

Role of A-SAA in monitoring subclinical inflammation and in colchicine dosage in familial Mediterranean fever

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Abstract

Objectives

1) To compare the sensitivity of serum amyloid A protein (A-SAA) and other acute phase proteins (APPs) in determining subclinical inflammation in patients with familial Mediterranean fever (FMF) during an attack-free period; 2) to define those clinical, laboratory features that may modify the A-SAA level; and 3) to evaluate the effect of an increase in the colchicine dose on the A-SAA level.

Methods

A-SAA, CRP, ESR, fibrinogen and ferritin levels were measured in 183 patients [88 F, 95 M; median age 11.0 years (1.0-20.0)] with FMF during an attack-free period. Mutational analysis was available in 157 patients. The colchicine dose was increased in 26 randomly chosen patients with no attacks within the last year; laboratory studies were repeated at the end of the second month.

Results

During an attack-free period, the median A-SAA level was 74 (6-1,500) mg/L; other APPs were within normal ranges in 49-93% of the patients. Age, gender, age at onset, age at diagnosis, the duration of treatment and the frequency of attacks had no significant effect on the A-SAA level. Homozygous and compound heterozygous patients had higher A-SAA levels than heterozygous patients [129 mg/L (8-1,500) versus 29 mg/L (6-216); $p < 0.005$]. There was a dramatic decrease in the A-SAA level [from 244 mg/L (16-1,400) to 35.5 mg/L (8-1,120); $p < 0.001$] and an increase in the hemoglobin (1.89 ± 0.10 mmol/L to 1.98 ± 0.19 mmol/L; $p < 0.005$) after the increase in colchicine dose in 26 patients.

Conclusion

Subclinical inflammation continues during an attack-free period in FMF patients. A-SAA was the best marker of subclinical inflammation. Patients who are homozygous or compound heterozygotes of MEFV mutations had higher A-SAA levels. An increase in the colchicine dose resulted in a dramatic decrease in A-SAA and an increase in hemoglobin level. These findings favor the use of A-SAA in drug monitoring.

Key words

Familial Mediterranean fever, serum amyloid A protein, C-reactive protein, erythrocyte sedimentation rate, acute phase proteins, subclinical inflammation, colchicine.

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Introduction

Familial Mediterranean fever (FMF) is an autosomal recessive disorder characterized by recurrent episodes of fever and serosal inflammation manifested by sterile peritonitis, arthritis, and pleurisy. The disease primarily affects Turkish, North African Arab, Jewish and Armenian populations. The diagnosis is based on clinical criteria (1). In 1997 two independent groups (the French FMF Consortium and the International FMF Consortium) have identified the gene associated with FMF, located on chromosome 16 (16p13.3) (2, 3). Although FMF is a periodic disease, i.e. patients are symptom-free between the attacks, recent data have suggested that a continuous subclinical inflammation might nevertheless be present. In a recent study by Tunca *et al.* CRP and SAA levels were higher in patients with FMF and their healthy first degree relatives (parents, children and siblings) than in controls (4).

Amyloidosis of the AA type, mainly renal, is the major complication of FMF (5). Colchicine not only reduces the frequency and severity of FMF attacks, but also prevents amyloidosis (6, 7). There are unexplained ethnic differences in the rates of amyloidosis (5, 8-10). Genotype-phenotype correlation is not well established. Although early data suggested that M694V mutation was a risk factor for amyloidosis, there are also patients with FMF who have renal amyloidosis and do not carry the M694V homozygous genotype (11-14). Un-identified genetic and environmental factors might have a protective or deteriorating effect on renal amyloidosis.

The serum amyloid A (SAA) protein family is known to contain a number of differentially expressed apolipoproteins which are synthesised primarily by the liver. A-SAA is a major acute phase protein (APP) which is highly conserved in evolution. Its concentration can increase to more than 1000-fold during inflammation (15). Its sensitivity was shown to be equal to or more than that of C-reactive protein (CRP) in several studies; persistently high A-SAA levels were correlated with rheumatic disease activity, the

rapid progression of secondary amyloidosis and a poor outcome in patients undergoing certain surgical interventions (16, 17). Amyloid A protein is the principal component of secondary amyloid plaques.

The aim of this study was: 1) to compare the sensitivity of A-SAA and other acute phase proteins in determining subclinical inflammation in patients with FMF; 2) to define clinical, laboratory features modifying A-SAA level; and 3) to evaluate the effect of an increase in the colchicine dose on the A-SAA level.

Patients and methods

In this cross-sectional study we evaluated 194 patients with FMF (Fig. 1). The diagnosis of FMF was established according to previously described criteria (1). Eleven patients were excluded due to parental refusal in 4 patients, the presence of another disease in 7 patients (vesico-ureteral reflux in 3 patients, nephrolithiasis in 2 patients, thalassemia major in 1 patient, and chronic hepatitis B infection in 1 patient). The clinical data were recorded on a standardised form that included age, gender, familial consanguinity, history of the disease, age at onset of symptom(s), presence of fever, abdominal pain, pleurisy, joint pain, colchicine dose (patients were divided into three groups: 0.014 - 0.025 mg/kg, 0.025 - 0.050 mg/kg and > 0.050 mg/kg) daily dose of colchicine ranged between 0.5-2.0 mg/day, and the duration and frequency of attacks before and after the use of colchicine. The blood counts, erythrocyte sedimentation rate (ESR, normal < 20 mm/hour), CRP (normal: 0-8,000 mg/L), fibrinogen (normal 1.4-4.3 g/L), ferritin (normal: 7-140mg/L), and A-SAA (normal: <10 mg/L) levels during an attack-free period were measured.

Any patient with a disease other than FMF or who had experienced an FMF attack, infection or physical trauma within the last 14 days was excluded. Ten healthy volunteers constituted the control group.

Mutation analysis

Our procedure for mutation analysis

consists of two steps. The hot spot exon 10, which harbours 14 mutations, is first analysed by denaturing gradient gel electrophoresis (DGGE). According to the band pattern, subsequent analysis is carried out either by restriction endonuclease enzyme digestion or sequencing. Furthermore, E148Q in exon 2 is analysed by restriction endonuclease enzyme digestion. Mutation analysis was performed in 157 patients.

Effect of colchicine dose

There were 80 patients who were symptom-free for 1 year, but with high A-SAA levels (Fig. 1). The dose was increased by 0.5 mg/day in 26 randomly chosen, symptom-free patients (maximum dose of colchicine was 2.0 mg/day). A-SAA, CRP, ESR, ferritin and fibrinogen levels were re-measured two months after the increase in colchicine dose. Informed consent was obtained from the parents.

A-SAA levels were measured by a solid phase sandwich ELISA method (Biosource International Cytoscreen, Human SAA Immunoassay Kit).

Statistical analysis

Student's t-test, the Mann-Whitney U-test or Kruskal Wallis (with Bonferroni correction) tests were used to compare the means between independent groups. The paired sample t-test and Wilcoxon matched pairs test were used to analyse the effect of an increase in colchicine dose. Correlation analysis was done by Spearman non-parametric correlation analysis. A p value < 0.05 was considered to indicate statistical significance. Data were expressed as the medians (minimum – maximum) if unequally distributed; and as the means \pm SD (standard deviation) if equally distributed.

Results

Demographic characteristics

183 FMF patients [88 female, 95 male; median age 11.0 years (1.0-20.0)] were enrolled in the study. The median age of the patients at the onset of symptoms was 3.5 years (6 month-18 years). The median age at diagnosis was 7 years (range: 1-18 years). Eighty patients

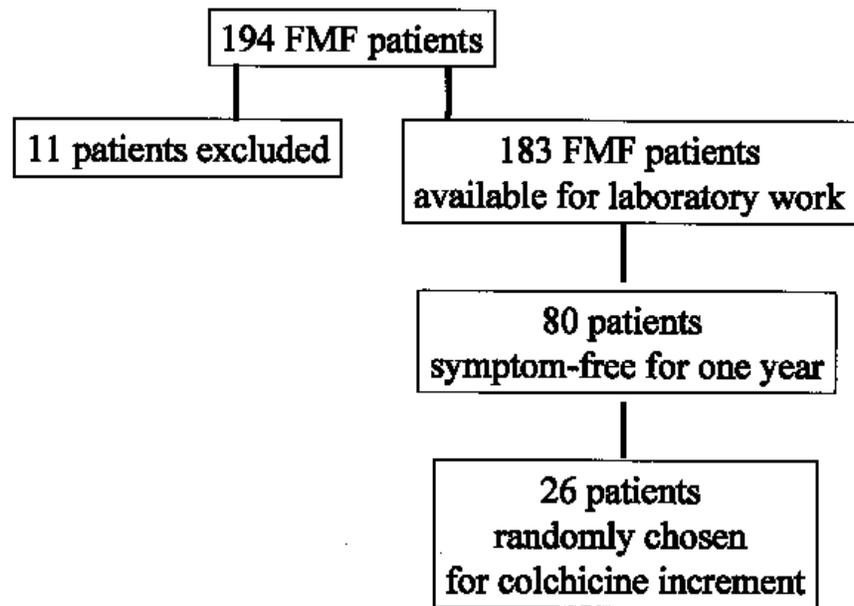


Fig. 1. Flow chart of the patients studied.

(43.7%) had a positive family history for FMF and 45 patients (24.6%) had first degree consanguinity. Fever (93%), abdominal pain (95%), arthritis/arthritis (80%) and chest pain (46%) were the leading symptoms during the attacks. The frequency of attacks at diagnosis was < 1 per month, 1-2 per month and > 2 per month in 48.1% (n=88), 32.2% (n=59), and 19.7% (n=36) patients, respectively. After the onset of colchicine therapy 80 patients had no symptoms within the last 12 months, 57 patients had less than one episode per month, and 17 patients had more than 1 episode per month. Eleven patients (6%) with a typical history of FMF had renal amyloidosis at the time of diagnosis.

MEFV mutation analysis

The distribution of MEFV mutation is shown in Table I. M694V and M680I compromised 76.5% of the alleles. Seventy-seven (49%) patients were homozygous, 42 (27%) patients were compound heterozygous, 20 (13%) patients were heterozygous; and 18 patients (11%) were (-/-) for M694V, M680I, V726A, M694I and E148Q mutations.

Laboratory values

Values at the time of entry into the study were as follows: hemoglobin

1.94 (1.35-2.56) mmol/L, hematocrit 37.3% (26.7-48.1), WBC 7.8 (3.6-17.9) $\times 10^9/L$, platelet 296 (128-675) $\times 10^9/L$, ESR 20 (3-66) mm/hour, CRP 2,700 (100-197,800) mg/L, fibrinogen 3.17 (1.81-6.24) g/L, and ferritin 27.1(3.3-291.0) mg/L. ESR, CRP, fibrinogen and ferritin levels were within normal ranges in 49.2% (n=90); 62.3% (114); 85.3% (156) and 93.0% (n=170) patients respectively. A-SAA levels were < 10 mg/L in 10 volunteers. The median A-SAA level in FMF patients was 74 (6-1,500) mg/L.

Factors affecting the A-SAA level

Age, gender, age of onset, age of diagnosis, duration of treatment, frequency of attacks had no significant effect on A-SAA level. Homozygous and compound heterozygous patients had higher A-SAA levels than the heterozygous patients [129 mg/L (8-1,500) vs 29 mg/L (6-216) respectively, (p < 0.005)] (Table II, Fig. 2A). The A-SAA level was lower in patients on colchicine therapy than in those without treatment [58 mg/L (6-1,500) vs 245 mg/L (24-1,400), p < 0.005] (Table II, Fig. 2B). Interestingly, the colchicine dose (in mg/kg) had no statistically significant effect on the A-SAA level, even in 80 patients who were symptom-free for the last 12 months (Table II, Fig. 2C).

A-SAA was also higher in patients with amyloidosis [196 mg/L (22-1,500) vs 74 mg/L (6-1,500); $p > 0.05$]. There was no correlation of acute phase proteins other than A-SAA with homozygosity.

Spearman correlation analysis showed that the correlation coefficient between A-SAA and CRP was 0.541 ($p=0.01$). The coefficient was less than 0.5 for fibrinogen ($r=0.478$; $p=0.01$), ESR ($r=0.355$; $p=0.01$) and ferritin ($r=0.274$;

$p=0.01$).

Effect of an increase in colchicine dose. Acute phase proteins were measured 2 months after an increase in the colchicine dose in 26 patients who were symptom-free for the last 12 months. The daily dose of colchicine before the increase was 1.0 mg in 18 patients and 1.5 mg in 8 patients. Before the increase in colchicine dose the ESR, CRP, fibrinogen and ferritin levels were normal in 53.8% ($n=14$), 65.4% ($n=17$), 65.4% ($n=17$) and 100% ($n=26$) of patients respectively. The complete blood count, ESR, CRP, ferritin, fibrinogen and A-SAA levels before and after dose incrementation are summarised in Table III. The hemoglobin level increased from 1.89 ± 0.10 mmol/L to 1.98 ± 0.19 mmol/L ($p<0.005$). The A-SAA level decreased from 244(16-1,400) mg/L to 35.5 (8-1.120) mg/L ($p<0.001$). ESR, CRP and ferritin levels also decreased significantly.

Table I. MEFV mutation frequency and genotypes in patients with FMF (157 patients, 314 alleles).

		n	%
Mutation frequency	M694V	199	63.4
	M680I	41	13.1
	V726A	13	4.1
	E148Q	4	1.3
	M694I	1	0.3
	Unidentified allele	56	17.8
Total		314	100.0
Genotypes			
Homozygous		77	49.0
	M694V / M694V	73	46.5
	M680I / M680I	3	1.9
	E148Q / E148Q	1	0.6
Compound heterozygous		42	26.8
	M694V / M680I	30	19.1
	M694V / V726A	10	6.4
	M694V / M694I	1	0.6
	V726A / E148Q	1	0.6
Heterozygous		20	12.7
	M694V / unidentified	12	7.6
	M680I / unidentified	5	3.2
	V726A / unidentified	2	1.3
	E148Q / unidentified	1	0.6
Unidentified / unidentified (-/-)	18	11.5	
Total		157	100.0

Table II. Effects of MEFV mutation and colchicine treatment on A-SAA level.

Characteristics	n	A- SAA level mg/L [median (min-max)]	p	
MEFV mutation	(+/+)	119	129 (8-1,500)	< 0.005
	(+/-)	20	29 (6-216)	
	(-/-)	18	36.5 (10-1,000)	
	Unidentified	26	184 (12-1,080)	
Colchicine treatment	On therapy	154	58 (6-1,500)	< 0.005
	Without therapy	29	245 (24-1,400)	
Colchicine dose (154 patients)	0.014 - 0.025 mg/kg	38	65.5 (16-1,240)	> 0.05
	0.026 - 0.050 mg/kg	83	58 (6-1,200)	
	> 0.050 mg/kg	31	49.5 (8-1,000)	
Patients with no symptoms within the last 12 months (80 patients)	0.014 - 0.025 mg/kg	24	68 (8-1,000)	> 0.05
	0.026 - 0.050 mg/kg	39	57 (11-1,000)	
	> 0.050 mg/kg	17	53 (6-1,100)	

Discussion

In our study M694V, M680I and V726A were the major mutations (80.6%). Since the diagnosis was based on clinical criteria, heterozygous patients and patients who had no mutations were also included. These patients were thought to probably have other mutations. Genotype-phenotype relation is not well established. Dewalle *et al.* had shown that the M694V homozygous genotype was associated with a more severe form of the disease, i.e. earlier age at onset, higher prevalence of pleurisy, higher frequency of arthritis and a higher frequency of amyloidosis in patients who did not have regular colchicine therapy (11). It was later shown that not all patients with amyloidosis carried the M694V mutation (12-14).

The homozygous and compound heterozygous groups had higher A-SAA levels than the heterozygous (+/-) patients and those with unidentified mutations (-/-). Among these patients the number who carried the non-M694V allele was too small to conduct a statistical analysis. Whether the higher level of A-SAA is attributable to M694V mutation or to other environ-

mental and/or genetic factors merits further studies. It might also be speculated that the unidentified mutations cause milder phenotypes or that some of the heterozygotes were over-diagnosed. Recently Cazeneuve *et al.* studied 137 Armenian patients living in Armenia (18). They showed that the SAA1 / genotype was associated with a 7-fold increased risk for renal amyloidosis. Whether this observation is reproducible in other geographic areas and whether the SAA1, SAA2 genotypes affect the A-SAA level deserve further studies.

In our series almost 50% of the patients (80/183) had not experienced an attack within the last 12 months. We have shown that ESR, CRP, fibrinogen and ferritin levels during an attack-free period were within normal ranges in 49.2% (n=90); 62.3% (n=114); 85.3% (n=156) and 93.0% (n=170) of patients respectively. In other words, the sensitivity of ESR, CRP, fibrinogen and ferritin in detecting subclinical inflammation was only 51%, 38%, 15% and 7% respectively. In a recent study by Korkmaz *et al.*, ESR, CRP, fibrinogen and ferritin levels during an attack-free period were above normal in 52% (22/42), 34% (34/44), 4% (1/24) and 0% (0/25) of patients (19). The percentages are very similar to those of our study. The A-SAA level was not measured by Korkmaz *et al.* In our study, on the other hand, A-SAA was increased up to 150 times the normal level [74 (6-1,500) mg/L]; it was above normal in > 95% of patients.

We have investigated whether we could identify an ideal colchicine dose (mg/kg/day) for a better control of subclinical inflammation. The patients were divided into three groups: 0.014-0.025 mg/kg, 0.025- 0.050 mg/kg and >0.050 mg/kg. A-SAA levels were not significantly different between groups. Individual factors might affect the serum level of the drug. It is hypothesised that the P-glycoprotein pump determines the level of colchicine within the granulocytes (20). Since we were not able to measure serum colchicine levels, one should not interpret this data as evidence to recommend the minimal colchicine dose at which clinical remis-

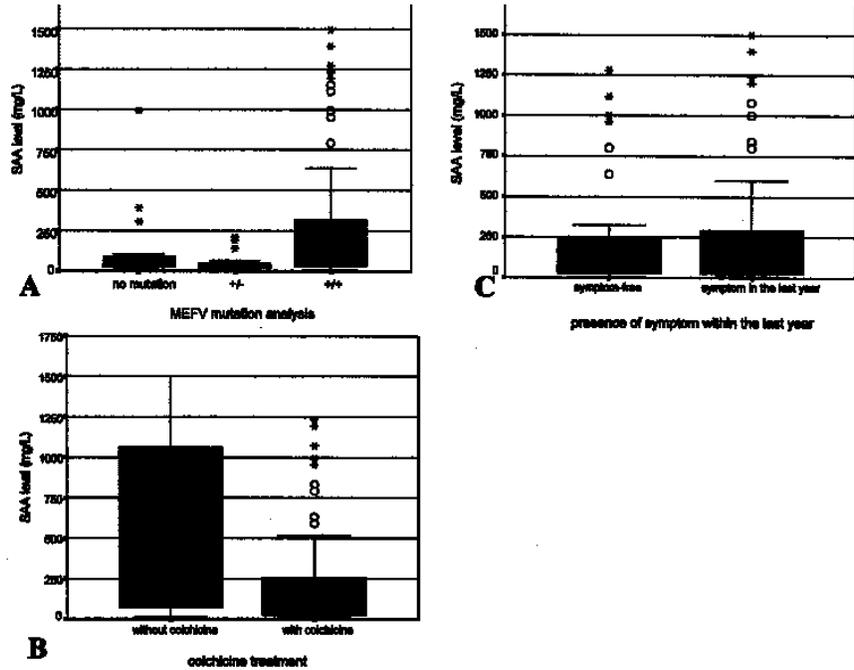


Fig. 2. A-SAA levels according to (A) MEFV mutation analysis; (B) colchicine treatment; and (C) the presence of symptoms within the last year. The lower and upper margins of the boxes present the 25th and 50th percentiles, the thick bars present the median. Outliers and extremes are indicated by circles and asterisks.

Table III. Effects of increase in colchicine dose on laboratory values in 26 randomly chosen patients who were symptom free for the last 12 months.

Laboratory	Before	After	P
Hemoglobin (mmol/L)	1.89 ± 0.10	1.98 ± 0.19	< 0.005*
Hematocrit (%)	36.9 (34.0-41.5)	38.6 (30.1-44.0)	< 0.05**
WBC (x10 ⁹ /L)	8.0 ± 2.6	8.1 ± 1.9	>0.05*
Platelet (x10 ⁹ /L)	288 (198-516)	288 (189-379)	> 0.05**
ESR (mm/hour)	30(10-60)	17 (3-54)	< 0.001**
CRP (µg/L)	12,200 (1,000-39,700)	1,000 (1,000-51,000)	< 0.005**
Fibrinogen (g/L)	3.47 ± 0.76	3.32 ± 0.80	> 0.10*
Ferritin (µg/L)	46.4 ± 23.4	25.7 ± 18.8	< 0.05*
A-SAA (mg/L)	244 (16-1,400)	35.5 (8-1,120)	< 0.001**

Data were expressed as medians (minimum – maximum) if unequally distributed and as means ± SD if equally distributed.

*Paired sample t test; **Wilcoxon matched pairs tests.

sion (cessation of attacks) is achieved. We increased the colchicine dose in symptom-free patients, but who had ongoing inflammation (increased A-SAA). A-SAA levels decreased significantly in all patients. Interestingly other APPs also decreased significantly. But their 'pre-dose increment levels' were within the normal range in 53-100% of patients, which confirms the limitation of these proteins in determining subclinical inflammation. An

early outcome of better control of subclinical inflammation was a significant increase in the hemoglobin level within 2 months. Thus a decrease in A-SAA level has clinical effects within even a short period.

Subclinical inflammation might have numerous side effects, especially in children, including loss of appetite, and impairment of physical and mental development. Zemer *et al.* analysed the compulsory medical examination of

patients prior to military service at age 17. They showed that the mean height of the colchicine-treated cohort with FMF had increased compared to the cohort of FMF patients who had not received colchicine (7).

Compliance is not always optimum. Some patients use less than the recommended dose of colchicine once the frequency and severity of attacks subsides, and a few may even stop therapy. A-SAA levels can also be used to detect patients with poor compliance. In conclusion, the elevated A-SAA levels seen during attack-free periods suggested that subclinical inflammation can be continuous. A-SAA was the best marker of subclinical inflammation in FMF patients. Patients who are homozygous or compound heterozygotes of MEFV mutations had higher A-SAA levels. An increase in the colchicine dose resulted in a dramatic decrease in the A-SAA level and an increase in hemoglobin, thus A-SAA may also be used in drug monitoring. To determine the long-term effects (growth, intellectual functions, risk for cardiovascular disease, etc.) of a better control of subclinical inflammation, larger epidemiological and longitudinal studies are warranted.

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