

Comparative studies of serum and synovial fluid C reactive protein concentrations

I F ROWE,¹ J SHELDON,² P G RICHES,² AND A C S KEAT¹

From the Departments of ¹Rheumatology and ²Chemical Immunology, Westminster Hospital, London

SUMMARY The relation between serum and synovial fluid (SF) C reactive protein (CRP) concentrations was investigated in a variety of arthritides, including rheumatoid arthritis (RA), psoriatic arthritis, reactive arthritis, and osteoarthritis. SF CRP levels were significantly reduced compared with serum levels in the inflammatory arthritides, but there was good correlation between serum and SF values. SF CRP values were all at the lower limit of the detectable range in osteoarthritis. In patients with RA or psoriatic arthritis followed up serially through an exacerbation of arthritis, changes in SF CRP reflected closely changes in serum CRP. In patients with RA SF/serum ratios of proteins of different molecular weight were used to derive a regression equation between SF/serum ratio and molecular mass. SF/serum values for CRP were significantly less than predicted from its molecular weight, suggesting that CRP is either being selectively bound in synovium or specifically consumed in SF and may be playing an important part in the inflammatory process in RA.

Key words: acute phase response, rheumatoid arthritis, psoriatic arthritis, reactive arthritis.

C reactive protein (CRP) is the classical acute phase reactant, circulating concentrations of which may increase up to 1000-fold in response to a wide variety of tissue damaging processes.¹ Raised levels occur in certain forms of inflammatory arthropathy, including rheumatoid arthritis (RA)² and psoriatic arthritis.³ In RA serum CRP levels correlate well with disease activity and are a useful aid in the clinical management of this condition.^{2,4}

CRP is produced exclusively in the liver; control of synthesis being mediated by a family of polypeptides known as interleukin 1. The CRP molecule has calcium dependent binding sites for phosphorylcholine residues^{5,6} through which it can bind in vitro to a variety of autogenous and extrinsic ligands, including lipoproteins,⁷ chromatin,⁸ and micro-organisms such as pneumococci. Once bound to a ligand human CRP may promote opsonisation or activate the classical complement pathway.⁹

In man in vivo, however, CRP has not been demonstrated in a complexed form with any ligand in the circulation¹⁰ or in synovial fluid (SF),¹¹ and its true biological role remains unknown. The stable

conservation through evolution of the structure and ligand binding specificity of CRP suggests that it has an important function, which is presumably beneficial. In contrast, there is some evidence to suggest that CRP may, in certain circumstances, promote or enhance inflammation through activation of the complement system,^{12,13} and intra-articular injection of CRP may exacerbate inflammation induced by poly-D-lysine in rabbit knee joints.¹⁴ We undertook to investigate the relation between CRP concentrations in serum and SF in RA, a number of other inflammatory arthritides, and in osteoarthritis in order to attempt to determine whether this protein is subserving a major role in the inflammatory process within the joint.

Patients and methods

Serum samples and SF specimens aspirated from one or both knee joints immediately after venepuncture were obtained from patients with different arthropathies (Table 1). Samples from 17 patients diagnosed as having RA according to the criteria of the American Rheumatism Association¹⁵ were studied, three patients being followed up serially from three to seven months, allowing collection of a total of 49 pairs of samples. Five patients with psoriatic

Table 1 Clinical details of patients studied

Disease	No of patients		Age (years)		Duration of disease		Treatment (Nos)	No of sera	No of SF	
	Total	Male	Female	Mean	Range	Mean				Range
Rheumatoid arthritis	17	2	15	64.4	55-73	13.1 years	1-30 years	NSAID alone (10) NSAID+pred (5)	31	49
Psoriatic arthritis	5	4	1	48.0	32-64	7.8 years	2-12 years	NSAID+D-pen (2) NSAID alone (4)	16	18
Reactive arthritis	13	13	—	26.6	19-40	3.5 months	8 days-12 months	NSAID+D-pen (1) NSAID alone (9)	13	13
Osteoarthritis	6	3	3	69.0	52-80	12.0 years	5-20 years	NSAID (6)	11	18
Pyrophosphate arthropathy	2	2	—	52*	80*	1 week*	—	NSAID (2)	2	2
Acute gout	1	1	—	55*	—	1 week*	—	NSAID	1	2
Septic arthritis/RA	1	1	—	72*	—	2 weeks*	—	NSAID	1	1
Parvovirus arthropathy	1	—	1	32*	—	2 weeks*	—	NSAID	1	1

*Values for individual patients. D-pen=D-penicillamine; NSAID=non-steroidal anti-inflammatory drug; pred=prednisolone; RA=rheumatoid arthritis; SF=synovial fluid.

arthritis as described by Moll and Wright¹⁶ were studied, four with undetectable rheumatoid factor and one with a weak positive Rose-Waaler of 1/8; one patient was followed up serially for nine months. Thirteen patients with reactive arthritis secondary to non-gonococcal urethritis were studied retrospectively. Details of patients with osteoarthritis and individuals with other arthropathies are given in Table 1.

SF samples were collected in plain glass containers. On 10 occasions the SF sample was divided and part put into a plain tube and part into a tube containing ethylenediaminetetra-acetate (EDTA) to give a final concentration of 0.75-1 mg/ml (2.5-3.5 mmol/l). To assess stability of CRP in SF aliquots of one SF specimen were left at room temperature or at 4°C for up to 72 hours before assaying for CRP concentration. Samples were centrifuged and stored at -20°C before analysis. CRP, α_1 acid glycoprotein, transferrin, caeruloplasmin, and α_2 macroglobulin concentrations in serum and SF samples were measured by rate nephelometry using the Beckman automated immunochemistry system (Brea, California, USA). Albumin concentrations were measured by modified automated immunoprecipitation (Technicon Autoanalyser II, Technicon, Tarrytown, New York, USA). Coefficients of variation within and between assays did not exceed 10%.

Statistical analysis of differences between matched serum/SF pairs was sought by Wilcoxon's matched pairs signed ranks test, and correlation between serum and SF samples was sought using the Spearman rank correlation coefficient. SF/serum ratios for CRP were compared with ratios for other proteins using the Mann-Whitney U test. SF/serum ratios for different proteins were used to derive a regression equation and multiple R value between SF/serum values and relative molecular mass. Differences between the SF/serum values for CRP predicted by this equation and those obtained were analysed by Student's *t* test.

Results

No significant differences in CRP concentrations were detected between SF collected in plain containers and in those containing EDTA. In addition, no significant change in CRP concentration in SF occurred either when samples were left at room temperature or at 4°C for up to 72 hours before assaying, or after freezing and thawing samples.

DIFFERENCES BETWEEN SERUM AND SF CRP VALUES

Raised serum CRP concentrations were found in patients with RA, psoriatic arthritis, reactive arthri-

Table 2 Serum and synovial fluid CRP values in different arthropathies

Disease	CRP values (mg/l)			
	Serum		SF	
	Median	Range	Median	Range
Rheumatoid arthritis	51	<6-158	15	<6-49
Psoriatic arthritis	66	12-157	27	7-96
Reactive arthritis	14	<6-107	8	<6-43
Osteoarthritis	<6	<6-35	all <6	
Pyrophosphate arthropathy	98*		14*	
	30*		26*	
Acute gout	131*		R knee 64*	
			L knee 30*	
Septic arthritis/RA	259*		167*	
Parvovirus arthropathy	<6*		<6*	

*Values for individual patients.

tis, pyrophosphate arthropathy, and acute gout but not in the patient with parvovirus arthropathy (Table 2). In no case was the CRP concentration in SF greater than that in serum. SF CRP values from patients with RA were significantly reduced compared with paired serum values (Wilcoxon $p < 0.0001$) (Fig. 1). Although fewer numbers were studied, a significant reduction in SF CRP compared with serum CRP in patients with psoriatic arthritis ($p < 0.05$) and reactive arthritis ($p < 0.05$) was also shown. Minor increases of serum CRP were observed in a few patients with osteoarthritis, but in all cases the SF CRP was < 6 mg/l.

CORRELATION BETWEEN SERUM AND SF CRP LEVELS

In samples from patients with RA the serum CRP values correlated with the SF CRP values (Spearman $r = 0.74$) (Fig. 2). It is noteworthy that in a few cases when samples of SF were obtained from both knees and compared with the same serum value a considerable difference was present (Fig. 2). Correlation between serum and SF CRP was also observed in samples from patients with psoriatic arthritis ($r = 0.80$) and reactive arthritis ($r = 0.82$).

KINETICS OF CHANGE OF SERUM CRP COMPARED WITH SF CRP

In patients with RA or psoriatic arthritis followed up serially over several months the SF CRP values mirrored closely the serum values (Fig. 3).

SF/SERUM CRP RATIOS COMPARED WITH THOSE OF OTHER PROTEINS

The SF/serum ratio of CRP concentrations was determined for each pair of samples. No significant correlation was found between serum CRP concen-

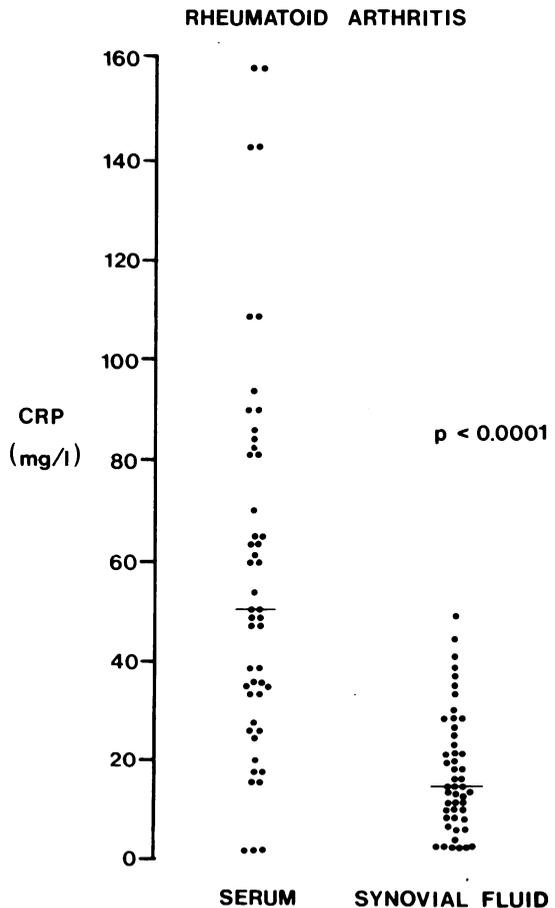


Fig. 1 Serum and SF CRP values in 49 paired samples from patients with RA. Horizontal bars indicate the median values in each group.

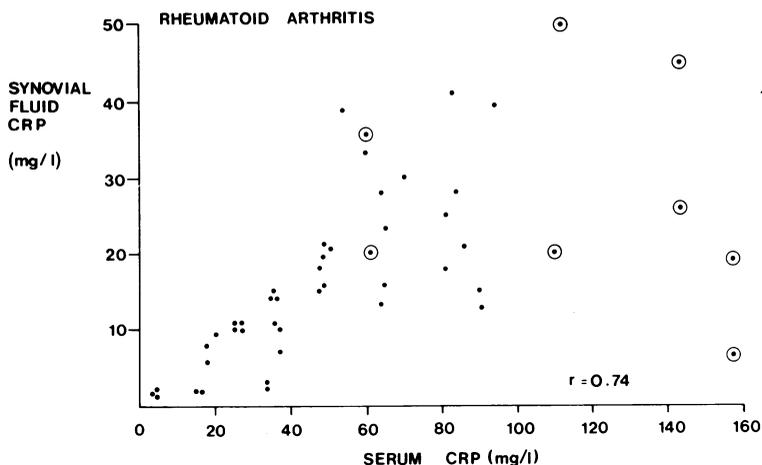


Fig. 2 Correlation between serum and SF CRP values in RA. Ringed values indicate cases in which there was a marked discrepancy in SF CRP concentration between each knee.

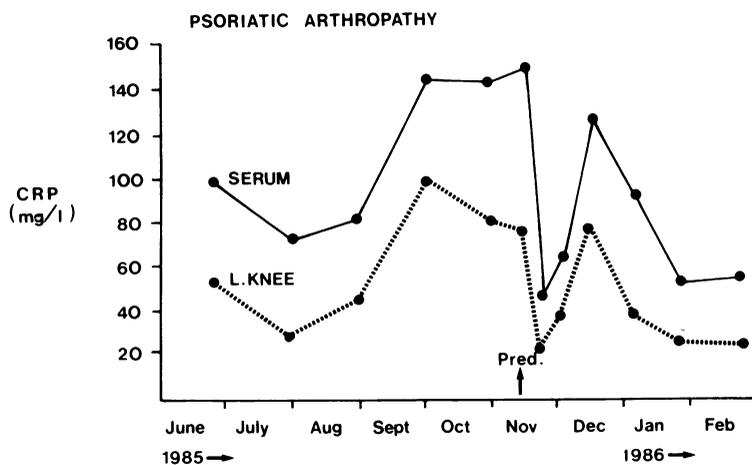


Fig. 3 Serum and left knee SF CRP concentrations in a 41 year old man with psoriatic arthritis followed up over a nine month period. The arrow indicates commencement of prednisolone therapy.

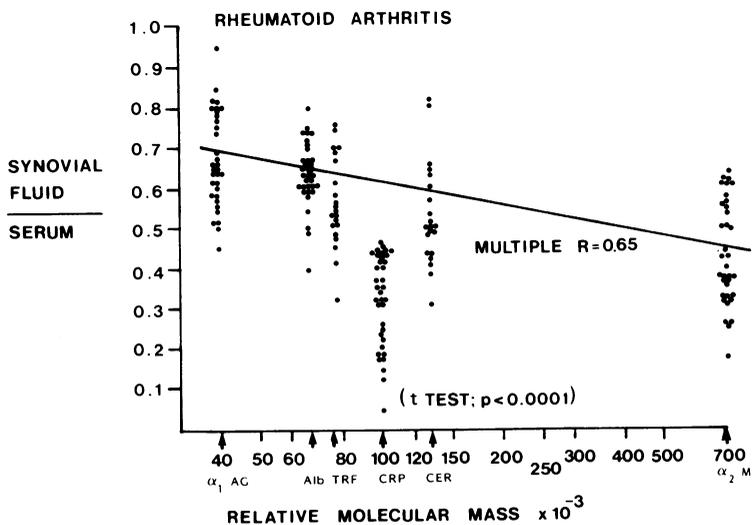


Fig. 4 SF/serum ratios of proteins of different molecular weight. Values for α_1 acid glycoprotein (α_1 AG), albumin (Alb), transferrin (TRF), caeruloplasmin (CER), and α_2 macroglobulin (α_2 M) were used to derive a regression equation between SF/serum ratio and relative molecular mass. SF/serum values for C reactive protein (CRP) were significantly less than predicted from its molecular weight.

tration and SF/serum CRP ratio in any of the inflammatory arthritides studied. The same samples from patients with RA were used to obtain SF/serum ratios of proteins of different molecular weight (α_1 acid glycoprotein, albumin, transferrin, caeruloplasmin, and α_2 macroglobulin; relative molecular mass 40 000, 66 290, 76 800, 132 000, and 725 000 daltons respectively) and thus derive a regression equation between SF/serum ratio and relative molecular mass (protein ratio = $-0.2 \times \log_{10}$ relative molecular mass + 1.61; multiple R = 0.65) (Fig. 4). SF/serum values for CRP were significantly less than predicted from its molecular weight (105 500 daltons) (*t* test, $p < 0.0001$). It was also found that SF/serum values for CRP were significantly less than those for transferrin or caeruloplasmin (Mann-Whitney U test, $p < 0.0001$), the two proteins of closest molecular weight to CRP that were studied.

Discussion

The results of this study confirm previous reports of raised serum CRP concentrations in RA² and psoriatic arthritis,³ and show that raised levels may occur in patients with reactive arthritis secondary to non-gonococcal urethritis. We have demonstrated that SF CRP concentrations in these inflammatory arthritides are significantly reduced compared with those in serum, but in each condition there is a good correlation between serum and SF values. In a few cases with RA, and one with acute gout, a considerable difference between SF CRP values was noted in samples obtained at the same time from each knee. Prospective objective studies of the degree of inflammation of each joint were not performed in this study, but it would be of interest in these circumstances to know which was the more severely affected joint clinically. The low SF CRP values in patients with osteoarthritis are not unexpected in view of the generally low serum CRP concentrations in this condition.

Serial samples were not available from patients with reactive arthritis, but studies on patients followed up serially through an exacerbation of RA or psoriatic arthritis indicate that SF CRP values mirror closely serum levels—best demonstrated in a patient with psoriatic arthritis with essentially monoarticular disease affecting the knee. The half life of CRP is rapid (3–7 hours)^{17, 18} compared with most other plasma proteins, and although patients were not investigated more frequently than weekly, there was no evidence in our study of a time lag before SF CRP concentration reflected a change in serum level, or evidence of relative accumulation of CRP within the joint.

Many plasma proteins are present in reduced

concentrations in SF,^{19, 20} and passage of plasma proteins from blood to SF is related to molecular size.²¹ It is of interest that there was no significant correlation between serum CRP value and the SF/serum ratio in any of the inflammatory arthritides studied, suggesting that the SF/serum CRP ratio is not dependent on the degree of inflammation in the joint. It was important to determine whether the finding of reduced levels of CRP in SF compared with serum was purely the result of diffusion of CRP into the SF or whether this also indicated specific depletion of CRP within the joint. In SF and serum samples from patients with RA the relative concentrations of a variety of other proteins of different molecular weight were therefore studied. These data show that in RA the relative concentration of CRP in SF compared with serum is significantly less than would be predicted from its molecular weight and therefore suggests that CRP is either being selectively bound in synovium or specifically consumed in SF. It is noteworthy that analysis of the relative concentrations of IgG in SF and serum from these patients with RA showed significantly higher concentrations of IgG in SF than would be predicted from its molecular weight (data not shown), consistent with the known local production of IgG in rheumatoid synovium. It would be of interest to perform studies on the relative concentrations of proteins in SF and serum in the other inflammatory arthritides to determine whether similar depletion of CRP in SF is found.

There have been few studies of CRP in human SF or synovium. Highton *et al* noted increased amounts of CRP in the cell pellet after centrifuging SF, and in preliminary studies CRP was found on the surface of cells in SF from patients with RA.¹¹ Gitlin *et al* reported the presence of CRP immunohistochemically in the nuclei of synoviocytes in RA synovium.²² This finding is of interest in the light of recent studies demonstrating that CRP binds with high affinity to chromatin and nucleosome core particles⁸ and can mediate solubilisation of nuclear DNA by complement *in vitro*.²³ Our findings would be consistent with binding of CRP to nuclei, to the surface of cells, or to other sites. Although our observations do not indicate any mechanism by which CRP may be involved in promoting inflammation or facilitating processes of resolution and repair, they suggest that CRP may be playing an important part in the inflammatory process in RA. Further studies of CRP in RA and other forms of inflammatory arthritis would therefore be of considerable interest and potential importance in the further understanding of these conditions.

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