Serum α_1 antichymotrypsin concentration as a marker of disease activity in rheumatoid arthritis

M D CHARD,[1] J CALVIN,[2] C P PRICE,[2] T E CAWSTON,[1] AND B L HAZLEMAN[1]

From the [1]*Rheumatology Research Unit and the* [2]*Department of Clinical Biochemistry, Addenbrooke's Hospital, Hills Road, Cambridge*

SUMMARY   Serum α_1 antichymotrypsin (α_1ACT), C reactive protein (CRP), orosomucoid, and erythrocyte sedimentation rate (ESR) were measured sequentially in 20 patients with rheumatoid arthritis (RA) treated with gold or penicillamine. Pain score, morning stiffness, grip strength, and articular index were measured and a Mallya score calculated. Based on a total of 148 sets of observations, significant correlations were found between α_1ACT and other variables ($p < 0.001$ except morning stiffness at $p < 0.05$). The actual correlation coefficients indicated a closer association with the other laboratory tests, CRP (0·62), orosomucoid (0·69), and ESR (0·61), than with clinical measurements: pain score (0·38), articular index (0·41), grip strength (−0·3), morning stiffness (0·19), and Mallya score (0·5). Sequential data on individual patients showed differing patterns of change in the variables indicating the importance of measuring more than one acute phase protein (APP), especially when CRP is inappropriately low. Serum α_1ACT concentration does reflect disease activity in RA. Its potential advantages are discussed.

Key words: C reactive protein, acute phase proteins.

Serum acute phase proteins (APPs) rise during the course of an acute inflammatory reaction but may also be raised in chronic inflammatory conditions such as rheumatoid arthritis (RA).

Measurement of these proteins in RA has been shown to be of value in assessing disease activity, the response to treatment, and prognosis.[1-3] The APPs traditionally measured would appear to have been chosen more on the availability of existing measuring techniques than on theoretical grounds.

Production of APPs in the liver is thought to be stimulated by the cytokine interleukin 1.[4] Despite this common inducer, individual APPs do not rise in parallel in some disease,[5] indicating that secondary control factors may be operating. In addition, some patients do not respond as expected. C reactive protein (CRP), the most widely used APP,[6] may be normal even though active RA is indicated on clinical grounds or by the concentration of other APPs.[7] It has therefore been advocated that measurement of a number of APPs may have advantages.[8]

Some APPs that have been used, such as fibrinogen and caeruloplasmin, usually show only a modest rise. In addition, their concentration and also those of haptoglobin, orosomucoid (α_1 acid glycoprotein), and α_1 antitrypsin are affected by various factors not directly related to inflammation.[7] More recently serum amyloid A protein (SAAP) measurement has been reported as being a useful APP to measure, behaving similarly to CRP but with some possible advantage.[9] As production is more quickly switched on by inflammation, producing a large response, small fluctuations in disease activity and other events such as minor infection may give rise to difficulties in the interpretation of a rise in concentration.

α_1 Antichymotrypsin (α_1ACT) is a serine protease inhibitor which also rises in inflammatory conditions. Measurement of this APP has theoretical advantages over most others. It increases more quickly and to a greater extent than other APPs, apart from CRP or SAAP,[10] but being less sensitive than these two may be less altered by minor events. In addition, its concentration remains raised for a long period and so a significant inflammatory episode is unlikely to be missed. Concentrations are not affected by genetic variation, sex hormones, or

corticosteroid administration, and renal function has no significant effect.[11-13] Also, from a practical point of view, because the concentration does not rise as dramatically as that of CRP the assay systems can avoid costly and time consuming dilution steps.

Therefore, α_1ACT measurement may be a useful APP to measure in assessing RA. This study was undertaken to find out if the serum α_1ACT correlates with clinical and laboratory measures of disease activity in RA.

Patients and methods

A group of 20 outpatients (14 women, six men) with classical or definite RA (American Rheumatism Association criteria) were studied. All were seen initially when they had active disease while taking a non-steroidal anti-inflammatory drug, and were starting a second line agent (gold or penicillamine). Each was then seen on several further occasions while undergoing treatment over subsequent months when the disease activity might have been changing in response to their treatment. They were seen on a mean of seven (range 4–14) occasions. This resulted in a total of 148 sets of observations.

Details of age, duration of RA, seropositivity,

and drug treatment were recorded. Disease activity was assessed on each occasion clinically by the following: pain score, using a 10 cm visual analogue scale; duration of morning stiffness; grip strength (mean of three measurements); articular index.[14] Blood was taken for measurement of the haemoglobin, ESR (Westergren), CRP, orosomucoid, and α_1ACT. CRP was measured with a Beckman rate nephelometer (Beckman RIIC, High Wycombe, UK). Orosomucoid and α_1ACT were measured by immunoturbidimetric assays on an Instrumentation Laboratory multistat centrifugal analyser.[15 16] The normal range of α_1ACT was determined from 128 healthy blood donors (0·35–0·64 g/l). The Mallya score for each patient was calculated at each visit as described previously.[17]

Associations between the serum concentration of α_1ACT and the clinical and laboratory variables were determined using the Spearman rank correlation test. Analysis of variance was used to check for any bias effect on the overall results by any individual patient.

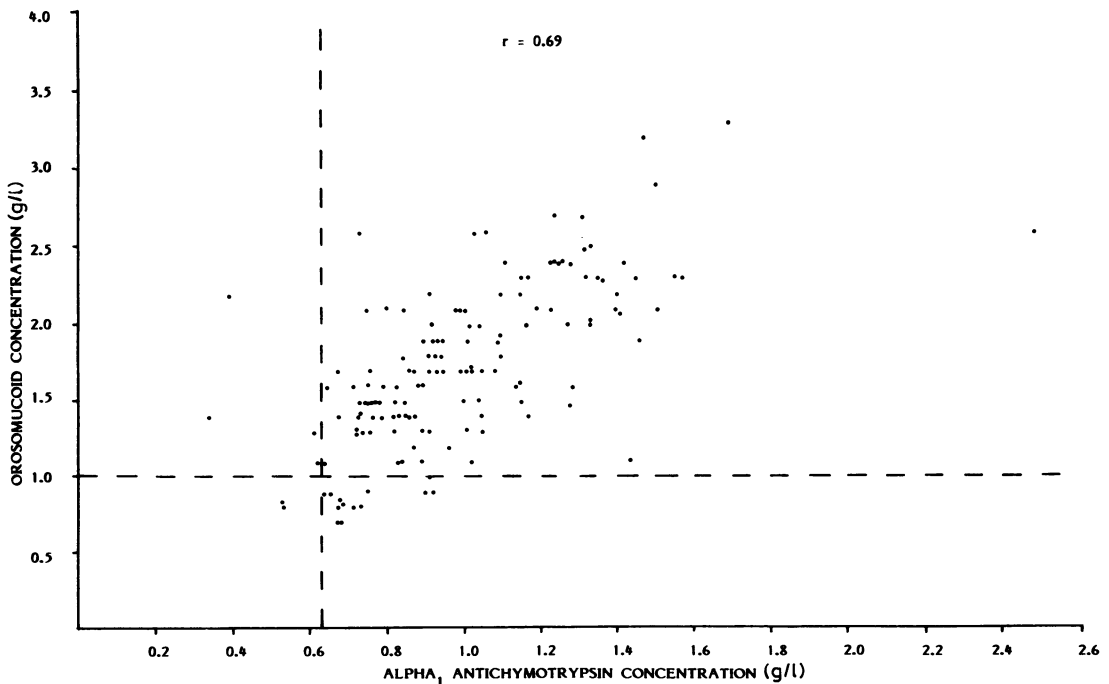## Results

The mean age of the patients was 51 (range 32–68)

Fig. 1   *Correlation of α_1 antichymotrypsin and orosomucoid concentration in rheumatoid arthritis.*

years. Eighteen had seropositive disease and the mean duration of RA was five (range 0·5–12) years.

Table 1 shows the correlation coefficients for the measured α_1ACT concentration. There was a close association between α_1ACT and the other laboratory variables. This occurred over values both within and above the normal ranges as illustrated by the plot against orosomucoid (Fig. 1). There was overall a good correlation with CRP but a raised concentration of α_1ACT was not infrequently found in the presence of a normal CRP (Fig. 2). A somewhat similar pattern was seen when comparing α_1ACT with ESR (Fig. 3). By comparison, a normal α_1ACT

was rarely found in the presence of either a raised CRP concentration or ESR. These findings were obtained in both gold and penicillamine treated patients.

The results from individual patients were shown not to affect the overall associations, which remained highly significant. None of the other laboratory parameters showed a significantly greater association with the clinical variables.

Although correlations with clinical variables were less marked, apart from the composite Mallya score, they were significant at the level of $p<0\cdot001$ with the exception of morning stiffness ($p<0\cdot05$). A normal concentration of α_1ACT was rarely found in the presence of clinically active disease as shown by the plot against the articular index (Fig. 4).

The sequential measurements on individual patients provided additional information which is illustrated by data on four selected patients (Figs 5a–d). Although there was broad agreement between the variables in most patients as disease activity changed (Fig. 5, pattern 1), there were some patients where there were differences. In three patients α_1ACT took longer to settle than the other variables (Fig. 5, pattern 2). On seven occasions in six patients CRP was normal despite other evidence of active disease (Fig. 5, pattern 3). In a further

Table 1   *Correlation between α_1 antichymotrypsin (α_1ACT) and other variables (n=148)*

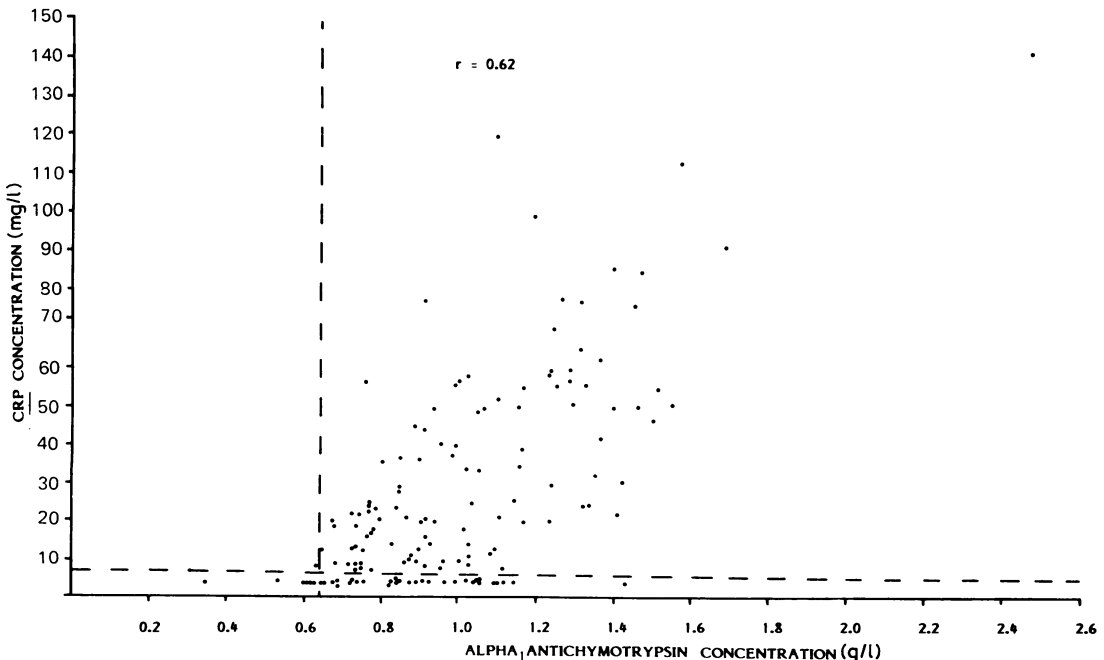| Variable	Correlation coefficient (r)	Significance (p)
ESR	0·61	<0·001
CRP	0·62	<0·001
Orosomucoid	0·69	<0·001
Pain score	0·38	<0·001
Ritchie articular index	0·41	<0·001
Grip strength	−0·30	<0·001
Morning stiffness	0·19	<0·05
Mallya score	0·50	<0·001

Fig. 2   *Correlation of α_1 antichymotrypsin and C reactive protein concentration in rheumatoid arthritis.*

three patients CRP was persistently normal in the presence of a raised α_1ACT and other evidence of variably active RA (Fig. 5, pattern 4). These differences help to explain the not infrequent occasions when a raised α_1ACT was found in the group results in the presence of a normal CRP.
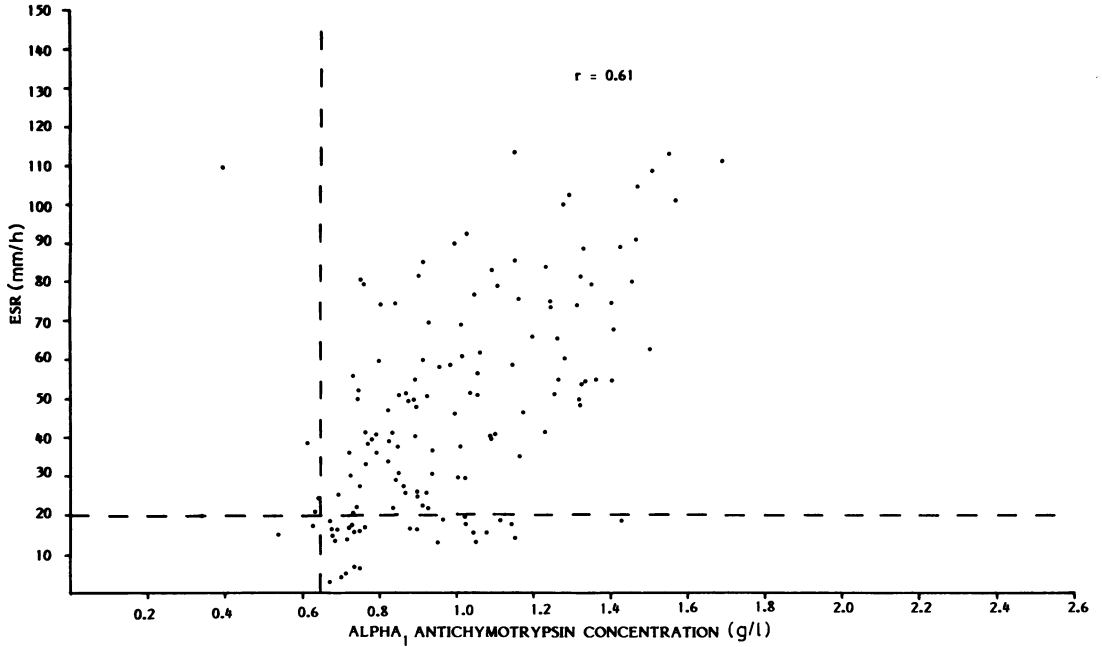
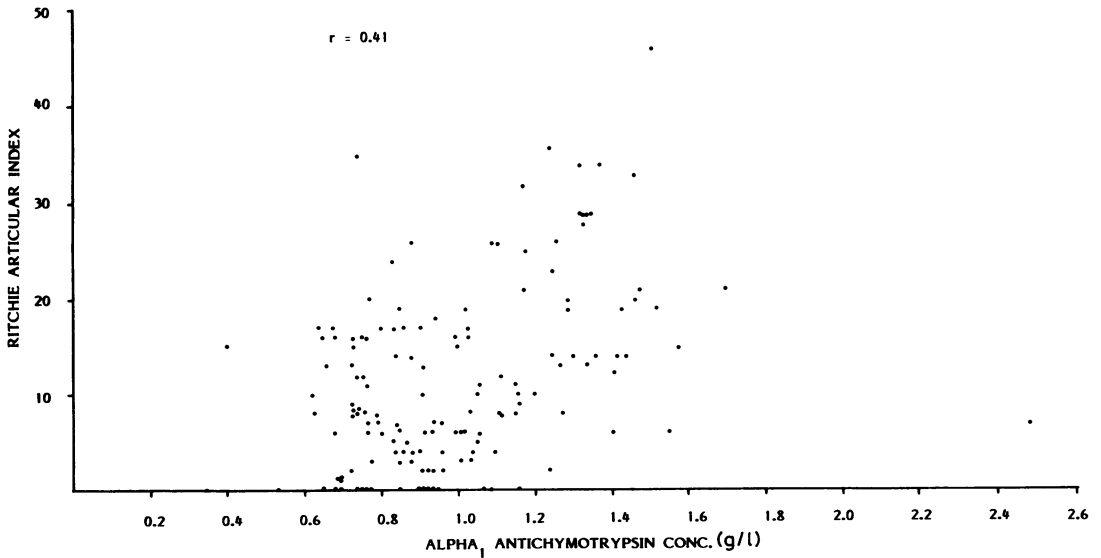Fig. 3   *Correlation of α_1 antichymotrypsin concentration and ESR in rheumatoid arthritis.*

Fig. 4   *Correlation of α_1 antichymotrypsin concentration and Ritchie articular index in rheumatoid arthritis.*
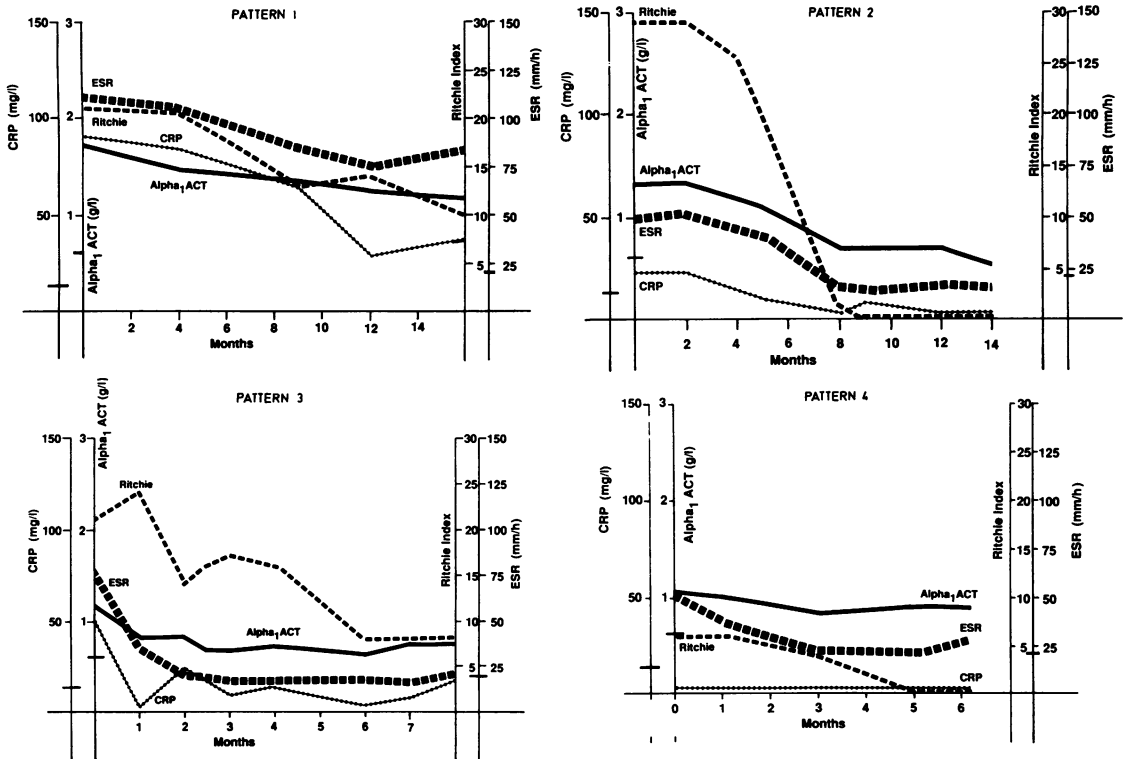
Fig. 5 *Sequential changes in laboratory and clinical variables in individual patients showing different patterns of association.*

Discussion

The findings confirm that the serum concentration of α_1ACT does correlate with the activity of RA as indicated by both clinical and laboratory parameters. It is perhaps not surprising that the concentration correlates better with the objective laboratory derived data than with the more subjective clinical measurements, though all associations were significant.

Pooled measurements of multiple sequential assessments on a small group of patients with active disease starting second line drug treatment were used, rather than single assessments on a large patient group. This avoided patients with minor or static late disease and reflected the clinical situation where APP measurement is likely to be of most benefit. The possibility that individual patient results could have favourably biased the findings was excluded.

Assessment of sequential measurements on individual patients gave insight into the variations in the patterns of change of the parameters. α_1ACT changed along with the other measures of disease activity but, especially in some cases, appears to take longer to settle to normal when RA is treated, particularly compared with CRP. It is known that in acute inflammation α_1ACT settles more slowly than CRP owing to its longer half life, and it may take longer for production to be switched off. An analogous situation appears also to occur over a longer period of months in the chronic inflammation condition of RA, particularly in some patients. The CRP concentration only reflects what has been happening within days of its measurement whereas α_1ACT shows what has happened for up to several weeks. Therefore CRP tends to indicate only the present situation and α_1ACT has more historical value, both kinds of information being useful when monitoring a chronic disease like RA. In addition, some patients had inappropriately low CRP concentrations and α_1ACT appeared to be a better measure of the disease activity. Although in most patients this occurred only once or twice for no

obvious reason (there was no indication that the method used to measure CRP was at fault), in several patients the CRP concentration was persistently low. It may be that CRP production is defective in these patients. Indeed this agrees with the finding that CRP concentration is not always as high as expected in chronic disease.[7] These findings help to show that the controlling mechanisms for production of APPs must be complex. It also agrees with the assertion that in inflammatory diseases, especially those of a chronic nature, measurement of a number of APPs is more useful than reliance on one.[8]

The ESR is the most popular laboratory test of inflammation, but it is well known that it may be affected by a number of factors, including age, sex, and state, size, and concentration of the red blood cells. The concentration of APPs in serum may be affected, in most cases, by factors other than the severity of an inflammatory process. Fibrinogen is produced by platelets as well as the liver, is consumed by coagulation processes, and is removed from the circulation in disseminated intravascular coagulation.[18] Haptoglobin concentrations vary considerably in normal subjects as a result of genetic variation.[19] Disorders of erythropoiesis and haemolysis will also affect concentrations. Activation of complement by immune complexes will consume these serum components.[20] At best the rise in caeruloplasmin is only modest and production is affected by oestrogen. The concentration of α_1 antitrypsin is affected by genetic variation and oestrogen production and is probably more useful as a 'liver marker' than an APP.[7] Orosomucoid is a good marker of inflammation, but concentrations may be affected by corticosteroid administration and renal function.[7]

Although CRP is the most widely used APP,[6] interpretation of CRP concentrations is not without at least some potential problems. The production of this protein is sensitive to inflammation even of a mild nature,[21] yet concentrations may be lower than expected in chronic inflammation,[7] both of which can give rise to problems of interpretation as previously mentioned. SAAP concentration also changes rapidly in response to interleukin 1 as a result of inflammation.[4] There are those who believe it has advantages over CRP in RA monitoring,[9 22] but the problems of relying on such a sensitive indicator of inflammation are similar.

The serum concentration of α_1ACT is believed to be a reliable indicator of the mass of inflamed tissue and is neither affected by genetic variation nor shows any increase in catabolic rates in disease states.[7] In addition, it is unaffected by corticosteroids, sex hormones, or the glomerular filtration rate

of the kidneys.[11-13] It does not achieve as great an increase as CRP or SAAP but it is more marked than other APPs. It is a less sensitive marker of inflammation than CRP or SAAP, but potentially misleading swings due to trivial inflammation are avoided. Theoretically it would appear likely that α_1ACT would have a useful role in monitoring disease activity in RA, and this is confirmed by the present study. Changes do sometimes lag behind those of other parameters and so individual values should be interpreted with caution in such situations, sequential measurements being of most value. It would appear to be especially useful, however, where there is uncertainty about the significance of a CRP concentration. The place of α_1ACT would appear to be as an additional APP to CRP (or SAAP), being preferred to other such proteins which have been traditionally measured as it is only affected by the inflammatory process itself.

References

1 McConkey B, Crockson R A, Crockson A P. The assessment of rheumatoid arthritis. A study based on measurements of the serum acute-phase reactants. *Q J Med* 1972; **41:** 115–25.

2 McConkey B, Crockson R A, Crockson A P, Wilkinson A R. The effects of some anti-inflammatory drugs on the acute-phase proteins in rheumatoid arthritis. *Q J Med* 1973; **42:** 785–91.

3 McConkey B, Davies P, Crockson R A, *et al*. Effects of gold, dapsone and prednisolone on serum C-reactive protein and haptoglobin and the erythrocyte sedimentation rate in rheumatoid arthritis. *Ann Rheum Dis* 1979; **38:** 141–4.

4 Sipe J D, Vogel S N, Sztein M B, Skinner M, Cohen A S. The role of interleukin-1 in acute phase serum amyloid A (SAA) and serum amyloid P (SAP) biosynthesis. *Ann NY Acad Sci* 1982; **389:** 137–50.

5 Pepys M B. Serum C-reactive protein, serum amyloid p-component and serum amyloid A protein in autoimmune disease. *Clinical Immunology and Allergy* 1981; **1:** 77–101.

6 Pepys M B. C-reactive protein fifty years on. *Lancet* 1981; i; 653–7.

7 Laurell C B. Acute phase proteins—a group of protective proteins. In: Price C P, Albert K G M M, eds. *Recent advances in clinical biochemistry*. Edinburgh: Churchill Livingstone, 1985: 103–24.

8 Laurent M R, Panayi G S, Shepherd P. Circulating immune complexes, serum immunoglobulins and acute phase proteins in psoriasis and psoriatic arthritis. *Ann Rheum Dis* 1981; **40:** 66–9.

9 Chambers R E, Macfarlane D G, Whicher J T, Dieppe P A. Serum amyloid-A protein concentration in rheumatoid arthritis and its role in monitoring disease activity. *Ann Rheum Dis* 1983; **42:** 665–7.

10 Johansson B G. Plasma protein as diagnostic aids. Methods and clinical applications. In: Blomback B, Hanson L A, eds. *Plasma proteins*. Chichester: Wiley, 1979: 351–4.

11 Docci D, Turci F. Serum alpha-1-acid glycoprotein levels in uraemic patients on haemodialysis. *Nephron* 1983; **33:** 77.

12 Docci D, Bilancioni R, Pistocchi E, *et al*. Serum alpha-1-acid glycoprotein in renal failure. *Nephron* 1985; **39:** 160–3.

13 Calvin J, Neale G, Fotherby K, Price C. The relative merits of acute phase proteins in the recognition of inflammatory conditions. *Ann Clin Biochem* 1988; **25:** 60–6.

14 Ritchie D M, Boyle J A, McInnes J M, *et al*. Clinical studies with an articular index for the assessment of joint tenderness in patients with rheumatoid arthritis. *Q J Med* 1968; **147**: 393–406.

15 Calvin J, Hallworth M J, Burrin J M, Price C P. Experience with the measurement of specific proteins using the multistat microcentrifuge analyser. In: *Proceedings of the fluorescence-nephelometry workshop*. Benelux: Instrumentation Laboratory, 1982.

16 Calvin J, Price C P. Measurement of serum $\alpha_1$antichymotrypsin by immunoturbidimetry. *Ann Clin Biochem* 1986; **23**: 206–9.

17 Mallya R K, Mace B E W. The assessment of disease activity in rheumatoid arthritis using a multivariate analysis. *Rheumatology and Rehabilitation* 1981; **20**: 14–17.

18 Laurell C B. The use of electro-immuno assay for determining specific proteins as a supplement to agarose gel electrophoresis. *J Clin Pathol* 1975; **28** (suppl 6): 22–6.

19 Giblett E. Haptoglobin. In: Allison A C, ed. *Structure and function of plasma proteins*. New York: Plenum Press, 1974: 133–68.

20 Whicher J T. The value of complement assays in clinical biochemistry. *Clin Chem* 1978; **24**: 7–22.

21 Fischer C C, Gill C, Forrester M G, Nakamura R. Quantitation of acute phase proteins post-operatively. *Am J Clin Pathol* 1976; **66**: 840–6.

22 Grindulis K A, Scott D L, Robinson M W, Bacon P A, McConkey B. Serum amyloid A protein during the treatment of rheumatoid arthritis with second-line drugs. *Br J Rheumatol* 1985; **24**: 158–63.