Acute-phase proteins and serum immunoglobulins in ankylosing spondylitis

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SUMMARY The erythrocyte sedimentation rate (ESR) and the serum acute-phase proteins (APP), C-reactive protein (CRP), fibrinogen, 9th component of complement (C9), and α_1 antitrypsin were measured on 231 occasions in 80 patients with ankylosing spondylitis and compared with those in 30 controls. APP levels did not correlate with clinical assessment of disease activity. However, there were significant correlations between CRP, C9, and fibrinogen ($p < 0.01$), suggesting that these APP may be more reliable indicators of disease activity. The mean values of the APP in those patients with a peripheral arthritis were significantly higher than in those with pelvispondylitis alone for ESR ($p < 0.01$), CRP ($p < 0.01$), and fibrinogen ($p < 0.05$). The only significant difference between those patients with an iritis and those with only pelvispondylitis was an elevated CRP in the iritis group ($p < 0.01$). This suggests that a peripheral arthritis is the most important cause of an elevated ESR or APP in ankylosing spondylitis. Serum immunoglobulins were also measured and they showed a significant elevation of IgA in all 3 patient groups, there being no difference between each group. Serum IgG was raised only in those patients with an iritis or peripheral arthritis, the IgM levels being within the normal range for all patient groups.

The dominant lesion in ankylosing spondylitis (AS) is an inflammatory arthritis of the spinal and sacroiliac joints. However, it is complicated by peripheral arthritis in 50% of cases, iritis in 30%, and aortitis in 5%.¹ Clinical assessment of spinal disease activity is difficult because there are few reliable signs, and there is a need for a reliable laboratory measurement of disease activity.

In rheumatoid arthritis the erythrocyte sedimentation rate (ESR) is the commonly used laboratory measure of disease activity, and its level usually correlates with clinical assessment of disease activity. C-reactive protein (CRP), an acute-phase protein, has been found to respond to changes in clinical activity more closely than the ESR.²

In AS there is a poor correlation between clinical disease activity and the ESR. Even though it tends to be elevated early in the disease, often patients with florid spondylitis may have a normal ESR, and, conversely, clinically quiescent patients may have an elevated ESR. In AS the ESR has been regarded as an aid to diagnosis but of little value in prognosis and response to therapy.³ However, the ESR is a non-specific test influenced by a large number of factors

unrelated to inflammation,⁴ and it may be of greater value to measure acute-phase proteins.

Serum immunoglobulins have also been shown to be elevated in AS,⁵⁻⁷ the most significant being an elevated IgA. IgA levels have also been shown to correlate with a raised ESR and CRP,⁸ suggesting that they may have a role in the measurement of disease activity.

The aim of this study was to measure the acute-phase proteins fibrinogen, C-reactive protein, α_1 -antitrypsin, and the 9th component of complement and immunoglobulins G, A, and M, and to determine their correlation with clinical disease activity and extraspinal manifestations.

Materials and methods

Eighty patients who fulfilled the New York criteria for AS were studied.⁹ Their mean age was 42.3 years (range 22-67 years), with a mean disease duration of 13.9 years (range 1.4-36 years). There were 17 female patients. Patients with spondylitis secondary to inflammatory bowel disease, psoriasis, or Reiter's disease were excluded from this study. All patients were rheumatoid-factor-negative. There were 50 controls who consisted of patients with degenerative

joint disease or minor joint or soft tissue injuries, whose mean age was 39.3 years (range 19–60 years). There were 11 female controls.

Sixteen patients had only one assessment, 19 patients 2, 17 patients 3, 21 patients 4, and 7 patients 5 or more, making a total of 231 assessments. The length of follow-up varied from 3 to 18 months.

Disease activity. This was evaluated on the basis of early morning stiffness, pain score, dosage of anti-inflammatory medication, and the presence of a peripheral arthritis and/or iritis. Eleven patients had a peripheral arthritis and 8 an acute iritis when assessed. Comparisons were also made between 3 patients subgroups which consisted of pelvospondylitis only, pelvospondylitis with iritis, and pelvospondylitis with a peripheral arthritis.

Serum and plasma samples. Blood was taken at the time of assessment, the serum and plasma (in ethylene diamine tetra-acetic acid) being separated and aliquots stored at -70°C , being thawed only once.

Methods. The ESR was measured by the modified Westergren method.¹⁰ C-reactive protein, fibrinogen, α_1 -antitrypsin, 9th component of complement, and immunoglobulins G, A and M were measured by single radial immunodiffusion in agar with monospecific antisera (Behringwerke).¹¹

Statistics. Results were analysed by multiple linear regression and Student's *t* test for independent means.

Results

Acute-phase proteins. When mean levels of acute-phase proteins for each patient subgroup were examined, in the pelvospondylitis group there was a significant elevation of fibrinogen and the 9th component of complement (C9) ($p<0.01$) in comparison with the normal controls (table 1). The CRP levels were only marginally elevated ($p<0.05$), and the ESR and α_1 -antitrypsin levels were not significantly different from the controls. However, the elevation of acute-phase proteins was more marked in the subgroup of patients with a peripheral arthritis or iritis in comparison with the controls. In both subgroups there was a significant elevation of CRP ($p<0.01$), fibrinogen, and C9 ($p<0.01$ for the iritis subgroup and $p<0.001$ for the peripheral arthritis subgroup), and α_1 -antitrypsin ($p<0.05$). The ESR was not elevated in the iritis subgroup, but was increased in those patients with a peripheral arthritis ($p<0.01$). Differences between the 3 patient subgroups were also noted. There was a significant difference between those patients with a peripheral arthritis and those with pelvospondylitis for the ESR, CRP, C9 ($p<0.01$), and fibrinogen ($p<0.05$), the levels being

Table 1 Mean ESR and acute-phase protein levels in patients with ankylosing spondylitis. The groups consisted of controls, patients with pelvospondylitis, pelvospondylitis and acute iritis, and pelvospondylitis and peripheral arthritis.

	Controls, n=50 (1)	Pelvospondylitis, n=212 (2)	Pelvospondylitis and iritis, n=8 (3)	Pelvospondylitis and peripheral arthritis, n=11 (4)	p 2 vs. 1	p 3 vs. 1	p 4 vs. 1	p 3 vs. 2	p 4 vs. 2
ESR (mm/h)	9.2±4.7	18±11.7	18±13	44±12	NS	NS	0.01	NS	0.01
C-reactive protein (mg/dl)	0.5±0.2	1.5±1.4	2.9±2.6	3.1±2.3	0.05	0.01	0.01	0.01	0.01
Fibrinogen (mg/dl)	357±83	460±121	456±48	523±105	0.01	0.01	0.001	NS	0.05
C9 (% normal)	98±27	156±61	146±53	206±58	0.01	0.01	0.001	NS	0.01
α_1 -antitrypsin (mg/dl)	283±56	312±82	335±43	354±50	NS	0.05	0.05	NS	NS

Mean±standard deviation is given. NS=not significant. SI conversion = mg/dl × 10 = mg/l.

higher in the group with a peripheral arthritis. The differences between those with pelvospondylitis only and those also with iritis were significant only for CRP ($p<0.01$), this being higher in the iritis group.

Comparison between the subgroups with iritis and peripheral arthritis revealed a significant increase in the latter only for the ESR ($p<0.01$) and C9 ($p<0.05$).

Serum immunoglobulins. The mean immunoglobulin A (IgA) level was significantly elevated in all 3 patient subgroups in comparison with the controls ($p<0.001$), but there was no significant difference between the 3 patient subgroups (Table 2). In the patients with pelvospondylitis only, serum IgA concentration was 3.45 ± 1.48 g/l (mean $\pm$ standard deviation) in those with a CRP greater than 1.5 mg/dl (15 mg/l) and 2.54 ± 1.32 g/l when the CRP was less than 1.5 mg/dl (15 mg/l). This difference was statistically significant ($p<0.01$), although absolute levels of IgA and CRP did not correlate. Immunoglobulin G (IgG) was significantly elevated only in patients with iritis ($p<0.05$) or with peripheral arthritis ($p<0.02$) in comparison with normal persons. There was no significant difference for mean IgG levels between the patient subgroups. Serum IgM levels were not significantly elevated. The ratio of IgA to IgG was significantly elevated in all 3 patient groups ($p<0.05$), the normal ratio being 1.08 in normal controls, 1.43 in the pelvospondylitis subgroup, 1.52 in the patients with iritis, and 1.64 in those with peripheral arthritis. This disproportionate increase in IgA levels is also found in psoriasis and psoriatic arthritis but not in rheumatoid arthritis.¹²

Clinical disease activity. There were no correlations between the disease activity measurements of early morning stiffness, pain score, or dosage of anti-inflammatory medication, and the acute-phase proteins or immunoglobulins.

Correlations between acute-phase proteins and immunoglobulins. For all 3 patient subgroups there were significant correlations between C9 and CRP ($p<0.01$), C9 and fibrinogen ($p<0.01$), and CRP and fibrinogen ($p<0.05$). In the patients with a peripheral arthritis there were additional correlations between IgG and IgA ($p<0.01$), ESR and CRP ($p<0.01$), and ESR and C9 ($p<0.05$).

Longitudinal studies. When the results were examined longitudinally, the disparity between disease activity measurements and acute-phase proteins persisted. There were a few exceptions, however, in which they did correlate, but these were usually patients with a peripheral arthritis (data not shown).

Discussion

The acute-phase proteins (APP) are a group of nor-

Table 2 Mean immunoglobulin G, A, and M levels in patients with ankylosing spondylitis. The groups consist of controls, patients with pelvospondylitis, pelvospondylitis and acute iritis, and pelvospondylitis and peripheral arthritis.

	Controls, n=50 (1)	Pelvospondylitis, n=212 (2)	Pelvospondylitis +iritis, n=8 (3)	Pelvospondylitis +peripheral arthritis n=11 (4)	p 2 vs. 1	p 3 vs. 1	p 4 vs. 1	p 3 vs. 2	p 4 vs. 2
IgG (g/l)	13.1 ± 2.0	15.1 ± 7.9	16.0 ± 7.3	16.8 ± 7.9	NS	0.05	0.02	NS	NS
IgA (g/l)	1.91 ± 0.90	3.00 ± 1.49	3.28 ± 1.44	3.71 ± 1.35	0.01	0.001	0.001	NS	NS
IgM (g/l)	1.27 ± 0.43	1.31 ± 1.06	1.39 ± 0.96	1.52 ± 0.87	NS	NS	NS	NS	NS

Mean $\pm$ standard deviation is given. NS = not significant.

mal plasma proteins whose levels rise significantly during an inflammatory process. Stimuli for APP production include injury, surgery, bacterial infection, and administration of bacterial endotoxin. C-reactive protein (CRP), α_1 -antitrypsin (α_1 -AT), and fibrinogen have often been used in assessing disease activity in man.¹³ Normally CRP is absent or present in very low levels in normal serum, is usually detectable in serum within 24 hours of an inflammatory stimulus, and disappears with resolution of the inflammation.¹⁴ Persistence of CRP therefore usually indicates persistence of inflammation. Fibrinogen and α_1 -AT levels also rise rapidly after an inflammatory stimulus, but, whereas α_1 -AT rapidly returns to normal levels, increased fibrinogen levels may persist for a long time after resolution of the inflammation.¹⁵ Animal studies have shown that increased concentrations of APP are proportional to the severity of tissue damage.¹⁶ The 9th component of complement (C9) has only recently been recognised as an APP.¹⁷ Levels increase with turpentine-induced inflammation in animals¹⁸ and in some diseases C9 concentrations correlated with the ESR and α_1 -AT concentrations.^{19 20}

In this study there was no correlation between the APP levels and the clinical measurement of disease activity for the total patient group. A similar lack of correlation has been shown between clinical disease activity as measured by pain, and the ESR.²¹ This lack of correlation was also present in patients followed up longitudinally, only occasional patients showing a relationship between an APP and disease activity. The main reason for this lack of correlation is the difficulty in accurately assessing clinical disease activity in patients with only pelvispondylitis. Even though patients with a peripheral arthritis had higher mean levels of APP, there was not a significant correlation with disease activity.

The commonly used nonsteroidal anti-inflammatory drugs (NSAID) do not influence the ESR or APP,²² so that their levels would more accurately reflect the degree of inflammation. In contrast to this, symptoms are significantly modified by NSAID medication, and this would give an erroneous clinical assessment of disease activity. In the pelvispondylitis subgroup there were correlations between the APP CRP, C9, and fibrinogen. This implies that the elevation of these 3 APPs could be more representative of the amount of inflammation than the clinical assessment.

The mean APP levels for the disease subgroups were interesting in that there was a marked difference between those with and without a peripheral arthritis. The ESR, CRP, fibrinogen, and C9 were significantly elevated in the peripheral arthritis group in comparison with those with only pelvispondylitis.

Cowling *et al.*²³ found a correlation between ESR, CRP, and clinically assessed disease activity. This is in contrast to other reports that could not find an association between ESR and disease activity. One reason for this discrepancy might be that an important criterion in their classification of active disease was the presence of a peripheral arthritis. It is possible that the synovitis is responsible for the raised CRP and ESR rather than the pelvispondylitis.

Possibly because the synovitis produces a greater degree of inflammation than the pelvispondylitis it stimulates a greater APP response. It could also be a different type of inflammation, being the appropriate one to produce a significant APP response. In diseases in which several APP have been measured there is not always a simultaneous elevation of all APP. Examples of these observations are an elevation of haptoglobin, ceruloplasmin, and orosomucoid but not of α_1 -antitrypsin and CRP in systemic lupus erythematosus^{24 25} and elevation of different APP in the various subgroups of Behçet's syndrome.²⁶ This suggests that each APP requires a different type of inflammatory stimulus and could have a specific physiological function, and therefore in assessing inflammation in a disease several APP should be measured. Serial measurements in patients did not add any additional information about the relationship between APP and clinical disease activity.

A slightly different pattern of response was noted for the immunoglobulins in that the differences between the 3 disease groups were not as great as those for the APP. IgA is elevated in all groups, with higher levels in those with an iritis or peripheral arthritis. However, this difference is not significant, and absolute levels did not correlate with clinical disease activity. Several authors have shown an elevated IgA in ankylosing spondylitis.⁵⁻⁸ Veys *et al.*⁵ did not find any correlation between serum IgA and a peripheral arthritis, ESR, CRP, subjective complaints, or a radionuclide scan of the spine and pelvis. However, 2 studies found the highest mean levels of serum IgA to be in those patients with active disease, as measured by an ESR greater than 15 mm in 1 hour or a CRP greater than 15 mg/dl (150 mg/l).^{8 27} We also noted that in the pelvispondylitis group mean IgA levels were highest in those patients with a CRP greater than 15 mg/dl (150 mg/l). Mucosal-associated lymphoid tissue is an important source of serum IgA, especially the polymeric fraction. The elevated serum IgA in ankylosing spondylitis suggests that the inciting antigen may be acting at a mucosal surface, the gastrointestinal tract being a possibility.

Mean serum IgG levels have also been shown to be highest in those patients with active disease as measured by an elevated ESR and CRP.^{8 27} In our study the highest mean levels were associated with a

peripheral arthritis. Most reports have found serum IgM levels to be within normal limits.⁵⁻⁸

In conclusion, the acute-phase protein levels distinguish between the patients with pelvospondylitis alone and pelvospondylitis with a peripheral arthritis. However, the problem in defining active disease in patients with only pelvospondylitis still remains. In our study the fibrinogen, C9, and CRP correlated with each other and probably more accurately reflect inflammation than clinical assessment. They may be a more reliable method for measuring disease activity in patients with ankylosing spondylitis.

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