Clinical Rheumatology (2020) 39:249–253
https://doi.org/10.1007/s10067-019-04765-1

ORIGINAL ARTICLE

Serum amyloid A as a biomarker in differentiating attacks of familial Mediterranean fever from acute febrile infections

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Received: 10 April 2019 / Revised: 22 August 2019 / Accepted: 26 August 2019 / Published online: 4 September 2019

Abstract

Objective To determine the capability of serum amyloid A (SAA) in differentiating attacks of familial Mediterranean fever (FMF) from acute febrile upper respiratory tract infections.

Method Children diagnosed with FMF during febrile attacks were recorded as the patient group. The control group consisted of children with febrile upper respiratory tract infections. Complete blood count, serum amyloid A (SAA), C-reactive protein (CRP), and erythrocyte sedimentation rate were recorded in both groups during febrile episodes.

Results The cohort consisted of 28 children with FMF attack and 28 previously healthy children with acute febrile infection. While CRP and SAA levels were elevated in both groups, elevations during FMF attacks were significantly higher in the FMF group than in the control group. Median CRP was 85 mg/L in the FMF attack group and was 36 mg/L in the control group ($p = 0.001$). Median SAA was 497.5 mg/L in the FMF attack group and was 131.5 mg/L in the control group ($p < 0.001$). Correlation analyses showed that SAA and CRP were positively correlated in the FMF attack group ($r = 0.446$, $p = 0.01$). The best cut-off value for SAA in differentiating FMF attack from an acute febrile infection was 111.5 mg/L (sensitivity 100%, specificity 65.1%, area under curve (AUC) = 0.78, confidence interval 0.66–0.90, $p < 0.001$).

Conclusion Serum amyloid A is a sensitive but not specific marker for demonstrating inflammation in FMF. SAA levels rise substantially in febrile upper respiratory tract infections.

Key Points
- *SAA levels rise substantially in febrile upper respiratory tract infections.*
- *SAA is a sensitive but not specific method for demonstrating inflammation.*
- *SAA cut-off value for discriminating FMF attacks from febrile infection is 111.5 mg/L (sensitivity 100%, specificity 65.1%).*

Keywords Acute phase reactants · Familial Mediterranean fever · Infection · Serum amyloid A

Introduction

Familial Mediterranean fever (FMF) is an autoinflammatory disorder characterized by recurrent, self-limited attacks of fever, polyserositis, and systemic inflammation. It is caused by mutations in the *MEFV* gene and is the most common autoinflammatory disease in a growing group of these disorders caused by monogenic defects. It has the highest incidence in the Mediterranean basin and Middle East, FMF is thus highly endemic in Turks, Arabs, Armenians, and Sephardic Jews [1, 2]. Systemic inflammation is reflected by high acute phase reactants (APR) including C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and serum amyloid A (SAA) [1–4].

Serum amyloid A is an apolipoprotein and one of the major acute phase proteins synthesized by the liver. It is markedly elevated during FMF attacks and normalizes within 1–2 weeks. SAA is a precursor of the amyloid fibrils that are

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deposited in tissues in AA amyloidosis, the most severe complication of untreated FMF and other rheumatologic diseases [1, 5–7]. Chronic elevation of SAA is necessary for the development of AA amyloidosis so, in some centers, SAA is monitored routinely. Even though SAA is regarded as a highly specific laboratory test for FMF by some physicians, SAA is also elevated in acute and chronic infections [8].

Objectives

The primary objective of this study was to characterize the sensitivity and specificity of SAA in FMF and to determine a cut-off level that may help distinguish an FMF attack from a febrile upper respiratory tract infection. The secondary objective was to see whether any correlation exists between SAA, CRP, and ESR levels during an FMF attack or febrile infection.

Patients and methods

This cross-sectional diagnostic test study was conducted by the Pediatric Rheumatology Department of Health Sciences University at the Kanuni Sultan Süleyman Research and Training Hospital, Istanbul, Turkey, between July 2017 and October 2018. Patients with diagnosis of FMF with a clinically diagnosed acute attack were sequentially recorded as patient group. All FMF patients fulfilled both Tel-Hashomer and the Turkish pediatric FMF criteria [9, 10]. Complete blood count (CBC), APR including SAA, CRP, and ESR were obtained during an attack period. All FMF patients were examined by a pediatric rheumatologist who confirmed whether there was evidence of infection, in some cases by ordering additional tests such as urinalysis or chest X-ray. An FMF attack was defined as fever and abdominal and/or chest pain lasting for at least 12 h. Blood samples were collected once between the 24th and 48th hours of an attack. The control group was comprised of previously healthy children who were admitted to pediatric emergency department for at least 48 h of fever with accompanying signs and symptoms of an upper respiratory tract infection. Blood samples were obtained to test for the same parameters mentioned above. All children in the control group were examined by the same pediatrician. Since FMF is highly prevalent in our country, children with prior signs and symptoms compatible with FMF or with a family history of FMF were not included in the control group. Children in both arms were reevaluated 1 week later to document that the FMF attack or febrile infection was a transient process. Laboratory parameters were studied at the in-house laboratory with the exception of SAA. SAA samples were stored at − 70 °C and were later tested at an affiliated laboratory. CRP was measured using an immunoturbidimetric test with the Roche Diagnostics Cobas 8000 (Roche Diagnostics GmbH, Mannheim, Germany). SAA was studied by nephelometric test with Siemens BN ProSpec (Siemens AG, Marburg, Germany). Normal values for CRP was less than 5.0 mg/L; for SAA, less than 6.4 mg/L; and for ESR, less than 15 mm/h.

Informed consent was taken from the legal guardians of the children. The study was approved by local ethics committee and was performed according to the tenets of the Declaration of Helsinki. The variables were investigated using visual (histograms, probability plots) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk's test) to determine whether or not they were normally distributed. Descriptive analyses are presented using proportions, medians, and minimum and maximum values as appropriate. The Mann-Whitney *U* test was used to compare non-normally distributed continuous data between two groups. Spearman rank correlation test was used for correlational analyses of the data. We used the receiver operating characteristic (ROC) curve to demonstrate the sensitivity and specificity of the SAA and its optimal cut-off values for predicting FMF attacks. A p value < 0.05 was considered significant and the confidence interval (CI) was 95%. Statistical analyses were performed using the SPSS software package for Windows (version 22.0; SPSS, Chicago, IL, USA).

Results

The cohort consisted of 28 children with FMF attacks and 28 previously healthy children with acute febrile upper respiratory tract infection. In the FMF attack group, all patients had biallelic mutations in the *MEFV* gene; 26 patients were homozygous for the M694 V mutation and 2 patients were homozygous for the M680I mutation. Median age at the emergence of FMF symptoms was 1.25 (0.3–7.0) years and median age at the time of diagnosis was 2.75 (0.6–8.0) years. All FMF patients were treated with colchicine. Age and gender distribution at the time of enrollment in the study did not differ significantly between the groups. The median age of FMF patients and control patients were 4 (2–11) years and 4 (2–15) years, respectively. The cohort consisted of 18 boys and 10 girls in the FMF group and 20 boys and 8 girls in the control group. Clinical features in FMF attacks were fever (100%), abdominal pain (96.4%), chest pain (42.8%), and arthritis (25.0%). None of the patients had abdominal pain in the control group.

Laboratory parameters of both groups are shown in Table 1. Leukocyte count with differential and ESR did not differ in between the groups and both groups showed minimal leukocytosis with neutrophilia and mildly elevated ESR. While CRP and SAA levels were elevated in both groups, both parameters had significantly higher elevation in the FMF attack group. Median CRP was 85 mg/L (27.9–371.8)

Table 1 Laboratory parameters of the patient and the control groups

Parameter	FMF attack group *n*, 28 Median (min-max)	Control group *n*, 28 Median (min-max)	*p* value
Leukocyte (mm^3)	10,295 (5470–24,500)	12,050 (4800–23,800)	0.245
Absolute neutrophil percentage (%)	56 (30–81)	63 (29–88)	0.117
Absolute lymphocyte percentage (%)	31 (10–60)	23 (5–61)	0.072
Erythrocyte sedimentation rate (mm/h)	36 (9–78)	34 (9–74)	0.737
C-reactive protein (mg/L)	85.0 (27.9–371.8)	36.0 (1.3–200.2)	0.001
Serum amyloid A (mg/L)	497.5 (113–899)	131.5 (10.1–783)	< 0.001

in the FMF attack group and 36 mg/L (1.3–200.2) in the control group ($p = 0.001$). Median SAA was 497.5 mg/L (113–889) in the FMF attack group and 131.5 mg/L (10.1–783) in the control group ($p < 0.001$). SAA levels were above normal range in all cases in both arms, CRP levels were normal in 2 cases (3.6%), both in the control group. ESR was normal in 5 cases (8.9%), 3 were in the FMF attack group, and 2 were in the control group.

Correlation analyses demonstrated positive correlation between SAA and CRP levels in the FMF attack group ($r = 0.446$, $p = 0.01$). ESR did not correlate with SAA or with CRP levels. SAA and CRP showed increased positive correlation in the control group ($r = 0.649$, $p < 0.001$). Also, ESR was positively correlated both with CRP ($r = 0.72$, $p < 0.001$) and SAA ($r = 0.596$, $p = 0.001$) in the control group.

The capacity of SAA to discriminate FMF attacks from febrile infection was analyzed using ROC curve. The best cut-off value for SAA level was 111.5 mg/L (Fig. 1) (sensitivity 100%, specificity 65.1%, area under curve (AUC) = 0.78, CI 0.66–0.90, $p < 0.001$).

Discussion

We demonstrate that SAA is a sensitive, but not specific marker of inflammation in FMF patients. SAA levels were increased in 100%, CRP in 96.4%, and ESR in 91.1% of the total cohort. The cut-off value for discriminating FMF attacks from febrile infection was 111.5 mg/L with a sensitivity of 100% and specificity of 65.1%. CRP and SAA levels were increased in all FMF patients and they correlated moderately with each other but not with ESR during FMF attacks. In children without a definitive diagnosis of FMF, though with symptoms consistent with an FMF attack, measuring either CRP or SAA levels can be a useful part of the diagnostic evaluation provided that there is no evidence of infection.

Familial Mediterranean fever is a disease characterized by self-limited attacks of inflammation. It is known that FMF patients with exon 10 mutations have the most severe phenotype, especially those homozygous for the M694 V mutations. These patients frequently present with FMF at a young age, have more frequent attacks, and require higher doses of colchicine [1, 2, 11]. Özdel et al. compared the clinical and genetic features of pediatric FMF patients with late onset (> 8 years old at the first symptoms of FMF) and earlier onset (≤ 8 years old at the first symptoms of FMF) disease. They demonstrated that fever and M694 V homozygosity were less frequently detected in late onset FMF group [11]. In our cohort, all FMF patients had homozygous exon 10 mutations and median age at the first symptoms compatible with FMF was 1.25 years, further evidence that FMF patients with homozygous or compound heterozygous mutations in exon 10 present at much younger ages.

The most severe complication of FMF is the development of AA amyloidosis [5, 6]. It was once thought that FMF patients must have frequent attacks for years before the development of amyloidosis. However, it has been established more recently that patients with pathogenic mutations in the *MEFV* gene may develop amyloidosis even without FMF symptoms. This group of patients is referred to as phenotype II. FMF patients treated with colchicine to prevent attacks who continue to have elevated acute phase reactants due to subclinical inflammation continue to be at risk of amyloidosis. Many studies have thus aimed to find a biomarker to assess risk of amyloidosis development in FMF. SAA is the most studied biomarker [7, 12–14]. The main problem with SAA is that like all acute phase proteins, its level increases rapidly in infectious and sterile insults that are leading to inflammation.

We are aware of one prior study that compared SAA, CRP, and ESR in FMF patients with attack (36 patients) and children with acute infection (20 patients). The median values of three parameters were above normal ranges in both groups with mean SAA level of 720.9 mg/L in the FMF attack group and 862.9 mg/L in the acute infection group [15]. Korkmaz et al. measured CRP and ESR in 49 FMF attack patients and in 39 control group patients comprised of 29 juvenile idiopathic arthritis patients and 10 patients with infection. Mean CRP level in the FMF attack group was 139 mg/L and 118 mg/L in the control group and mean ESR level was 52 mm/h in the FMF attack group and 75 mm/h in the control group. They concluded that a marked acute phase response was present during FMF attacks that was comparable with the control

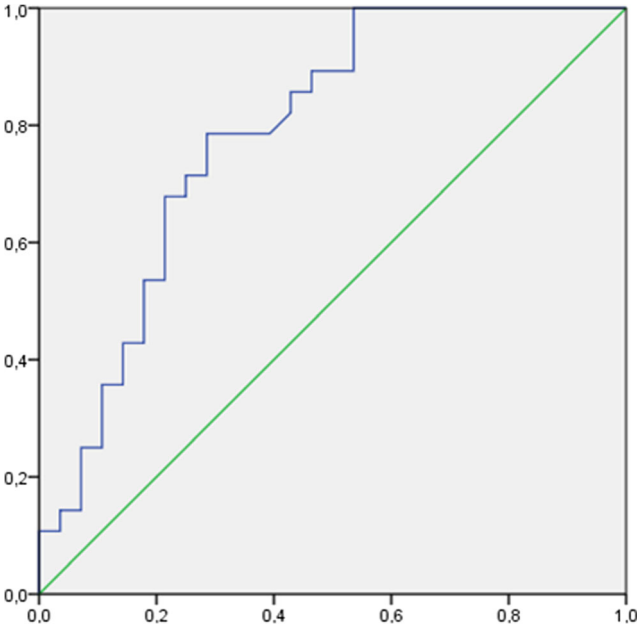

Fig. 1 Receiver operating characteristic curve of serum amyloid A. The largest AUC (area under the curve) was demonstrated for serum amyloid A (AUC = 0.768, $p < 0.0001$), which at the optimal cut off value determined using the Youden index at the level of 111.5 mg/L for SAA has a sensitivity of 100% and a specificity of 65.1%

group [16]. High median SAA, CRP, and ESR levels in both FMF attack group and the control group in our study once again confirms the paradigm that an acute phase response is universal, independent of the stimulus.

Another objective of this study was to see whether there was any correlation of APR in the FMF attack group and the control group. Yalçınkaya et al. compared APR (SAA, CRP, ESR) in FMF patients during attacks and during healthy periods and demonstrated that all APRs were increased during attacks with the following median levels: SAA 720.9 mg/L, CRP 45.5 mg/L, and ESR 41.5 mm/h. They found that serum SAA and CRP showed a positive correlation ($r = 0.557$) only during the attack-free period. The coefficient was less than 0.5 for other parameters during attacks and attack-free periods [15]. Yüksel et al. measured APR levels in 21 FMF patients during attacks and reported median CRP of 45.6 mg/L and ESR of 35 mm/h. The main objective of their study was to evaluate procalcitonin levels in FMF attack and attack-free periods. They concluded that procalcitonin levels were not affected by inflammation and that this could not be used as a marker for attack in FMF [17]. In our study, we report that SAA and CRP are positively correlated in the FMF attack group but that ESR was not correlated with either marker. There were no patients with high ESR and normal CRP or SAA in the FMF attack group. As ESR remains persistently elevated in chronic inflammatory conditions it is of little value of quantifying inflammation during FMF attacks.

To the best of our knowledge, this is one of the first studies that assessed the sensitivity and specificity of SAA to differentiate FMF attacks from febrile infection. We have specifically chosen FMF patients with exon 10 homozygous mutations and children with febrile upper respiratory tract infection to create relatively homogenous groups. The major limitation of this study is the small sample size of both groups. Additional studies with greater numbers of both patients and controls are required to determine whether SAA provides additional value compared to CRP in attack periods of suspected FMF patients or other autoinflammatory diseases. We conclude that SAA is sensitive but not specific in demonstrating inflammation in FMF. SAA levels increase both in FMF attacks and febrile infections of childhood. While contemporary medicine continues to offer an increasing array of diagnostic tests physical examination, careful history taking and clinical judgment continue to be the sine qua non in medicine.

Acknowledgments The authors would like to thank Dr. Raif S. Geha and Craig D. Platt for language editing and critical review of the manuscript.

Compliance with ethical standards

Disclosures None.

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