in nature, the origin and significance of the GGS are still conjectural at the present time.

Although CPI were found in 23% of all SF specimens in our study, we are not certain how this result may be applicable to SF in all settings. Some of our specimens, which were sent to us for crystal confirmation, may have had bias in sample selection. There might be occasional errors in our clinical diagnoses. However, we believe that rare sampling and diagnostic errors would not alter our general conclusions. There are still many questions about what CPI numbers may be meaningful, and about techniques for CPI identification in SF examination. In this preliminary study, the number or percentage of cells with CPI and actual number of each inclusion per cell were not estimated and those can be further evaluated in future studies with more detailed criteria.

In this study, the clinical significance of demonstration of CPI in SF specimens was evaluated and updated. Ninety seven SF specimens among 419 SF specimens were found to have CPI. CPI were a common finding on SF examination. CPI were more likely to be found in inflammatory arthropathy than noninflammatory. Among inflammatory arthropathy, CPI can favor non-crystal arthropathy than crystal arthropathy. Awareness of the presence of CPI is suggested as an addendum to routine SF analysis. Renewed investigation of the several types of CPI may add further to the understanding of joint disease.

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