ORIGINAL INVESTIGATIONS

Serum amyloid A as a marker of ankylosing spondylitis activity

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Serum amyloid A protein A (SAA) is a normal serum protein (serving as a precursor of fibrillar tissue protein AA), synthesized in the liver and a rapidly responding marker of the acute phase of inflammation. A constant high concentration of SAA is one of the factors in the development of AA-amyloidosis. As a rule, secondary amyloidosis develops in patients with long-term and poorly controlled inflammatory diseases, including rheumatic diseases, one of which is ankylosing spondylitis (AS).

Objective: *to assess the level of SAA in AS patients and its relationship with indicators of disease activity.*

Patients and methods. *The study included 124 patients with AS who met the modified New York 1984 criteria. The disease activity and functional status of patients were assessed according to the recommendations of Russian experts. SAA and CRP, ESR in blood serum were measured in all patients.*

Results and discussion. *The median SAA concentration was 12.5 mg/L [4; 71.6]. Of 124 patients, 31% had SAA levels <5 mg/L and 69% had >5 mg/L. A strong correlation was found between the levels of SAA and CRP (r=0.80, p<0.000001), no significant relationship was found between SAA and ESR (r=0.31, p=0.92). The correlation between the AS activity according to the BASDAI index and SAA was weak (r=0.3, p<0.002), the correlation with ASDAS-CRP was moderate (r=0.54, p<0.00001).*

Conclusion. *A statistically significant relationship was found between SAA and CRP levels, as well as the AS activity indices. Research has shown that SAA can be used as one of the markers of inflammation in AS.*

Key words: *ankylosing spondylitis; HLA-B27; SAA; CRP; ESR; secondary amyloidosis.*
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Ankylosing spondylitis (AS) is a chronic inflammatory disease of the spondyloarthritis group, affecting mainly young people of active working age. In some patients, due to high clinical and laboratory activity and insufficiently effective therapy, it can result in serious complications, such as secondary amyloidosis.

AA amyloidosis (formerly known as secondary AA amyloidosis) is a disease characterized by extracellular deposition of fibrils [1]. AA amyloid fibrils are created from an acute phase protein, serum amyloid A (SAA) protein, through the process of cleavage, misfolding, and aggregation into a highly ordered abnormal β-sheet conformation [2]. Amyloid fibrils bind with other fragments, including glycosaminoglycans and serum amyloid P component (SAP), forming deposits which disrupt the structure and function of tissues and organs [3]. AA is a blood plasma apolipoprotein, one of high-density lipoproteins, which is synthesized by hepatocytes as a result of changes in the transcriptional regulation caused by proinflammatory cytokines [4]. The mean SAA concentration in the blood plasma of healthy humans is 3 mg/L, but it may increase up to 2000 mg/L or more in the acute inflammation phase [5]. A persistently high SAA level is a prerequisite for the development of AA amyloidosis, however, for unknown reasons, amyloidosis affects only a small proportion of patients with chronic inflammatory diseases [6, 7]. In a retrospective analysis, the rate of histologically confirmed AA amyloidosis in AS was 5.7% [8], while the average life expectancy of patients after verification of the diagnosis of amyloidosis was from 30 to 60 months [1]. However, with intensified therapy, most of these patients have a favorable prognosis [9].

According to the literature, AA amyloidosis occurs more often in patients with an early onset of the disease, longer times from symptom onset to diagnosis confirmation, late initiation of therapy, persisting high clinical and laboratory activity, and involvement of peripheral joints, the synovium of which actively produces SAA in case of inflammation [1].

The main target organs of amyloidosis are the kidneys, liver, and spleen. In more than 90% of the cases, proteinuria, nephrotic syndrome, and/or impaired renal function prevail in the clinical findings at early stages of the disease. Without timely effective treatment of amyloidosis, this invariably leads to the terminal stage of renal failure.

In some patients, the onset of secondary amyloidosis can be suspected based on a change in laboratory findings: an increased level of creatine and urea, decreased glomerular filtration, onset of proteinuria. Meanwhile, it is known that subclinical AA amyloidosis can progress even without organ damage. However, it is believed that the identification of even asymptomatic amyloid deposits should justify prescribing a more effective therapy [1].

In case of long-term amyloidosis with clinically significant kidney damage, the prognosis is determined by the effectiveness of systemic anti-inflammatory therapy for the underlying disease. Based on the observation of the largest cohort of patients with AA amyloidosis, the median post-diagnosis survival period was 133 months [10]. At the same time, a definite correlation between the survival and tSAA concentration was found. In patients with blood serum SAA concentrations >155 mg/L, the risk of death was 17.7 times higher than in those with SAA levels <4 mg/L. With SAA concentrations from 4 to 9 mg/L, the mortality rate

was 4 times higher. Amyloid deposits regressed at an average SAA concentration of <10 mg/L, and patients with the protein level of <4 mg/L had the most favorable prognosis.

Thus, it can be assumed that the detection of a high SAA level will help detect secondary amyloidosis in AS patients early and optimize the therapy to prevent the development of this formidable complication and improve the prognosis.

The **objective** of our study is to assess the SAA level and its association with the AS activity markers.

Patients and Methods. The study included 124 AS patients matching the 1984 Modified New York Criteria who were admitted for inpatient treatment at Federal State Budgetary Scientific Institution V.A. Nasonova Research Institute for Rheumatology from February to November 2020 and signed an informed consent to participate in the study. The disease activity and the patients' functional status were assessed according to the guidelines of Russian experts [11]. All the patients underwent laboratory testing commonly used to diagnose and monitor AS. Additionally, the blood serum SAA was determined by nephelometry on a BN ProSpec analyzer (Siemens, Germany) using commercially available reagent kits. The normal SAA level was 5 mg/L. The general clinical profile of the patients is presented in Table 1.

In the examined group, there were only 22% more men than women, and the average disease duration was about 15 years. Almost all the patients showed HLA-B27 antigen, most of them had high activity, enthesitis, coxitis, and peripheral arthritis.

A statistical data analysis was performed on a PC using Microsoft Excel and Statistica 10 analytics software package for Windows (Statistica 12.0, StatSoft Inc., USA 2011). The normality of distribution of characteristics was tested using the Shapiro–Wilk test. As the distribution of the majority of parameters was not normal, the methods of nonparametric statistics were used to describe the quantitative variables with data reported as a median with an interquartile range (Me [25th; 75th percentiles]). The association of variables was examined using the Spearman rank correlation analysis. Differences with $p<0.05$ were considered statistically significant.

Table 1 General Clinical Profile of the Patients

Parameter	Value
Men/women	1.3/1
Age, years, M±σ: at enrollment at disease onset	 38.1±12.9 23.5±9.9
BASDAI, Me [25th; 75th percentiles]	5.5 [4.6; 6.2]
ASDAS-CRP, Me [25th; 75th percentiles]	2.6 [2.2; 3.2]
ESR, mm/h, Me [25th; 75th percentiles]	13 [7; 27]
CRP, mg/L, Me [25th; 75th percentiles]	6.7 [1.4; 24.9]
HLA-B27, %	91.1
Peripheral arthritis, %	56.4
Enthesitis, %	76.6
Coxitis, %	65.3

Note: BASDAI – Bath Ankylosing Spondylitis Disease Activity Index; ASDAS-CRP – Ankylosing Spondylitis Disease Activity Score based on the CRP level.

Results. The median SAA concentration was 12.5 mg/L [4; 71.6]. Out of 124 patients, 31% had SAA levels <5 mg/L, and 69% had SAA levels > 5 mg/L. In 17.5% of the cases, the SAA level was increased with normal CRP values, while only 2 patients showed the opposite: an increased CRP level with a normal SAA value. Furthermore, 50 (40.3%) patients with a normal ESR value had an increased SAA level, while 7 (5.6%) had an accelerated ESR and a normal SAA content. There were no differences in the mean SAA, CRP and ESR levels between men and women. Table 2 shows the clinical aspects of AS depending on the SAA level.

Patients with an increased SAA level and a shorter AS duration had higher ASDAS-CRP and CRP values, they were more likely to have peripheral arthritis compared to those with a normal blood serum SAA concentration.

Table 2. Clinical Aspects of AS Depending on the SAA Level

Parameter	SAA ⩽5 mg/L (n=38)	SAA >5 mg/L (n=86)	p
Sex (male/female)	1.4/1	1.3/1	
Age, years, M±σ: at enrollment at disease onset	 37.8±3.8 25.3±11.3	 38.2±12.6 22.7±9.1	 0.9 0.3
Disease duration, years, Me [25th; 75th percentiles]	12 [7; 19]	10 [2; 16]	0.0003
BASDAI, Me [25th; 75th percentiles]	5.2 [4.6; 6.2]	5.6 [4.5; 6.4]	0.3
ASDAS-CRP, Me [25th; 75th percentiles]	2.4 [2; 2.9]	2.9 [2.1; 3.2]	0.003
ESR, Me [25th; 75th percentiles]	9.5 [5; 20]	15 [9; 34]	0.7
CRP, Me [25th; 75th percentiles]	1.25 [0.3; 3.7]	14.6 [4.2; 36.5]	0.000001
HLA-B27, %	84.2	93	0.4
Peripheral arthritis, %	39.4	59.8	0.04
Enthesitis, %	84.2	65.2	0.3
Coxitis, %	63.1	66.2	0.3

A strong correlation was found between the SAA and CRP levels ($r=0.80$, $p<0.000001$), with no significant correlation between SAA and ESR ($r=0.31$, $p=0.92$). The correlation between the AS activity based on the BASDAI index and SAA was weak ($r=0.3$, $p<0.002$), and the correlation with ASDAS-CRP was moderate ($r=0.54$, $p<0.00001$).

Discussion. The SAA level in the setting of AS is understudied. One study reported a correlation between an increased amyloid protein level and the AS clinical activity, as well as acute phase inflammatory markers, ESR and CRP [12]. Our results also show a strong association between the SAA and CRP levels, with a weak association with BASDAI and a moderate one with ASDAS-CRP. There were no differences in the SAA level between men and women, which is consistent with the data obtained for early rheumatoid arthritis (RA) [13].

When examining the feasibility of assessing the ESR, CRP and SAA levels to predict the response to AS treatment with drugs from the tumor necrosis factor-α inhibitor group, it was found that patients with higher CRP and SAA values responded better to the therapy. It was suggested that these data may help monitor the disease activity and further prognosis for AS patients [14]. Similar data were also obtained for RA [13]. According to the study results, SAA is synthesized more actively than CRP and has a wider dynamic response range. During the acute inflammation phase, the serum amyloid level can increase up to 1000 times, and its degradation period is much shorter than that of CRP [15]. A moderate association was demonstrated between the SAA and CRP levels, regardless of treatment method or frequency of visits to the research center. It was suggested that an increased blood serum SAA level may be a more accurate RA activity marker than the CRP level, as well as a predictive factor for early RA. Therefore, it was proposed to use the blood serum SAA level as an alternative inflammation marker to assess the RA activity [13]. In our study, some patients had an increased SAA level with normal CRP concentration and ESR values, which also justifies the need to determine the amyloid protein in case of AS, especially in the absence of an acute phase response.

Conclusion. There was a statistically significant correlation between the SAA and CRP levels, as well as the AS activity indices. The study showed that SAA can be used as one of the inflammation markers in AS.

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Conflict of Interest Statement

The study was conducted as part of the "Development of Treatment Methods for Refractory Severe Ankylosing Spondylitis" pilot study (AAA-A20-120041390035-8).

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