

# Secondary (AA) Amyloidosis with Development of Nephrotic Syndrome

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## ABSTRACT

A 25-year-old Caucasian female with a history of irritable bowel syndrome, presented to the emergency room with worsening upper bilateral abdominal pain and fatigue that began two days before arrival. The patient described having mild intermittent lower back pain and worsening bilateral edema of her ankles that began several months prior. Blood and urine specimens were tested, with results indicating the presence of systemic inflammation and nephrotic syndrome. The patient was admitted to the hospital for further testing and observation. An ultrasound of the kidneys was negative for renal calculus. A gallium scan indicated localization of leukocytes in the kidneys, liver and spleen. A CT urogram indicated renal damage and a SAP scan indicated amyloid deposits in the kidneys, liver and spleen. The patient was diagnosed with amyloidosis and nephrotic syndrome. Corticosteroids were prescribed and testing to determine the underlining cause of amyloidosis was initiated.

**ABBREVIATIONS:** BUN - blood urea nitrogen, CBC - complete blood count, CRP - C-reactive protein, ECM - extracellular matrix, ESR - erythrocyte sedimentation rate, HLA - human leukocyte antigen, RBC - red blood cell, SAA - serum amyloid A, SAP - serum amyloid P, TNF - tumor necrosis factor, WBC - white blood cell

**INDEX TERMS:** Systemic Amyloidosis, Inducible SAA, AA Amyloidosis

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## Case History

A 25-year-old Caucasian female presented to the emergency room with worsening upper bilateral abdominal pain that began two days before arrival, and mild intermittent lower back pain starting several months prior. Both the abdominal and lower back pain were described as being dull and achy. The patient also stated that both of her ankles were swollen, and described that she first noticed the swelling around the same time that her lower back pain began. She denied having diarrhea, fever, hematuria, or nausea. She stated that she was diagnosed with irritable bowel syndrome one-year prior and has a three-year history of short-lived episodes of self-limiting fever. She denied any family history of kidney disease or irritable bowel syndrome.

During the physical exam, abdominal percussion revealed an enlarged spleen and costovertebral angle tenderness. Evaluation of the patient's ankles revealed bilateral 2+ pitting edema, auscultation of the abdomen revealed normal bowel sounds. Past laboratory results were reviewed, a one-year history of elevated serum amyloid A protein (SAA) was previously documented. Current blood results revealed elevations of the following inflammatory markers, C-reactive protein (CRP), SAA, and erythrocyte sedimentation rate (ESR). Blood results also indicated low serum albumin and increased creatinine. A urine analysis revealed large amounts of protein in the urine, but no blood or leukocytes were detected. Overall, the initial laboratory findings were suggestive of systemic inflammation and nephrotic syndrome.

Once admitted to the hospital, the results of a CT urogram indicated moderate renal damage. A gallium scan was then completed to determine the source of her inflammation, the scan revealed leukocyte

concentrations in the kidneys, liver and spleen. Due to her past history of elevated SAA, a serum amyloid P (SAP) scan was ordered to identify possible locations of amyloid fibril deposits. The scan located amyloid deposits in the kidneys, liver and spleen. A biopsy of the kidney was then taken to determine the type of amyloidosis present. First the kidney tissue was stained with Congo red stain, the tissue sample was positive for amyloid fibrils. Next, the kidney tissue sample was incubated with anti-AA antibodies, the antibodies had affinity to the amyloid fibrils, affirming the presence of AA amyloid antigens. Subsequent blood and urine testing was negative for free lambda or kappa antibody light chains. Without these light chains being present, amyloid light-chain (AL) amyloidosis was ruled out and amyloid A (AA) amyloidosis, also known as secondary amyloidosis, was confirmed.

Due to the patient's history of irritable bowel syndrome, testing for additional autoimmune diseases was initiated. Eventually, laboratory results, in combination with the patient's history and clinical exam, lead to the diagnosis of ankylosing spondylitis. Treatment with corticosteroids and tumor necrosis factor (TNF) blockers was initiated. Two days later, the patient was discharged from the hospital with instructions to follow-up with a rheumatologist within the week.

## BACKGROUND

As a response to infection or injury, the human body rapidly produces acute phase proteins. This systemic reaction provides a mechanism to help isolate pathogens, protect tissue and reduce the time required for healing.<sup>1</sup> Due to the rapid synthesis of acute phase proteins, they have become important diagnostic biomarkers in a variety of clinical presentations spanning from autoimmune disorders to bacterial infections.<sup>2,3,4</sup>

Two of the most commonly measured acute-phase proteins are SAA and CRP. Both proteins are rapidly produced by hepatocytes following infection or injury and are measurable in plasma samples.<sup>5</sup> In particular, SAA increases 1000-fold and can reach a plasma concentration of 2,000 mg/L.<sup>5, 6, 7</sup> Within 72 hours after this initial increase, SAA plasma concentrations decline and eventually return to baseline levels of less

than 5 mg/L in a span of 5-7 days after hepatocyte stimulation.<sup>1</sup> CRP follows a relatively similar time course, however unlike CRP, SAA kinetics better parallel the typical transition from innate to specific immune response, suggesting that SAA may be an important component in the development of adaptive immunity.<sup>8</sup>

SAA is a 104-amino acid protein encoded by the SAA1 and SAA2 human alleles.<sup>6</sup> In hepatocytes, production is stimulated by inflammatory cytokines IL-1 $\beta$  and IL-6, it can also be stimulated by lipopolysaccharide, a bacterial wall component.<sup>4</sup> However, synthesis does not only occur in the liver, but can also be located at sites of inflammation. Localized production of SAA in macrophages, endothelial cells and synoviocytes has been reported.<sup>4</sup>

While the precise role of SAA is unknown, scientific evidence suggests that it serves an important role in supporting localized immunologic response. SAA has affinity to both mononuclear and polymorphonuclear leukocytes, platelets and extracellular matrix (ECM) components.<sup>9-15</sup> It is reported to inhibit platelet aggregation, enhance leukocyte adhesion and bind to ECM proteins.<sup>9-15</sup> All of these functions support an acute immunological response, but if prolonged, these activities have the potential to damage tissue. The accumulation of amyloid fibril deposits most often affects the kidneys, liver and spleen.<sup>7</sup> These fibril deposits cause disruption of tissue architecture and function and because SAA contains a binding site for ECM proteins,<sup>14</sup> removal from tissue is challenging. Sustained elevation of SAA is most commonly due to either AL amyloidosis or AA amyloidosis. The more common type of amyloidosis is AL, also known as primary amyloidosis. AL amyloidosis results from an overproduction of amyloidogenic proteins called light chains, antibody fragments.<sup>7</sup> These light chain fragments induce amyloid deposits. Treatment of primary amyloidosis targets the B-cells that produce these light chain fragments. The prognosis of primary amyloidosis is dependent upon the patient response to the chemotherapy, and is often poor.

The less common type of amyloidosis is AA amyloidosis, also known as secondary amyloidosis. In this type of amyloidosis, amyloid production is increased due to an underlining inflammatory

response or infection, often caused by an autoimmune disease. If a patient is able to discover the source of their inflammation, they then have a greater chance for reducing amyloid deposits by treating the underlying disease. Unfortunately, not all secondary amyloidosis patients are able to identify the disease causing the elevated SAA response. With these patients, treatment is difficult and prognosis is poor.

### Correlation of Laboratory Results

Laboratory testing is critical for the diagnosis of amyloidosis, without the current available assays, AA amyloid patients would have a significantly reduced life span. In this case study, the patient demonstrated

key findings, seen in Table 1, that would be expected in most AA amyloidosis patients. A complete blood count (CBC) indicated neutrophilia, likely because SAA stimulates macrophages to produce granulocyte colony-stimulating factor, a cytokine that induces neutrophil production.<sup>17</sup> Mild thrombocytopenia was also present. The patient had an enlarged spleen due to deposits of amyloid fibrils, as a result, platelets became trapped in the enlarged spleen causing a reduction in the platelet count.<sup>18</sup> Additionally, due to the enlarged spleen, the patient's blood smear was positive for Howell-Jolly bodies, a marker for splenic dysfunction.

**Table 1.** Hematology and Chemistry Laboratory Results

Analyte	<sup>1</sup> Adm	Day 1	Day 2	<sup>2</sup> Ref Range
<b>Hematology</b>				
Neutrophils	69.6	70.2	69.8	38 – 70%
Lymphocytes	23.1	22.9	23.3	15 – 40%
Monocytes	5.6	5.4	5.2	2 – 11%
Eosinophils	1.2	1.0	1.2	0 – 6%
Basophils	0.5	0.5	0.5	0 – 2%
RBC count	5.9	5.7	5.9	4.7 – 6.1 x 10 <sup>12</sup> /L
Reticulocytes	0.6	0.6	0.6	0.5 – 2.0%
Platelets	111	119	109	150 – 400 x 10 <sup>9</sup> /L
<sup>3</sup> Sed Rate	29	25	18	0 – 15 mm/hr
WBC Count	14	14	13	4.8 – 10.8 x 10 <sup>9</sup> /L
Hematocrit	48	49	48	40 – 54%
Hemoglobin	17	17	17	14 – 18 g/dL
<b>Chemistry</b>				
Creatinine	3.1	3.5	3.0	0.6 – 1.2 mg/dL
BUN	27	26	26	5 – 25 mg/dL
C-Reactive Protein	45	40	21	0.5 mg/dL
Serum Amyloid-A	111	105	99	0.03 mg/dL
Serum Albumin	2.1	2.1	2.5	3.0 – 5.4 g/dL
<b>Additional Studies</b>				
<sup>4</sup> ANA	-	Negative	-	Autoimmune Marker
HLA-B27	-	Positive	-	Ankylosing Spondylitis
HLA-DR4	-	Negative	-	Sjorgens Syndrome
HLA-DR3	-	Negative	-	Rheumatoid Arthritis

<sup>1</sup>Adm = Admission, <sup>2</sup>Ref = Reference, <sup>3</sup>Sed = Sedimentation, <sup>4</sup>Anti-Nuclear Antibody

Chemistry tests revealed elevated acute phase proteins like SAA and CRP. Her ESR was also elevated due to liver synthesizes of fibrinogen in response to stress. Plasma fibrinogen facilitates erythrocyte aggregation; this increases blood viscosity and elevates ESR.<sup>19</sup>

Chemistry tests also indicated decreased serum albumin and increased creatinine. Because the patient's kidneys were damaged, large amounts of albumin protein escaped the blood and entered into the urine, this explains why the patient had

proteinuria. Decreased plasma albumin also provides an explanation for the patient's lower extremity edema. Low levels of plasma albumin would cause a change in blood osmotic pressure resulting in tissue edema. Her elevated creatinine and BUN both indicated kidney damage. Creatinine is a muscle waste product that is usually removed by kidney filtration. BUN is also a waste product, resulting from protein metabolism in the body, and is removed by kidney filtration. Because the patient had impaired glomeruli, there was a decrease in creatinine and BUN filtration causing an increase in plasma concentrations.

After blood and chemistry results were reviewed, scans were taken and indicated both kidney damage and the accumulation of leukocytes and amyloid fibrils in the kidneys, liver and enlarged spleen. Congo-red staining of the biopsied right kidney appeared apple-green under polarized light when viewed through a microscope, this confirmed the presence of amyloid deposits within the tissue. Staining with anti-AA antibodies to the biopsied tissue revealed the presence of secondary amyloid antigen providing a confirmation of secondary amyloidosis. Subsequent anti-nuclear antigen testing, used to screen for autoimmune disorders, was negative. However, human leukocyte antigen (HLA) testing was positive for HLA-B27.

HLA is an inherited gene complex present in all nucleated cells. This gene complex encodes for major histocompatibility complex cellular proteins, important components of the immune system. If HLA mutations occur, an autoimmune disease may result. Because of the strong association between HLA mutations and particular autoimmune diseases, the identification of an HLA mutation in this patient helped to establish the later diagnosis of ankylosing spondylitis.

## TREATMENT

With limited knowledge of the precise role of SAA, current therapies are tailored to the underlining condition that is responsible for the chronic overproduction of amyloid. The first principle of treatment is to reduce amyloid forming precursor proteins by better controlling the inflammation. The second principle is to support the organs most

effected by amyloid deposits.

In 1994, the National Amyloidosis Center reported that 1 in 3 patients suffering from chronic illness had developed secondary amyloidosis.<sup>7</sup> These illnesses included arthritis, Crohn's disease, ankylosing spondylitis, Familial Mediterranean Fever, chronic infections and certain types of cancer.<sup>7,19</sup> Treatments tailored to the individual disease included biologics like anti-TNF drugs, anti-IL-1 drugs, corticosteroids, colchicine and antibiotics.<sup>7,19</sup> With better management of the known underlining inflammatory conditions, fewer cases of secondary amyloidosis developed, and for those with the disease, prognosis was improved.

Unfortunately, not all patients with secondary amyloidosis have a diagnosed underlining inflammatory disease. For these patients, treatment is much more limited. Without reducing SAA, kidney failure is inevitable and prognosis is poor. Routine SAA blood tests are required to monitor plasma levels, as well as annual whole body SAP scans to monitor significant amyloid progression or regression.<sup>7,16</sup> For these patients, supporting the functions of the organs most affected is the goal.

## CONCLUSION

The immune system is indeed very sophisticated. Many of the mechanisms involved in an immune response remain unknown. Yet while the role of the immune system is critical to human survival, over activation can be destructive. Amyloidosis is an example of a disease where a normal response is elevated and sustained causing tissue and organ damage. Because the immune system is very complex, regulating any abnormality becomes extremely challenging. However, understanding the pathology of amyloidosis and the laboratory tests that provide the diagnosis, is critical for the start of treatment.

Fortunately for the patient, her stay at the hospital revealed an unknown autoimmune disease that was contributing to a heightened SAA response. The combination of having both irritable bowel disease and ankylosing spondylitis in this patient, resulted in sustained high level SAA production. After discharge, the patient was able to decrease and better manage her over active immune response using immunosup-

pressant medications.

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