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Performance of serum Amyloid A and C-reactive protein for disease control assessment in Familial Mediterranean Fever

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Data statement

The data that support the findings of this study are available from the corresponding author, IE, upon reasonable request.

Conflict of interest

The authors have no conflict of interest to disclose.

Clinical implications

Neither C-reactive protein (CPR) nor Serum Amyloid A (SAA) taken alone are good biomarkers to reflect past disease activity. CRP and SAA measurements may not always be necessary in FMF but can be useful for patients without clinically active disease or challenging control assessment.

Keywords: Biomarkers, Autoinflammatory diseases, Familial Mediterranean Fever, C-reactive protein, Serum amyloid A

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Familial Mediterranean Fever (FMF) is a monogenic auto-inflammatory disease responsible for periodic inflammatory flares. The most critical complication of FMF is AA Amyloidosis (1). It is caused by the deposition of serum Amyloid (SAA) proteins in the organs due to repeated or chronic high levels of SAA (2). European recommendations for the treatment of FMF suggests that C-reactive proteins (CRP) and SAA levels should be monitored (2,3). The assessment of disease control relies on both inflammatory markers and declared number of flares, which can be subjective. Since SAA is directly implicated in the pathogenesis of AA amyloidosis, it was suggested that it could be a better marker for disease control and risk of AA amyloidosis than CRP (4). While the use of CRP measure is cheap and wildly available, the measurement of SAA is more expensive and restricted to fewer medical centers. In this study, we aimed to evaluate the accuracy of SAA for reflecting disease activity in FMF and to compare it to CRP measurement.

We conducted a multicentric retrospective observational study using the JIR Cohort database (http://www.fondationres.org/fr/jircohorte - NTC02377245) in Versailles hospital and Lyon pediatric centers for auto-inflammatory diseases in France. All FMF patients <18 years-old with ≥1 concomitant measurement of CRP and SAA recorded in the database between 2009 and 2022 were included. For each visit, age, clinical data, CRP levels, SAA levels, number of inflammatory attacks 6 months before the visit and treatments were extracted. For each patient, genotype and sex were extracted. Visits during inflammatory attacks as defined by the physician were excluded. CRP and SAA levels were considered positive if >5mg/L and 10mg/L, respectively. Disease was considered active if ≥2 inflammatory attacks were recorded in the 6 previous months (5).

Numeric variables were expressed as median [range] and discrete outcomes as absolute and relative (%) frequencies. Groups were compared with unpaired Student t-test, or Mann-Whitney U test (continuous variables) and with chi-squared or Fisher's exact test (discrete variables). The alpha risk was set to 5%. ROC curves were performed to compare test performance of CRP and SAA to reflect the absence of disease activity defined as < 2 attacks in the last 6 months.

438 out-of-flare visits for 117 patients with FMF were identified (table 1). Diagnostic performances of CRP and SAA were poor to reflect active disease in the 6 previous months (AUC=0.67). There was no difference between CRP and SAA (p=0.9, figure 1). The positive predictive value for SAA>10mg/L and CRP>5mg/L were 73.6% and 69.9%, respectively.

Dissociated positive SAA levels (negative CRP, positive SAA) were encountered in 32 visits from 17 patients. There was no difference in sex, age or genotype between patients with at least one positive dissociated SAA and those without. In those patients, dissociated positive SAA visits amounted to 11 to 75% of total visits. In these visits, SAA was between 10 and 20mg/L in 68% of the visits. Active disease

in the last 6 months was significantly more likely in this group (52% vs 26%, p=0.012). There was no difference when SAA was between 10 and 20mg/L (0 vs 1.7 attacks/6 months, p=0.07). Positive predictive value for uncontrolled disease was 100% when SAA was >20mg/L.

Among the 229 visits with <2 attacks/6months, dissociated positive SAA occurred in 11 visits (5%), dissociated positive CRP in 25 visits (11%) and both SAA and CRP were positive in 32 visits (14%).

Interestingly, the use of interleukin-1 blockers was significantly more frequent in dissociated visits (31% vs 4%, p<0.01). However, 9 of the 10 visits occurred in the same highly treatment-resistant patient. It is therefore possible that this result is specific to the patient rather than to the group as a whole.

SAA is thought to be a better marker of subclinical inflammation than CRP because it is more frequently elevated when measured out-of-flare (6,7). Moreover, positive dissociated SAA is more frequent than positive dissociated CRP (7). We found that dissociated positive SAA is associated to a higher number of inflammatory flares in the 6 months preceding the visit. In this study, we have used a cutoff for SAA at 10mg/L as recommended by EULAR (2). This cutoff was adapted from a 2007 study showing that in FMF-related AA amyloidosis, amyloid deposits regressed in 60% of patients who maintained SAA levels <10mg/L (8). From this observation and given the potential severity of AA amyloidosis, it was used as a target of treatment. In our study, disease control was not different in visits with mildly dissociated positive SAA (SAA<20mg/L) compared to those with strictly negative CRP and SAA. These results suggest that SAA levels< 20mg/L could be associated with disease control.

There is no international consensus on the clinical definition of disease control. European recommendations state that the goal of treatment should be to prevent clinical flares. French guidelines define an uncontrolled disease as having ≥3 inflammatory flares/year . Therefore, we have used the definition of 2 flares/6 months. We found that there is an association between SAA levels and the number of flares >2/6 months, supporting its relevance.

Since the clinical criteria for uncontrolled disease is sufficient to state undertreatment, the systematic measurement of SAA is questioned. The monitoring of several inflammatory biomarkers could be used to define disease activity when patients are clinically inactive. In our study, when the disease was clinically controlled, CRP and/or SAA levels were elevated in 14% of visits, which supports the necessity to use both biomarkers to detect all patients with subclinical inflammation. The indication for treatment escalation in clinically inactive patients with isolated elevated SAA or CRP levels must be evaluated. It was suggested that SAA levels could be higher in case of non-compliance than of colchicine resistance, regardless of CRP levels (6,10). Our study suggests that SAA could also be a better biomarker than CRP in patients for which clinical control of the disease is difficult to assess (young children,

atypical clinical presentations) (Supplementary Fig.1). A cut-off of 20 mg/L seems to be in our study a relevant threshold for deciding about disease control.

In French guidelines, it is proposed to measure CRP levels during follow-up if there was no CRP/SAA dissociation at diagnosis (5). However, we found that CRP/SAA dissociation can occur at any time during follow-up. Therefore, we believe that there remains a place for repeated determination of SAA and CRP during follow-up of FMF.

This study has several limitations due to its retrospective methodology. First, the number of flares registered into the database relied on patients and their parents' memory, which could have been faulty (memory bias). Furthermore, the exact definition of inflammatory flares may not be consensual between physicians, and between physicians and patients. While the febrile episodes of arthritis, abdominal or chest pain may be obvious, the case of joint pain, myalgia and fatigue could account to discrepancies in the flare count. Finally, factors that could interfere with CRP and SAA levels (delay since last flares, other inflammatory or infectious symptoms, recent antibiotics treatment) may not have been systematically investigated, and should be addressed in further prospective studies.

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1
2

	N (%) or median [range]		
Number of patients	117		
Females	62 (53)		
Homozygotes/compound heterozygous	74 (62)		
M694V/M694V	50 (43)		
M694V/other	12 (10)		
Non M694V	12 (10)		
Heterozygous	43 (38)		
M694V	33 (29)		
Number of samples	438		
Age at analysis (years)	10 [0.5-18]		
CRP levels (mg/L)	1.2 [0-106]		
SAA levels (mg/L)	5 [0-600]		
Number of attacks in the previous 6 months	0.9 [0-19.91]		
Colchicine only	362 (83)		
Interleukin-1 blockers	26 (6)		
TNF blockers	10 (2)		
No treatment	40 (9)		
	≥1 SAA+/CRP-	**0 SAA+/CRP-**	**p-value**
Number of patients	17	100	
Females	10 (59)	53 (53)	0.8
sample/patients	6 [3-16]	3 [1-10]	**<0.001**
Positive dissociated SAA/patient	1 [1-9]		
Percentage of positive dissociated SAA	25 [11-75]		
Homozygous/Compound heterozygous	13 (76)	61 (60)	0.28
	SAA+/CRP-	**SAA-/CRP-**	
Number of visits	32	247	
Age at visit	10 [2-18]	11 [0.5-18]	0.9
CRP levels (mg/L)	1.65 [0-4.4]	0 [0-4.8)	**<0.001**
SAA levels (mg/L)	15.95 [10.1-65]	0 [0-9.75]	**<0.001**
Number of attacks in the previous 6 months	2 [0-18.25]	0 [0-16.25]	**0.004**
Treatment			
Colchicine only	20 (63)	204 (83)	**<0.01**
Interleukin-1 blockers	10 (31)	10 (4)	
TNF blockers	1 (3)	5 (2)	
No treatment	1 (3)	28 (11)	
FMF-related symptoms during visit	0	8 (3)	1

3

Table 1. Characteristics of FMF patients with at least one out-of-flare samples

CRP: C-reactive protein, SAA: Serum Amyloid A, FMF: Familial mediterranean fever. Values are n (%) or median [range]

Figure legends

Figure 1. ROC curves for CRP and SAA and active disease defined by number of attacks ≥2/6 months.