Serum amyloid protein A concentration in cryopyrin-associated periodic syndrome patients treated with interleukin-1 beta antagonist

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ABSTRACT

Objective. *Cryopyrin-associated periodic syndromes (CAPS) are a group of chronic, relapsing autoinflammatory disorders which may be complicated by systemic AA amyloidosis. The aim of our study was to evaluate serum amyloid protein A (SAA) level in CAPS patients treated with Interleukin-1 beta (IL-1β) antagonist and to correlate its level with treatment response.*

Methods. *All patients of CAPS Italian Register treated with IL-1β inhibitor were enrolled. SAA levels before starting therapy, and at last visit were evaluated. Patients were then divided in complete responders and partial responders.*

Results. *Twenty-five patients were enrolled. SAA level before starting therapy was increased (median 118.5 mg/L, IQR 96.4-252.8; normal value <6.4 mg/L), while at last visit SAA was significantly reduced (median 4.3 mg/L, IQR 2.3–12.7) (p<0.001). However 12 patients still presented SAA levels beyond normal range, 10/25 patients (40%) showed a complete response to treatment. Conversely, 15 patients presented only a partial response, of which 12 for increased SAA value and 3 for increased CRP value. Patients with partial response had SAA values significantly higher than patients with complete response (median 12.6 mg/L; IQR 8.3–20.0 vs. 2.7 mg/L; IQR 1.6–4.1, p<0.001).*

Conclusion. *Our results confirm the long term efficacy of anti IL-1β treatment in CAPS and the decrease of SAA levels; however 48% of patients still presented SAA elevation despite treatment. The real risk of these patients in developing amyloidosis is not clear but the persistent increase of SAA needs a close follow-up.*

Introduction

Autoinflammatory syndromes are a heterogeneous group of genetic diseases caused by dysregulation of the innate immune system, clinically characterized by recurrent febrile attacks and inflammatory symptoms, affecting the serosal surfaces, joints, skin, eyes, gastroenteric tube, central nervous system (1). During attacks, patients have a vigorous inflammation, with leukocytosis and elevated concentrations of acute-phase proteins, such as C-reactive protein (CRP) and serum amyloid protein A (SAA). Although all patients with chronic inflammatory conditions are at risk for developing systemic AA amyloidosis, the incidence varies widely between the different autoinflammatory syndromes. Before effective treatment was available, up to 60% of familial Mediterranean fever (FMF) patients developed AA amyloidosis (2). The reported incidence is about 25% in tumour necrosis factor (TNF) receptor associated periodic syndrome (TRAPS) patients and up to 35% of patients with Muckle-Wells syndrome (MWS) and chronic infantile neurologic cutaneous and articular syndrome (CINCA) (2), the most severe form of cryopyrin-associated periodic syndromes (CAPS) which comprise an overlapping severity spectrum diseases and are associated with mutations in NLRP3/CIAS1 (3, 4).

Previous studies have demonstrated that IL-1 β inhibitors (anakinra, canakinumab) are able to induce complete remission of clinical manifestations and suppression of markers of inflammation in the majority of patients, SSA included (5-7). Since amyloidosis is the most severe complication of CAPS, the aim of our study was to evaluate SAA level in CAPS long term treated patients with anti IL-1β therapy and to correlate its level with the response to the treatment, grading of disease activity and with the presence of genetic mutation.

Material and methods

All patients of the CAPS Italian Register affected by MWS or CINCA treated

with anakinra (IL-1 receptor antagonist) or canakinumab (IL-1 β monoclonal antibody) were enrolled.

Since 2004, the CAPS Italian Register collected at screening and follow-up visits, global disease activity and each of the following symptoms: urticarial skin rash, arthralgia/arthritis, myalgia, headache/migraine, conjunctivitis, fatigue or malaise, and other symptoms related or unrelated to CAPS (8). The assessment was performed with the use of a 5-point scale for disease activity: absent, minimal, mild, moderate or severe. Patients performed a global assessment of their symptoms together with assessments of each of the following symptoms: fever or chills, rash, joint or muscle pain, eye discomfort or redness, fatigue, headache, and other symptoms. The assessments were performed with the use of the same 5-point scale used by physicians. Blood samples were collected at screening and specified time points (every six months) for measurement of concentrations of C-reactive protein (CRP), serum amyloid-A protein (SAA) and for assessment of haematologic and biochemical markers.

We considered SAA levels and CRP before starting IL-1β inhibitors therapy (time 0), and at last visit.

A complete response to treatment (4), was defined as a global assessment of no or minimal disease activity by a physician, and a value for both serum CRP and SAA that was within the normal range (<0.8 mg/dL for CRP, <6.4 mg/L for SAA).

Partial response to IL-1β inhibitors (4), was defined as a global assessment of no or minimal disease activity by a physician, and persistent elevated inflammatory markers (CRP and/or SAA).

All patients underwent genetic analysis to identify NLRP3 mutations.

Categorical data are presented as number and percentages, continuous data as median and interquartile range (IQR). Differences between the continuous data were analysed with paired or unpaired non-parametric tests (Wilcoxon test and Mann-Whitney test, respectively), depending on the data. A *p*-value <0.05 was considered as statistically significant. The analysis was carried out using the SPSS 11 software.

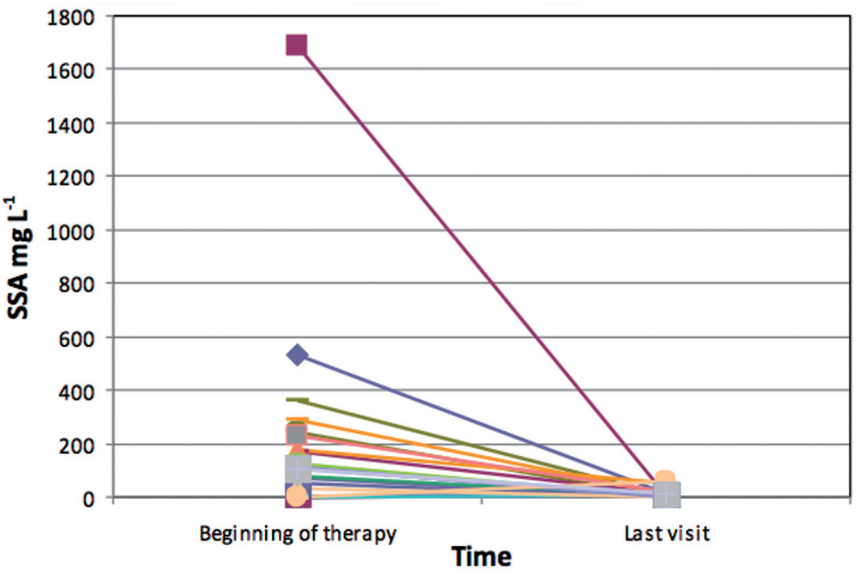

Fig. 1. SAA values before starting IL-1β inhibitor therapy and at the end of follow-up.
SAA: serum amyloid-A protein; normal value <6.4 mg/L.

Table I. Clinical and laboratory assessment at last visit.

Patients	Disease activity	CRP	SAA
10	no or minimal	n	n
10	no or minimal	n	↑
3	no or minimal	↑	n
2	no or minimal	↑	↑

CRP: C-reactive protein; SAA: serum amyloid-A protein; n: normal.
Normal value for CRP <0.8 mg/dL, normal value for SAA <6.4 mg/L

Results

Twenty-five patients (15 M, 10 F) were enrolled, aged 2 to 52 years (median 16.5 ys, IQR 11–26.5). Eighteen out of 25 treated patients, were treated with canakinumab (5 patients ab initio and 13 after a period with anakinra therapy) and 7 patients were still being treated with anakinra.

The mean duration of IL-1β inhibitor therapy was 60.0 months (IQR 24.0–96.0 months).

Eighteen out of 25 patients underwent SAA dosage before starting therapy; all of them presented elevation in SAA value (median 118.5 mg/L, IQR 96.4-252.8). At the end of follow-up these patients presented SAA values significantly reduced (median 4.3 mg/L, IQR 2.3–12.7) (Wilcoxon test: $p<0.001$) (Fig. 1). In the overall sample (25 patients), the median value of SAA at the end of follow-up was 5 mg/L, IQR 2.7–13.7.

At the last visit 10 out of 25 patients showed no or minimal disease activity with normal laboratory tests, including SAA (<6.4 mg/L), while 10 out of 25 patients had elevated SAA even if they presented no or minimal disease activity and normal CRP; median SAA value was 13.5 mg/L, IQR 10.5–20.3. Two out of 25 showed no or minimal disease activity, but high values of SAA (median 33.7 mg/L) and CRP (median 1.62 mg/dL); three out of 25 patients presented no or minimal disease activity, normal SAA values, but CRP values over the normal values (median 1 mg/dL) (Table I).

According to the Lachmann criteria (6), only 10/25 patients (40%) affected by CAPS showed a complete response to IL-1β inhibitors.

Patients with partial response to treatment (15/25) had SAA values at the end of follow-up significantly higher than patients with complete response to treatment (10/25), with median SAA of 12.6 mg/L (IQR 8.3-20.0) *vs*. 2.7 mg/L (IQR 1.6-4.1) (Mann-Whitney test: $p<0.001$) (Fig. 2).

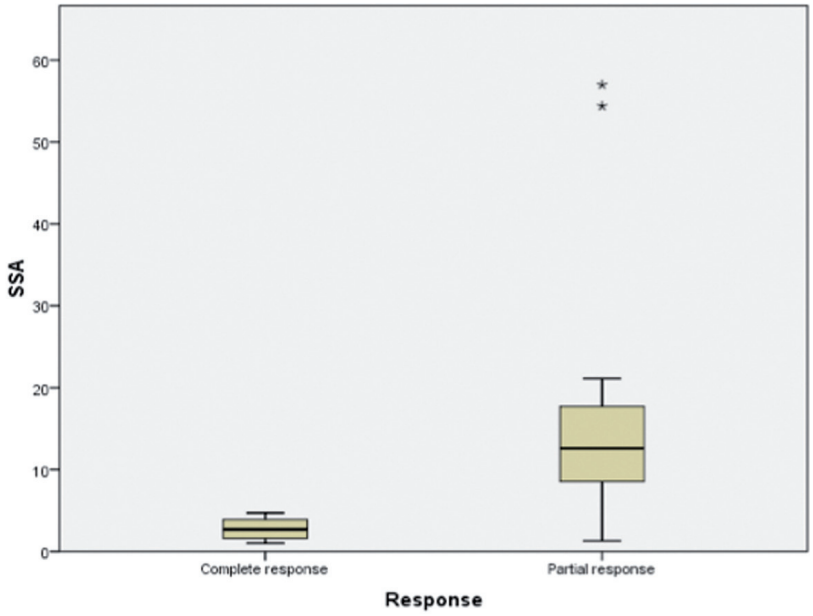

Fig. 2. SAA values of patients with partial response and with complete response to IL-1β inhibitor therapy. SAA: serum amyloid-A protein; normal value <6.4 mg/L.

Table II. Genetic background of our patients.

Patients	Diagnosis	Genetic mutations	Response
1	CINCA	N 477 K	PR
2	MWS	R 260 W	PR
3	CINCA	F 573 S	PR
4	CINCA	negative	PR
5	MWS	negative	PR
6	MWS	negative	CR
7	CINCA	negative	CR
8	CINCA	M 496 I	CR
9	CINCA	negative	CR
10	MWS	303 N	CR
11	MWS	V 198 M/ R 260W	PR
12	MWS	D 303 N	CR
13	MWS	T 348 M	CR
14	MWS	T 348 M	PR
15	CINCA	T 348 M	PR
16	CINCA	E 304 K	PR
17	MWS?	V 198 M	PR
18	CINCA	T 348 M	CR
19	CINCA	E 567 K	PR
20	CINCA	I 572 F	PR
21	CINCA	negative	PR
22	MWS	ASP 280 ASN	PR
23	MWS	V 198 M/ R 260W	CR
24	CINCA	negative	PR
25	MWS	E 525 K	CR

CINCA: chronic infantile neurologic cutaneous and articular syndrome; MWS: Muckle-Wells syndrome; CR: complete response; PR: partial response.

In 18 out of 25 patients a mutation of NLRP3 gene was detected. Nine out of 18 mutated patients and 3 out of 7 non-mutated patients had elevated SAA at the end of follow-up. Median value of SAA in the mutated patients was 6.7 mg/L (IQR 2.3-13.3), median value in no mutated patients was 5 mg/L (IQR 3.9-15.4); the difference was not statistically significant (Mann-Whitney test: p=0.5). The correlation between clinical phenotype and type of mutation is summarised in Table II.

Discussion

Systemic AA amyloidosis may complicate the course of autoinflammatory syndromes in a variable percentage of patients. It is characterised by extracellular deposition of proteolytic cleavage products of the acute phase reactant serum amyloid A (SAA) as insoluble fibrils in various tissues.

AA amyloid fibrils first accumulate in the spleen, kidney and liver, then also in the nervous system, testis, thyroid, adrenal glands and heart. Clinical presentation mostly consists of glomerular proteinuria of 0.5 g/day or more, nephrotic syndrome and/or progressive loss of renal function (9).

The median plasma concentration of SAA in healthy persons is 3 mg per liter, but the concentration can increase many times during the acute-phase response (10). Sustained overproduction of SAA is mandatory for the development of AA amyloidosis that occurs only in a small proportion of patients with chronic inflammatory disorders (11, 12).

Autoinflammatory diseases are characterised by a significant increase in SAA level during inflammatory bouts, which often persists also between attacks. The mechanism involved in SAA production is first of all the activation of the NLRP3 protein which results in its unfolding and assembly with the other protein components of the inflammasome. Oligomerisation induces cleavage of procaspase 1 to form the active caspase 1 that in turn cleaves pro IL-1β, which is released from cells. IL-1β in a proinflammatory cytokine that has pathological consequences, and has known to contribute to increased synthesis of SAA. An important part of the evidence for the role of IL-1β in autoinflammatory disorders is the efficacy of IL-1β blockade (13).

Protracted disease duration is expected to increase the risk of this complication. However, exposure to high levels of SAA even for decades may not result in clinically overt systemic AA amyloidosis. On the contrary, rapidly progressive kidney damage due to AA still occurs in children with FMF before colchicine treatment is established, particularly in some populations (14). To date, it is known that SAA genotype significantly affected the risk of development of AA: SAA1.1 genotype is as-

sociated with a seven-times-higher risk of amyloidosis as compared to the other SAA genotyped (9). In FMF patients, many studies have indicated that mutations in Exon 10, in the region between 680 and 694, and especially M694V homozygosity, are associated with amyloidosis (15-17). Concerning CAPS it is not known if IL-1β inhibitors are also useful for preventing amyloidosis and if there are genetic or clinical factors affecting amyloidosis risk.

In our series there is no correlation between SAA values and genetic background: half of mutated patients had elevated SAA as well as half of no mutated patients.

In the past decade the biologic therapy of CAPS with anti IL-1β therapies, anakinra first, then canakinumab, completely modified the natural history and outcome (3).

Previous reports stated that, in the majority of cases, the number of complete response to anti-IL1 therapy varies between 65% and 85% (5, 6), and the complete response is always associated with the normalization of SSA.

In our study median value of SAA in patients with partial response is significantly higher compared the patients with complete response (Fig. 2), but the elevation is mild and the risk of systemic amyloidosis overtime is problably very low. In fact, it is well-known that renal outcome and survival are strictly dependent on the residual inflammatory activity, and only SAA concentrations persistently over 50 mg/L has been associated with disease progression and worse prognosis (18).

On the other hand, if we erase SAA from the definition of response we found, interestingly, that the median SAA in patients with complete response and in patients with partial response are not significantly different (6.5 *vs*. 5 mg/dL). With this analysis 20/25 patients (80%) have a complete response to IL-1 β inhibitors, only 8 out of 25 patients (32%) have a complete response if SAA levels were within normal range (data not shown). We also found that CRP and SAA react differently: in fact only 2 out of 5 patients with high CRP during canakinumab treatment also have high SAA levels.

These data, in contrast, could suggest that role of SAA could be considered not so crucial in order to define clinical response among CAPS patients.

In conclusion, our results confirm the extreme efficacy of long term anti IL-1β treatment in CAPS, in fact at the end of follow-up all patients were still in clinical remission, although in 60% of patients the suppression of inflammation is not complete because of elevation of SAA and/or CRP. In almost half of patients (48%) with CAPS treated with anti IL-1β therapies, the SAA remained elevated although with a median value only twice respect normal range (13.65 mg/L); although the role of SAA in evaluation of clinical response among CAPS patients remains to be elucidated its persistent elevation, in our opinion, needs a close clinical follow-up.

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