

Review

# Serum Amyloid A (SAA): a Concise Review of Biology, Assay Methods and Clinical Usefulness

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Serum amyloid A (SAA) is a family of proteins encoded in a multigene complex. Acute phase isotypes SAA1 and SAA2 are synthesized in response to inflammatory cytokines. SAA and C-reactive protein (CRP) are now the most sensitive indicators for assessing inflammatory activity. In viral infection and kidney allograft rejection, SAA proved more useful than CRP. Development of convenient assay methods for SAA will facilitate its use in clinical laboratories.

**Key words:** Serum amyloid A; Inflammation; Assay method; Clinical significance; C-reactive protein.

## Introduction

Serum amyloid A (SAA) was discovered a quarter of a century ago as a plasma component which shares antigenicity with amyloid AA, the chief fibrillar component in reactive AA amyloid deposits (1-3). Although its exact biochemical nature was unclear, it was soon noticed that this component increases its plasma concentrations in inflammatory conditions (4). Then, SAA was identified as an apolipoprotein of high density lipoprotein (HDL) (5). To date, the medical profession has paid attention to SAA not only as a fibrillogenic precursor in AA amyloidosis but also in its role in inflammatory reactions and lipoprotein metabolism or atherogenesis (6-9). In spite of such interests and expectation as a useful inflammatory marker, SAA has not been widely used as a routine clinical test. This is mostly due to technical difficulties in large scale purification of SAA, stable production of antibodies with high titer, devel-

opment of an assay system, and standardization of the assay, as mentioned below. The author's group solved those problems and established an assay system suitable for the clinical laboratory (10). This method was the first to become commercially available for wide use. A second, developed by a research group in Germany, is now available from an American manufacturer. Thus, SAA is coming out of a limited research area, i.e. amyloidogenesis or atherogenesis and spreading to a general clinical field. This review will first assess biological and pathological aspects of SAA, and then examine the assay methods for SAA and their clinical usefulness.

## Biochemical and Physiological Aspects of SAA

### Structure

SAA, a polymorphic protein, consists of several genetically determined isotypes (7, 11-13). In humans, an individual has three isotypes in plasma, SAA1, SAA2, and SAA4. The gene coding SAA3 is not expressed (14). SAA1 and SAA2 are plasma precursors of amyloid fibrils and behave as an acute phase reactant (APR) in plasma. Generally, and also in this review, these acute phase isotypes are referred to as SAA. Although SAA1 is not distinguished from SAA2 by antibodies used in the assay, it is believed to comprise 70% or more of plasma SAA concentration (11). SAA1 and SAA2 have three ( $\alpha$ ,  $\beta$ ,  $\gamma$ ) and two ( $\alpha$ ,  $\beta$ ) common allelic variants, respectively (15, 16) (Figure 1). There is a difference in frequency of SAA1 alleles between races. Three alleles appear evenly in the Japanese population, while  $\alpha$  is dominant (80-90%) and  $\gamma$  is rare in the Caucasian population (17-19). For SAA2,  $\alpha$  is dominant (80-90%) and distribution is similar among races (20, 21).

The acute phase SAA is a single polypeptide with

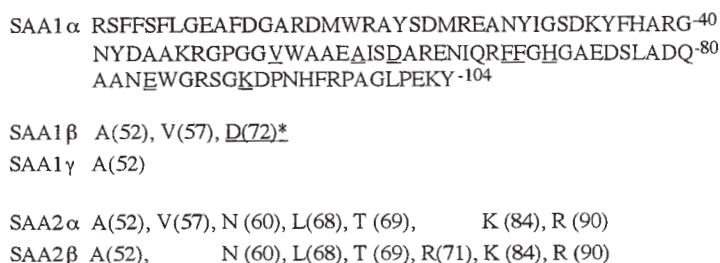


Fig. 1 The primary structure of acute phase SAA. Amino acid sequence of SAA1 $\alpha$  is shown. Residues substituted among isotypes are underlined. For isotypes other than SAA1 $\alpha$ , only residues different from SAA1a are shown. \* The reported protein (SAA1 $\beta$ ) has Asp as residue 72 (104). Genomic DNA of

SAA1 $\beta$ , however which was separately reported (12), indicates Gly at this position. A project recommending new nomenclature of SAA is ongoing and will resolve this discrepancy.

104 amino acid residues, which often lack amino-terminal arginine because of a posttranslational modification. Although the exact tertiary structure of SAA is unknown, the critical role of the amino terminal 10 residues has been noted in amyloid fibril formation and HDL binding (22, 23). SAA4 is a constitutive apolipoprotein in HDL and it does not behave like APR (24, 25). SAA4 has 55% homology in its primary structure with acute phase SAA (13). For measurement of acute phase SAA, cross-reactivity of antibodies with SAA4 should be avoided, since the physiological concentration of SAA4 is 10-times higher than that of acute phase SAA.

#### Synthesis and metabolism (Figure 2)

SAA synthesis is induced in various tissues by the stimulation of inflammation-related cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor- $\alpha$  (TNF $\alpha$ ) (26–28). Most of plasma SAA probably originates in the liver. Previous investigation has demonstrated extrahepatic synthesis of SAA (29), but its extent and biological significance is unknown. Immediately after SAA is released from the cells, it binds with HDL and circulates throughout the system. In the circulation, SAA rapidly clears; its half life in mice is 30–50 minutes (30), considerably faster than that of apolipoprotein A-I (apoA1), the main protein constituent of HDL. SAA may be attached or internalized into phagocytic cells as HDL-associated forms (31), and degraded by lysosomal proteinase there (32–34). Whether considerable amounts of SAA stay and function as an HDL-free form in tissues is unknown. In drawn plasma, at

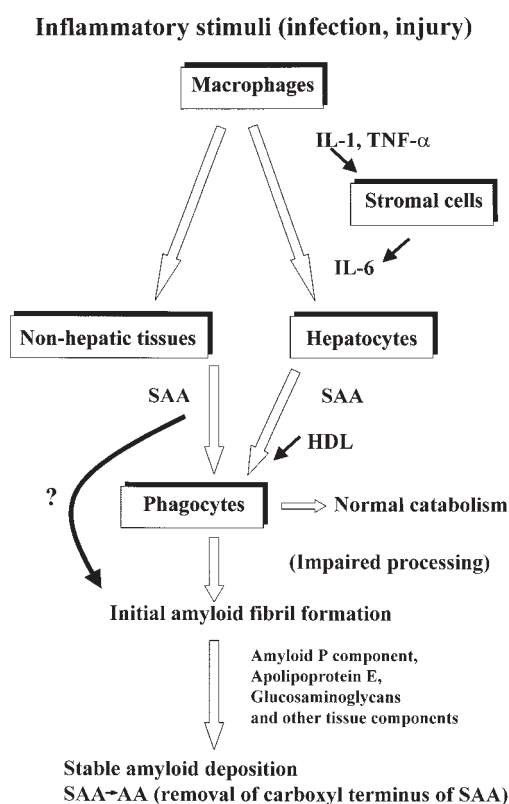


Fig. 2 Synthesis of SAA and catabolic pathway leading to amyloidogenesis.

least, almost all SAA is present in high molecular form (most in HDL and little in other lipoproteins). This phenomenon is important, because most SAA assays are directed to SAA in HDL, not SAA monomer, which reacts with antibodies differently from the lipid-bound form.

#### Functional and Pathological Aspects of SAA

##### Inflammation

Since plasma SAA concentration changes from 1 to 1000 mg/l corresponding to inflammatory activity, some researchers have proposed that SAA may play a critical role in the process of inflammatory reactions. Among these roles are the inhibition of antibody production by lymphocytes (35), inhibition of platelet agglutination (36), inhibition of oxidative burst reaction in neutrophils (37), induction of collagenase (38), and chemotaxis for neutrophils and monocytes (39). These imply that SAA functions to preserve homeostasis by preventing further progress of injuries or by repairing damaged tissues. However, some findings suggest a proinflammatory role of SAA (40, 41).

##### Lipoprotein metabolism and atherogenesis

Inflammatory conditions greatly alter HDL composition, reducing levels of apoA1 and apoAII, and increasing those of SAA (8, 9, 42). Reduction of apoAs is due to lowered synthesis in the liver and probably displacement from HDL by SAA, which is observed as an *in vitro* phenomenon (43). ApoA1 plays a central role in the reverse cholesterol transport system, which may be essential for preventing atherogenesis. Some propose that SAA may be increased in HDL in order to compensate for defects in this system, which may be impaired by apoA1 reduction in inflammation. Experimental findings support the notion that SAA-rich HDL gains high affinity binding to macrophages where cholesterol should be removed (44), and that SAA enhances lecithin:cholesterol acyltransferase, a key enzyme for cholesterol esterification (45). SAA is expressed also in the artery wall (46, 47) and is present in atherosclerotic lesions (48). We showed experimentally that SAA-loaded LDL has increased non-specific affinity with skin fibroblasts and macrophage-like cells (49). It is now under discussion whether SAA promotes or prevents atherogenesis (8, 9, 50).

##### Amyloid precursor

SAA, or more precisely, its amino-terminal 50–76 residues, form insoluble fibrils in the extracellular spaces. This disorder, reactive amyloidosis, develops in chronic inflammatory diseases such as rheumatoid arthritis (RA) and tuberculosis (6, 7). Approximately 10% of RA patients are believed to have amyloid deposits, according to the studies using gastroendoscopic biopsy (51). Amyloid deposits are systemic and progressive; in advanced stages they cause severe diarrhea and renal failure (52, 53). Prolonged hyperproduction

of precursor SAA in underlying diseases is essential for development of amyloidosis, but not sufficient by itself. Impaired processing of SAA, that is, disturbance of SAA degrading system (54, 55) and participation of other components, may be another factor (Figure 2) (56). Recent studies genetically link SAA1 allele ( $\gamma$ ) with susceptibility to reactive amyloidosis in Japanese patients with RA (17).

Currently, the most practical way to prevent or delay progressive amyloid deposition is by reducing inflammatory activity, represented by plasma SAA levels. Viewed in these terms, frequent monitoring of SAA levels is recommended in patients with chronic inflammatory disorders or in those who have been diagnosed with amyloidosis.

## Assay Method for SAA

### Materials

Large scale preparation of SAA requires significant amounts of acute phase serum, but a steady supply is difficult to obtain. An *E. coli* expression system has been shown to generate satisfactory amounts of human SAA (57). Our own group is now utilizing such recombinant SAA for immunization and will soon use it as an assay standard. Not a few researchers had difficulty in producing antibodies either polyclonal or monoclonal, with high avidity against the SAA antigen (58). This may be due to the hydrophobic nature of purified SAA and high homology of the structure of SAA in various species (7). Frequent immunization with large amounts of antigens is necessary for the production of polyclonal antibodies. Rats, which are believed to lack mature SAA proteins, seem to have a fair immune response against human SAA. In fact, rat-derived monoclonal anti-SAA antibodies were successfully produced and utilized in this laboratory (59).

Similarly to other apolipoproteins, purified protein is not suitable for the assay standard in any immunoassay for SAA when non-denatured serum or plasma are used (60). Therefore, acute phase HDL, SAA content of which is determined by electrophoresis and then pro-

tein staining (Figure 3), is used in most assays (60). Not surprisingly, there are significant variations in the absolute values of SAA among the assay methods, according to the international survey (61). These variations are due not only to different estimation of SAA content when one compares in-house standards one with the other, but also to absence of parallel responses in in-house standards. At this moment, each assay should maintain its own SAA values, which should compare with international standards, which the World Health Organization has recently approved (61).

To avoid the use of insoluble SAA proteins, a recombinant SAA2 was produced as a relatively soluble form, and was used for immunization and as the assay standard in a recent report (62). Interestingly, the SAA2 standard was found to work well in the presence of 25 % propan-2-ol in the enzyme-linked immunosorbent assay.

### Assay system

Various assay methods for SAA such as radioimmunoassay, radial immunodiffusion, and enzyme-linked immunosorbent assay (ELISA) have been reported. However, these are mostly for use in research laboratories. To introduce SAA measurements to clinical laboratories, an automated method, either fully or partially, is desirable. Also, the method should be rapid enough to be utilized in acute diseases such as acute myocardial infarction and kidney allograft rejection. Concentrations of serum SAA in patients' samples are more than 10 mg/l, which do not always require highly sensitive assays like ELISA. We developed an automated latex agglutination immunoassay, the kinetic photometry of anti-SAA coated latex particles (10), which is now commercially available. This allows us to determine SAA levels within 10 minutes. Originally, use of the reagent for this assay was limited to a specific analyzer. Later, however, the manufacturer developed a reagent which can be implemented on commonly used automated analyzers (63). Another system, introducing latex technology to the original nephelometric immunoassay (64), is now available worldwide.

ELISA is also easy to use. A couple of manual methods are commercially available. An automated method has been reported (65), but has not been widely used. A unique method, "direct binding ELISA", is worth noting (66). This method utilizes the preferential binding of SAA to the plastic surface of microtiter plates. This property of SAA is, in general a disadvantage. A significant fraction of SAA can be lost because of its attachment to the inner surface of sample containers, tubes, reaction cuvettes, and the like. ELISA is expected to measure low levels of SAA because of its high level of sensitivity. We have developed a highly sensitive ELISA, the detection limit of which is 100 ng/l of SAA, by the use of monoclonal and polyclonal antibodies (67). This method is now being utilized for measurement of SAA in body fluids other than plasma, and supernatant of cell cultures (Table 1). In fact, production of SAA pro-

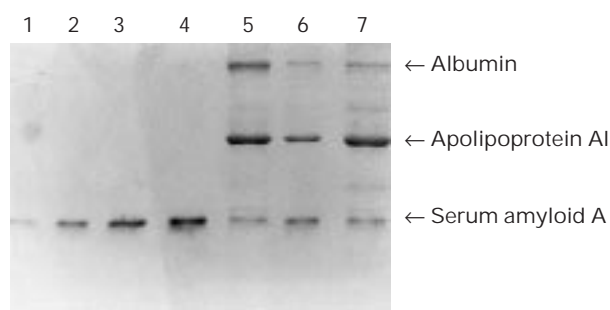


Fig. 3 Determination of SAA contents in acute phase HDL. Purified SAA (110, 220, 330, and 440 mg/l in lane 1 to 4, respectively) and acute phase HDL (lane 5 to 7) were subjected to SDS-PAGE. SAA contents in the HDL were determined against calibration with purified SAA. Such HDL can be used as the primary standard for the SAA assay. This analysis can also be used to study the accuracy of SAA immunoassays (10).

Tab. 1 SAA levels measured by the sensitive ELISA.

Samples	SAA concentrations*
Serum (healthy)	0.5–10 mg/l < 3 mg/l (90 %)
Urine (healthy)	–0.8 µg/l < 0.08 µg/l (80 %)
Cerebrospinal fluid (healthy)	1.0 µg/l (median) < 10 µg/l (90 %)
Culture media of HepG2 cells (stimulated)	10–100 µg/l

\*standardized using the standard used in the current nephelometric assay.

tein by a human monocytoic cell line THP-1, where SAA mRNA has been quantified but SAA protein has not been measured (68), can be evaluated quantitatively (our unpublished findings).

Thus, the assay method should be chosen depending on the intended purpose (Table 2). For bedside testing, a rapid qualitative test has been developed in our laboratory (unpublished). The test, based also on latex agglutination, can detect SAA at levels of 20 mg/l in whole blood, a condition which indicates the presence of inflammation.

#### Clinical Implications of SAA Test

##### *First class acute phase reactant*

In inflammatory states several parameters appear in plasma and in rather strict sequence (27, 28, 69, 70). After the occurrence of inflammatory stimuli, cytokines such as TNF $\alpha$ , IL-1 and IL-6 are increased within 2–3 hours. SAA and C-reactive protein (CRP) are next. These latter two start to elevate in 3–6 hours, reach their peaks in 2–3 days, and return to the basal levels at 5–7 days (Figure 4). These two are called first class APR because they are the most sensitive plasma proteins indicating inflammatory activity. Meanwhile, the other APRs, such as  $\alpha_1$ -antitrypsin,  $\alpha_1$ -acid glycoprotein, ceruloplasmin and haptoglobin reach their peaks in 3–5 days.

The SAA of healthy subjects is below 10 mg/l, mostly below 3 mg/l. SAA levels show no difference between genders. In the elderly, however, the levels are slightly

Tab. 2 The measurement of SAA concentration.

Assay method	Detection limit	Time for assay completion
Latex agglutination nephelometry or turbidimetry (automated and commercially available)	0.5–3.0 mg/l*	10 min
Enzyme-linked immunosorbent assay (in house)	100 ng/l	5 h
Latex agglutination slide test	20 mg/l	5 min

\*depending on the apparatus used

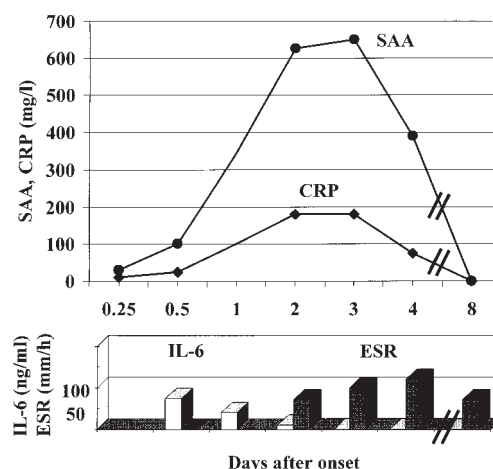


Fig. 4 Changes of SAA, C-reactive protein, interleukin-6 and erythrocyte sedimentation ratio in a patient with acute myocardial infarction.

higher (71). The concentration can be increased to several thousand mg/l, depending on the severity of inflammation. Basically, any inflammatory conditions, including infection, trauma and invasive malignant disease, can cause the elevation of plasma SAA (72–82). However, the magnitude of elevation varies among disorders. SAA increases moderately to markedly (100–1000 mg/l) in bacterial and fungal infections, invasive malignant diseases, tissue injuries like acute myocardial infarction, and autoimmune diseases such as rheumatoid arthritis and vasculitis. Mild elevation (10–100 mg/l) is often seen in viral infections, systemic lupus erythematosus and localized inflammation or tissue injuries like cystitis and cerebral infarction. An increase of SAA to high levels in these disease groups may indicate involvement of bacterial infection or severe tissue injuries.

##### *Advantages compared with CRP test (Figure 5)*

Disorders mentioned above, of course, can be detected by the CRP test because the synthesis of both SAA and CRP may be regulated by similar cytokines. For many years, CRP has been widely known as the most reliable inflammatory marker. Here, discussion focuses on those situations in which the SAA test is more useful than the CRP test.

The amplitude of the increase of SAA is similar to or greater than that of CRP. Sometimes, as in patients with severe burns, there is a greater response of SAA (83). In viral infections, SAA levels are often slightly elevated when CRP is at or below the detection level. Some re-



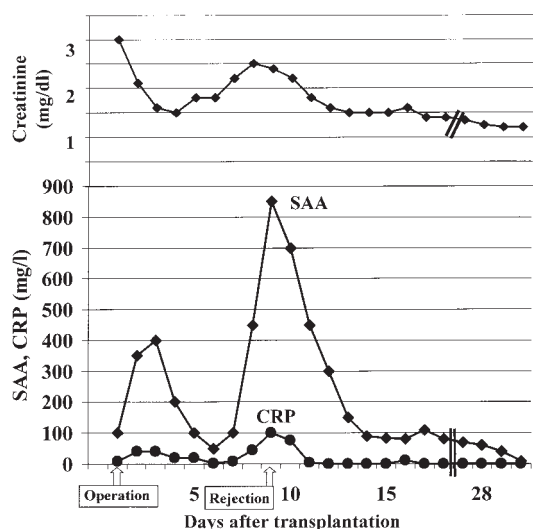


Fig. 5 Relationship between values of SAA and CRP concentration.

searchers have proposed using SAA measurements in testing for infections caused by adenovirus, measles, mumps, varicella, rubella, influenza and others (84–86). In these conditions, clear elevation of CRP may indicate involvement of bacterial infections or tissue injuries. Since the physiological level of SAA is approximately ten times higher than that of CRP, it may be easier to detect slightly elevated SAA than CRP. Another possibility, according to a recent report, is that the mRNA of SAA has a longer half life than that of CRP in cultured hepatoma cells (87). CRP may return to the basal level faster than SAA. Whichever it is, the SAA test is recommended in conditions in which CRP shows poor response.

A recent report proposes that SAA predicts more accurately poor prognosis of elderly inpatients than does CRP (88). Indeed, SAA probably will detect some events such as minor infection and ischemic tissue injury, which may occur in the elderly and patients with chronic disorders such as diabetes mellitus and hemodialysis. Slightly elevated CRP has been proposed as a risk factor or as an indicator of disease outcome in cardiovascular disorders (89–92). The published data for SAA, on the other hand, are scanty.

The most prominent discrepancy between SAA and CRP has been seen in patients who undergo kidney transplantations (Figure 6) (93–97). At the rejection of a transplant, SAA is markedly elevated along with serum creatinine. Elevation of creatinine, though the best confirmation of the body's rejection of the kidney, is often very slight and needs careful observation. Since SAA changes are dramatic enough to be recognized easily, patients should be monitored by both SAA and creatinine. In contrast, CRP responds poorly to such rejections, not only in early phases but throughout subsequent days. Thus, CRP's low levels contrast with SAA's high responses. One reason for this poor response of CRP may be the therapeutic use of cyclosporin (98) and corticosteroids, though the mechanism is unknown. In fact, relatively low CRP levels have been observed in patients with cystic fibrosis (99), systemic lupus ery-

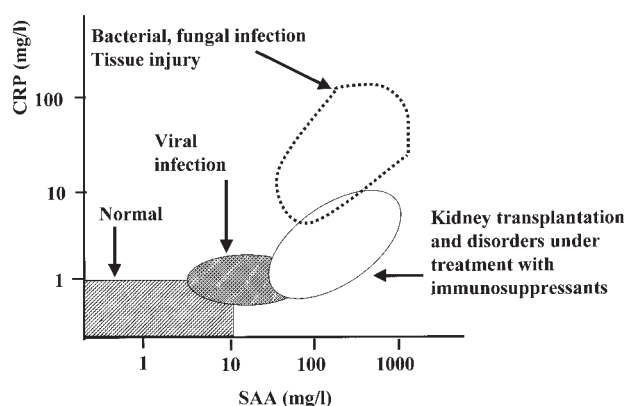


Fig. 6 SAA, CRP and creatinine concentrations in a patient with kidney transplant. The patient was given cyclosporin and a low dose of corticosteroid throughout the whole period and a high dose corticosteroid at rejection. Note that CRP levels are low throughout.

thematosus, and bronchial asthma under corticosteroid therapy (100). The same is observed in patients with psoriasis and corticosteroid resistant glomerulonephropathy, when they are administered cyclosporin (our unpublished findings).

There are no data to indicate the relationship between CRP and SAA when other immunosuppressive drugs are used. Thus, at least measuring the SAA levels is strongly recommended for patients who are administered cyclosporin and corticosteroids, i.e. in transplantation of liver and bone marrow (101, 102). In Crohn's disease and ulcerative colitis, SAA is superior to CRP as an activity indicator (103). Both the effect of therapies and an influence of the disorders themselves, especially autoimmune diseases, on both SAA and CRP concentrations should be assessed further.

## Conclusions

SAA is not only pathogenic for reactive amyloidosis but it also plays roles in the modulation of inflammation and atherosclerosis. SAA can detect inflammatory conditions, more sensitively than any other acute phase reactant. In time, the availability of assay methods and their wide use should corroborate these SAA values.

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