Comparison of serum amyloid A protein and C-reactive protein concentrations in cancer and non-malignant disease

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SUMMARY Serum amyloid A (SAA) concentrations correlate well with C-reactive protein (CRP) concentrations. However, SAA is sometimes raised in disease when CRP is normal. This appears to occur more often in certain diseases such as rheumatoid arthritis, primary biliary cirrhosis and chronic active hepatitis. SAA concentrations did not distinguish between cancer with and without metastases as previously indicated, although mean concentrations were higher in more advanced tumours. Despite the higher sensitivity of SAA over CRP in the inflammatory response, SAA has little advantage over CRP in the assessment of malignant disease.

Serum amyloid A was first found in serum because it cross-reacted immunologically with antiserum raised against amyloid A protein purified from amyloid deposits.^{1–3} SAA has been shown to have a molecular weight of approximately 14 000 daltons although in serum it was shown to have an effective molecular weight of 85 000 to 200 000 daltons depending on the conditions.^{1–4} This has been shown to be due to binding to high density lipoproteins (HDL), of which SAA is considered to be an apolipoprotein.^{5,6} SAA is thought to be a precursor of amyloid fibrils² and is synthesised in the liver.⁷

SAA has been demonstrated to be an acute phase reactant, its serum concentration increasing greatly in response to tissue damage or inflammation.^{8,9} SAA has been reported to be a biochemical marker that discriminates between disseminated and local or regional neoplastic disease.^{10,11} SAA has also been reported to be raised in a variety of chronic inflammatory diseases such as rheumatoid arthritis and systemic lupus erythematosus,^{12,13} inflammatory bowel disease,¹⁴ as well as in myelomatosis and benign monoclonal gammopathy.¹⁵ SAA has been observed to be moderately raised in pregnancy and old age¹⁶ although a more recent report has failed to confirm the increase in old age.¹⁷

C-reactive protein (CRP) is another better known acute phase reactant and serum concentrations of this protein have been shown to correlate with SAA.⁹ In this study concentrations of SAA and CRP were examined in blood samples from patients with

a variety of diseases to see if SAA measurement was more informative than CRP especially in the assessment of malignant disease and its metastatic and local spread. The possible clearance of SAA by the kidney has also been examined.

Patients and material and methods

PATIENTS

Serum samples were obtained from blood donors (35 women and 35 men) and asymptomatic elderly men, aged 56–83 yr recalled 1–2 years after trans-urethral resection for benign prostatic hypertrophy (BPH) to observe any age-dependent effect on the normal concentrations. Serial samples were taken from seven patients undergoing elective cholecystectomy to observe the rate of change of SAA and CRP concentrations in response to wounding. Random samples were obtained from 86 patients with chronic liver disease, benign inflammatory gastrointestinal disease (29 patients), and rheumatoid arthritis (53 patients), burn injury (20 patients) and paraplegia (22 patients). Samples were studied from patients with a variety of cancers prior to treatment. They included 233 with lung cancer, 109 bladder cancer, 64 colorectal cancer, and 75 from gastric cancer. Seventeen samples were from breast cancer prior to treatment, nine from recurrent breast cancer, and 25 from women who were tumour-free after treatment; 47 samples were tested prior to the treatment of chronic lymphocytic leukaemia (CLL); 25 samples prior to the treatment of Hodgkin's disease (HD) and 26 after treatment and 72 sera prior

to the treatment of myelomatosis.

Urine samples were collected from patients with a variety of inflammatory conditions and known tubular proteinuria. Samples were stored at -20°C .

PROTEIN MEASUREMENT

SAA was measured by a modified single radial immunodiffusion (RID) method as developed by Dr J Whicher (personal communication 1982) based on the method of Mancini *et al.*¹⁸ In this method the plates were prepared normally with agarose (1% wt/vol, HSA Litex) in barbitone buffer pH 8.6 containing polyethylene glycol (PEG, 5% wt/vol) and goat antihuman SAA antiserum ($4\ \mu\text{l}/\text{cm}^2$; provided by Atlantic Antibodies, Scarborough, Maine, USA). After introducing the samples and standards into the wells, the plates were then incubated at 37°C for 36 h before extensive washing and pressing followed by staining with Coomassie blue 0.25% wt/vol in methanol/acetic acid/water (5/1/5) and destaining with ethanol/acetic acid/water (3/1/6).

A standard SAA (prepared for the European Amyloid Research Society) was obtained from Dr J Whicher (Department of Chemical Pathology, Bristol Royal Infirmary, Bristol BS2 8HW).

Freezing and thawing (ten times) did not appear to significantly alter the concentrations of SAA in serum from normal subjects nor was there any difference in the values obtained in serum and plasma from the same donor, although serum gave clearer precipitin rings.

CRP and α_1 -acid glycoprotein (AGP) and IgG were measured by radial immunodiffusion (RID) incubated at room temperature for 48 h using antisera and standards obtained from Behringwerke AG (Marburg, Lahn, West Germany).

IMMUNOELECTROPHORETIC METHODS

SAA was measured using "rocket" immunoelectrophoresis¹⁹ at 37°C using antiserum ($3\ \mu\text{l}/\text{cm}^2$) and PEG (5% wt/vol) with agarose (1% wt/vol, HSA Litex) in barbitone buffer (0.02 M; pH 8.6). Crossed immunoelectrophoresis²⁰ was performed at 37°C or 15°C with PEG (5% wt/vol) and agarose (1% wt/vol HSA Litex) in barbitone buffer (0.02 M; pH 8.6). Intermediate gels containing antiserum to HDL (Behringwerke AG, Marburg, Lahn, W Germany) were used in some experiments.

Results

SAA concentrations in serum samples as measured by RID were compared with those obtained using rocket immunoelectrophoresis. The correlation coefficient between results was very high ($r = 0.98$;

with a slope $\text{SAA (rocket)} = 0.76 \times \text{SAA (RID)} + 41\ \text{mg/l}$) although the concentrations were lower than those using RID at very high concentrations of SAA. The change in binding properties of SAA for HDL at 37°C was shown when for comparison the first dimension electrophoresis was run at 15°C and 37°C . At 15°C the SAA coelectrophoresed with HDL (α -lipoprotein) as shown by an intermediate gel containing antiserum to HDL in crossed immunoelectrophoresis, and at 37°C the SAA electrophoresed at a much slower rate (in the β_1 - β_2 region).

The concentrations of SAA in 35 women and 35 men blood donors were 18 ± 13 and $17 \pm 12\ \text{mg/l}$ (mean $\pm$ SD) respectively. In 24 older men (follow-up of BPH) aged 56–83 yr the concentrations were $23 \pm 9\ \text{mg/l}$ and they showed no significant increase with age. From the data an arbitrary upper limit of normal was taken as $60\ \text{mg/l}$ although one normal serum examined had higher concentrations than the set limit. The arbitrary limit was calculated as the mean plus three standard deviations. This was the same criteria that established the upper limit of $10\ \text{mg/l}$ for CRP for the same set of blood donors ($1.8 \pm 2.4\ \text{mg/l}$).

In seven patients the change in serum concentrations was followed over several days after elective cholecystectomy and the rates of change of SAA and CRP concentrations were found to correlate ($r = 0.52$; based on the logarithms of SAA and CRP to allow for their skewed distribution). An example of the rate of change in blood concentrations (Fig. 1) shows that the blood half-life for SAA

Fig. 1 Comparison of the response of SAA and CRP concentrations in a patient after an uncomplicated cholecystectomy.

Table 1 Incidence of raised serum CRP concentrations in patients with inflammatory disease who have normal (<60 mg/l), moderately raised (60-400 mg/l), or greatly raised SAA concentrations (>400 mg/l).

	SAA (mg/l)		
	<60	60-400	>400
Liver disease			
PBC	0/16	2/15	6/8
CAH	0/22	0/9	3/3
AC	0/9	3/4	0/0
Rheumatoid arthritis	3/10	12/21	21/22
Burn injury	0/1	0/1	18/18
Paraplegia	1/11	2/5	6/6
Inflammatory G-I disease	0/15	4/9	5/5

(1.1 ± 0.3 days) was normally shorter than that for CRP (2.2 ± 0.9 days).

Low concentrations of SAA (50 mg/l) could be detected in the urine from 11 out of a total of 39 patients with a variety of both chronic and acute renal diseases. SAA occurred most commonly in patients with chronic or acute pyelonephritis (7 of 11). Those patients with detectable SAA were all within the group of 28 patients with urinary IgG concentrations > 90 mg/l, whilst SAA was not found in 17 patients with lower IgG concentrations. In 20 patients with burns, 19 of whom had raised acute phase reactants in the blood (Table 1), only four had detectable concentrations in the urine (in two it was 140 and 460 mg/l). SAA was not detectable in the urine of 20 patients with paraplegia, the majority of whom had chronic urinary infections of varying severity.

SERUM CONCENTRATIONS IN NON-MALIGNANT DISEASE

Serum concentrations of SAA and CRP were measured in chronic active hepatitis (CAH), primary biliary cirrhosis (PBC), and alcoholic cirrhosis (AC) and benign inflammatory gastrointestinal diseases, as well as rheumatoid arthritis. It was found that in some patients the concentrations of SAA were higher than the designated upper limit of normal whilst the CRP concentrations were not raised. These diseases were compared to the concentrations in patients with burn injury in whom there was usually a pronounced acute phase reaction and to patients with paraplegia of many years duration who had chronic inflammation due to pressure sores or other causes. The association of these two indices of the inflammatory response (SAA and CRP) is shown in Table 1.

SERUM CONCENTRATIONS IN MALIGNANT DISEASE

SAA was measured in lymphoma, myelomatosis, and chronic lymphocytic leukaemia (CLL). There

Fig. 2 Distribution of SAA and CRP concentrations in patients with myelomatosis.

was a good correlation between CRP and SAA in all diseases examined, the results for myelomatosis in Fig. 2 ($r = 0.70$) are typical of these findings. The level of serum SAA was also found to correlate well with serum AGP in patients with myelomatosis ($r = 0.76$, for AGP ν logSAA). The pretreatment serum samples from patients with stage I and II Hodgkin's disease (HD) showed a larger number of patients with raised concentrations of both SAA (8 out of 13) and CRP (5 out of 13) than the corresponding samples for the same stage in remission (2 out of 13 with raised SAA and 0 out of 13 with raised CRP). The pretreatment samples from 47 patients with CLL (stage 0, 29; stage I, 6; stage II, 8; stage III & IV, 6) had a low frequency of raised acute phase reactants (8 with raised SAA, and 6 with a raised CRP). Pretreatment HD samples of stage III and IV had a high proportion with raised SAA (8 out of 11) and raised CRP (8 out of 11).

In carcinomas there was again a high correlation between the levels of logCRP and logSAA ($r = 0.77$). Comparison of the number of patients with raised or normal concentrations of SAA reveals that about 21% of the 579 patients with normal CRP concentrations had a raised SAA, although this was higher in some types of cancer than others. This dissociation is not as great as that found in some non-malignant diseases such as rheumatoid arthritis or PBC where it was approx 50%. Table 2 shows that a greatly increased SAA concentration, found in 172 cancer patients, was with only two exceptions accompanied by a raised CRP concentration. How-

Table 2 Incidence of raised serum CRP concentrations in patients with malignant disease who have normal, moderately raised or greatly raised SAA concentrations.

	SAA (mg/l)		
	<60	60-400	>400
Lung cancer	4/56	53/77	99/100
Bladder cancer	4/51	11/27	31/31
Colorectal cancer	0/22	10/26	16/16
Gastric cancer	1/26	7/24	24/25

ever, there was a group of patients (154) with moderately raised SAA, of whom 47% have CRP concentrations within the normal range.

The concentrations of SAA in various types of solid tumours (lung, bladder, stomach, and colorectal) were compared with regard to stage. It can be seen from Fig. 3 that the more advanced stages had a higher proportion of raised SAA concentrations (normally accompanied with a raised CRP). The SAA concentrations of even the earliest and most advanced stages were shown to overlap. This overlap was present in all the cancers examined in this series. The increase in median SAA concentrations of different stage III of stomach and lung cancer and HD is shown in Table 3. Primary breast cancer stages I-III were found to be a poor stimulus for the acute proteins and SAA concentrations were not raised in 17 pretreatment patients or 24 patients who were subsequently tumour-free after surgery,

Fig. 3 A comparison of serum amyloid A concentrations in solid malignancies at different stages (○ = raised CRP >10 mg/l; ● = normal CRP <10 mg/l). The transverse dotted line represents the mean SAA level for each of the groups of patients with cancer at different stages.

Table 3 Median concentrations of SAA in serum from patients at different stages of malignant disease.

	SAA (mg/l) (No of patients)			
	Stage I	II	III	IV
Lung cancer	88(48)	130(32)	275(114)	230(18)
Gastric cancer	140(13)		62(28)	465(34)
Pretreatment Hodgkin's disease	25(14)		560(11)	
Posttreatment Hodgkin's disease	19(13)		42(13)	

but SAA concentrations were moderately raised in three out of six patients with recurrences.

Metastatic colorectal (Dukes "D"; Fig. 3) had a much higher median SAA level than non-metastatic cancer (Duke's A+B). Nevertheless, there was still considerable overlap between the groups. Similarly 25 patients with metastatic gastric cancer had a median concentration of 480 mg/l whilst 50 non-metastatic patients had a median SAA concentration of 90 mg/l. However the range of SAA concentrations in each group was similar (with metastases 22-5500 mg/l; without metastases 12-4600 mg/l). Many of the stage IV gastric cancer patients had liver metastases and not all of them had significantly raised concentrations of SAA. Patients with non-metastatic prostatic cancer had a median SAA concentration of 25 mg/l whilst 19 patients with metastatic disease had only a slightly higher median SAA concentration (38 mg/l).

Discussion

Concentrations of SAA were found to correlate well with CRP in all the diseases examined and also in serial samples. This agrees well with previous data on groups of patients and also on serial SAA concentrations.^{8,9,13} The serial measurements either used an injection of etiocholanolone (a naturally occurring steroid metabolite which has the property of being a non-antigenic human pyrogen) to produce an acute phase response⁹ or were on patients after elective surgery.⁸ In experiments on serial measurements of SAA, in this study in patients after cholecystectomy, the time required for SAA concentrations to halve their original value (mean 1.1 days) was shorter than that of CRP (mean 2.2 days). The half-life of SAA has been measured at 40-60 min²¹ in normal mice injected with ¹²⁵I-labelled material which was considerably shorter than the half-life of labelled apo A-1 or other apolipoproteins. Thus, the slower blood half-life observed after wounding is presumably due to the combined effects of the decreased rates of synthesis as the inflammatory response waned and possibly a fixed rate of

clearance. This is in agreement with the suggestion that the putative inducer of SAA synthesis in liver, interleukin 1, is required for continued SAA synthesis. It may be that CRP like SAA is induced by interleukin 1.²² The dissociation of the concentrations of these two acute phase proteins, in some patients, may be due to differences in their rates of clearance from the blood. The fact that SAA has been shown to have a much shorter half-life in mice than other apolipoproteins is interesting in view of the reduction in binding or change in structure that appears to occur between HDL and SAA at higher temperatures, and it may be possible that SAA is only bound firmly to HDL at lower temperatures (eg 15°C). Concentrations of SAA increased up to 200fold during the acute phase reaction and can reach approximately 5 g/l. The measurement was based on the standard of the European Amyloid Research Society and cannot be compared directly with values obtained in radioimmunoassays (normal levels by radioimmunoassay obtained by other researchers are <220 ng/ml;¹⁰). It was likely that SAA concentrations were a more sensitive indicator of inflammation than CRP because of the more rapid increase in SAA and the greater increase in concentrations obtained after the same stimulus. It was found that the group of elderly men (BPH recalls) did not have an increased SAA despite previous reports that the elderly subjects can exhibit raised SAA.^{23 16} Our result agrees with the more recent observations on large numbers of elderly people in which no increase in SAA with age was observed.¹⁷ The reason for the different results obtained by other investigators^{23 16} may be due to differences in the specificities of the immunoassays employed. However, the finding that there was no increase makes it logical to compare patients with diseases that have a different age distribution.

The finding that SAA could be detected in the urine of patients with pyelonephritis is probably consistent with an increased high-molecular-weight proteinuria and the acute inflammatory response in the serum of patients with pyelonephritis.²³ Although most patients with burn injury did not have detectable concentrations of SAA in their urine there were two exceptions with particularly high concentrations which cannot be explained by an associated glomerular defect. It was possible that the protein was a fragment of SAA reacting with the antisera.

The distribution of patients according to whether SAA and CRP were raised or normal was similar in all types of cancer examined, although lung cancer displayed the highest proportion of patients with raised SAA and normal CRP concentrations. Generally the percentage with raised SAA and normal

CRP was approx 10–30% and the converse (normal SAA and raised CRP) was rare. Amongst the non-malignant diseases, however, PBC and rheumatoid arthritis and to a lesser extent CAH showed a much higher proportion of patients with raised SAA and normal CRP. Thus despite reports to the contrary¹⁶ it would appear that chronic liver disease can cause an increase in SAA although concentrations attained were not as high as those in some acute inflammatory states and CRP was not often raised. It may be that the increased SAA found in patients with PBC may be due to the often associated systemic disorders. It has been suggested that the occurrence of amyloid deposits correlated with the incidence of very high SAA concentrations in certain diseases although this is not the only requirement for the deposition of amyloid.¹⁴

In the haematological malignancies, CLL was found to give CRP and SAA concentrations in the normal range, a finding in agreement with previous reports¹¹ although the patients examined were generally of an early stage.

Our results do not match the clear separation between SAA concentrations of disseminated cancer and local or regional cancer that were reported previously.¹¹ The reason for this is not clear. The occurrence of infection in some malignancies can cause an increase in CRP;²² however, infection was present in very few of the patients in this study, mainly it was limited to those patients with CLL and myelomatosis who presented with raised acute phase reactant proteins. In the majority of cancer patients with an acute phase response; this is attributable to the reaction to products of tissue destruction.²⁴

In all the diseases we studied SAA appeared to be more sensitive an index of an acute phase response than CRP independent of the cause of the inflammatory reaction. Furthermore the claims that high concentrations of SAA are associated with the presence of distant metastases are not substantiated. The introduction of an improved assay may facilitate SAA measurement but is unlikely to change the general picture in neoplastic disease.

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