Percentage of glycosylated serum ferritin remains low throughout the course of adult onset Still's disease

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Abstract

***Objective*—To determine the evolution of levels of total serum ferritin and percentage of the glycosylated form in patients with adult onset Still's disease (AOSD) at the time of diagnosis and during follow up.**

***Methods*—All patients with AOSD were tested at the time of diagnosis and during follow up. Total serum ferritin levels were analysed by immunoassay, and the percentage of glycosylated ferritin was determined by methods using Sepharose-Con A.**

***Results*—14 patients (eight women, six men) with AOSD were enrolled. At the time of diagnosis, mean (SD) age was 36 (16) years. Mean initial total serum ferritin was 6350 (1300) µg/l (normal <250 µg/l). The mean initial percentage of glycosylated ferritin was 14.7 (13)% (normal >50%). Mean follow up time was 37 (35) months. At the time of the last examination all patients were in remission except one, who presented a chronic articular form. Total serum ferritin remained high in this single patient and was normal in the 13 others, with a mean of 98 (73) µg/l. In all patients the percentage of glycosylated ferritin remained low, with a mean of 16 (16)%.**

***Conclusion*—Total serum ferritin is a marker of the active phase of AOSD. The percentage of glycosylated ferritin is low both in the active phase and in remission. Further studies are needed to confirm these data and to determine their specificity for AOSD before considering any possible use of a low percentage of glycosylated ferritin as a diagnostic tool in suspected AOSD, especially when atypical or previously treated.**

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Adult onset Still's disease (AOSD) is an inflammatory disorder of unknown cause. Clinical manifestations of AOSD include high peak fever, rash, articular involvement, serositis, and sore throat. To date, there are no specific biological abnormalities to confirm the diagnosis of AOSD. Leucocyte count with percentage of polymorphonuclear cells is the only biological parameter included in the major Yamaguchi criteria of AOSD.[1] Ferritin is the major iron storage protein found in all tissues. It contains 24 subunits of two types, L (liver) and H (heart), with a molecular weight of 19 kDa and 21 kDa respectively.[2] Different combinations of L or H subunits comprise the various isoferritins seen on isoelectric focusing. The proportion of these two subunits varies according to the organ involved.[3] In most healthy subjects serum ferritin is acid, with a high proportion of ferritin which binds to concanavalin A (Con A). The microheterogeneity of serum ferritin is due to glycosylation rather than to variation in the proportions of H and L subunits.[3] In 1994 Van Reeth *et al* investigated levels of total serum ferritin and isoferritin profiles in different inflammatory diseases.[4] Markedly high levels of total serum ferritin with a low percentage of glycosylated ferritin were noted in the active phases of AOSD. In the other diseases, total serum ferritin remained within normal ranges with no decrease in the percentage of glycosylated ferritin. In this paper, one patient only, had repeated determinations. During remission, total serum ferritin returned to normal values, though the percentage of glycosylated ferritin remained low.[4]

Our purpose was to investigate the serum levels of total ferritin and the percentage of its glycosylated form in a large series of patients at time of the first flare of AOSD and during follow up.

Patients and methods

PATIENT ELIGIBILITY CRITERIA

In this retrospective study, patients with AOSD were recruited from two different centres. Diagnosis of AOSD was based upon Yamaguchi criteria—namely, having five criteria with two or more major criteria (fever >39°C during two weeks, arthritis or arthralgias for more than seven days, typical salmon pick rash, leucocytosis $>10 \times 10^9$/l with 80% polymorphonuclear cells).[1]

MEASUREMENT OF TOTAL SERUM FERRITIN AND GLYCOSYLATED FERRITIN

For each patient, total serum ferritin and percentage of glycosylated ferritin were determined at the time of diagnosis and repeated during follow up. Technical procedures were provided in a single laboratory (Biochimie A, Bichat Hospital) to ensure reproducibility and comparison of results before treatment and during follow up. Total serum ferritin concentration was determined on a Stratus Fluorimetric Enzyme Immuno Assay System. This procedure is a sandwich immunoassay methodology (Dade Behring, Paris La défense, France) using the double antibody technique:

*Table 1 Main characteristics of the patients with adult onset Still's disease at the time of diagnosis. Fever, articular involvement, and cutaneous rash were present in all patients**

Case	*Age (years)*	*Polynuclear cells (×10⁹/l)*
1	47	18
2	25	14.4
3	55	14.8
4	35	19.8
5	54	16.8
6	21	10.1
7	35	15.3
8	18	10.4
9	17	11.3
10	31	11.6
11	20	12.3
12	69	17.4
13	35	8.1
14	37	2.2

*Fever >39°C during two weeks, arthritis or arthralgias for more than seven days, typical salmon pick rash (Yamaguchi classification[1]).

rabbit antihuman ferritin and enzyme labelled Fab' fragment of rabbit antiferritin IgG.

Glycosylated ferritin was determined according to the method of Worwood *et al*[5] with minor modifications. The heterogeneity of ferritin due to glycosylation was investigated by the different affinity of ferritin for a vegetable lectin, Con A. Lectins are proteins that interact specifically with some sugar residues. Con A presents a great affinity for methyl α-D-mannopyranosides, less for α-D-mannose, and even less for α-D-glucose and fructose residues. Mammalian glycoproteins contain neither glucose nor fructose. Con A essentially recognises mannosyl residues when this sugar is accessible.[5–6] Binding of serum ferritin was measured by incubating serum with Con A Sepharose 4B (Pharmacia Biotech. Europ., Saclay, France) and then mixing on a roller mixer for two hours at room temperature. The sample was centrifuged at 3000 rpm for 15 minutes and unbound ferritin was recovered in the supernatant. Similarly, serum was incubated with Sepharose 4B and the ferritin measured in the supernatant corresponded to the total serum ferritin. Glycosylated ferritin was obtained from the difference between total ferritin and unbound ferritin. Duplicate samples were used. For serum specimens with a high total serum ferritin concentration, it was necessary to use a different volume of serum buffer and gel to determine the best portion of working range. Glycosylated ferritin was determined for a pool of serum stored at −80°C to define the normal values of the laboratory. The results were expressed as a percentage of total serum ferritin. Normal values of total serum ferritin are 10–250 µg/l and percentage of glycosylated ferritin was 50–80%. These results confirm previous data of Cazzola *et al*.[7]

Table 2 Total serum ferritin concentrations and percentage of glycosylated ferritin at the time of the first flare and at the time of the last examination in adult onset Still's disease

	At time of the first flare		*At the time of the last examination*		
Patient No	*Total serum ferritin (µg/l)*	*Percentage of GF¶*	*Total serum ferritin (µg/l)*	*Percentage of GF*	*Follow up (months)*
1	956	10	35	2	22
2	428	18	10	9	20
3	4 250	10	151	41	59
4	7 780	14	147	12	5
5	10 760	20	184	49	38
6	4 200	14	17	3	14
7	6 000	3	73	32	20
8	1 784	10	107	10	30
9	127	32	40	4	94
10	94	3	57	2	6
11	49 910	2	128	32	10
12	815	16	259	6	57
13	1 400	2	69	6	22
14*	483	50	703	20	127
Mean (SD)	6 350 (1300)	14.7 (13)	98 (73)†	16 (16)‡	37 (35)

*Patient with chronic articular form.
†For the 13 patients in remission at the time of the last examination.
‡No significant difference between the mean percentage of glycosylated ferritin at the time of the last examination and at the time of the first flare.
¶GF = glycosylated ferritin.

STATISTICAL ANALYSIS

Results were expressed as mean (SD). Statistical analysis was carried out using a paired Student's *t* test for comparisons of the percentage of glycosylated ferritin at the time of diagnosis and during follow up. A level of $p<0.05$ was accepted as significant.

Results

Fourteen patients (eight women, six men) with AOSD were included. Table 1 shows their main characteristics. At the time of diagnosis, their mean age was 35.6 (15.8) years (range 17–69). At the time of the first flare of AOSD, inflammatory parameters, including erythrocyte sedimentation rate, C reactive protein, and fibrinogenaemia, were increased in all patients. Liver enzymes were two to four times higher than the normal range in four patients and 20 times higher in one patient with negative serological tests for various viral hepatitis. No correlation was found between liver cytolysis and serum ferritin levels in the study group (data not shown).

Table 2 shows concentrations of total serum ferritin and glycosylated ferritin. Initially, two patients had a normal level of ferritin. Mean initial total serum ferritin was 6350 (1300) µg/l (range 94–49 910, median 1600). Mean initial percentage of glycosylated form was 14.7 (13)% (range 2–50, median 12%). Different treatments were used to achieve remission of AOSD assessed by the doctor: intravenous immunoglobulin with non-steroidal anti-inflammatory drugs (NSAIDS) (four patients) as recently reported,[8] intravenous immunoglobulin with steroids (three), and methotrexate (one), steroids alone (two) or associated with NSAIDs (one), with methotrexate (one), NSAIDs alone (one), steroids with cyclophosphamide changed later into cyclosporin A (one). Eight patients had a single flare of AOSD, two had two flares, two others had three flares, one had four flares and another one had a chronic destructive articular form of the disease. At the time of the last examination the mean follow up was 37 (35) months (range 5 months–10 years, median 22 months).

At this time 13 patients had achieved clinical remission. Thirteen patients then had normal inflammatory parameters and normal total serum ferritin with a mean of 98 (73) µg/l (range 10–259, median 73). Total serum ferritin remained high (703 µg/l) in the one patient with a chronic form of the disease. At the time

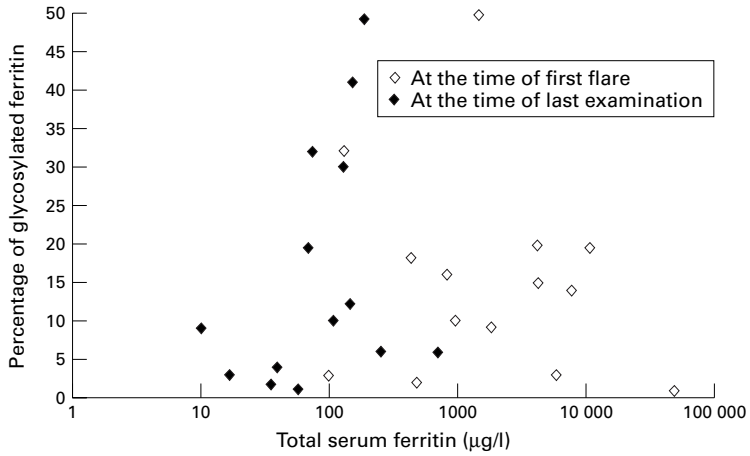

Figure 1 Percentage of glycosylated ferritin and total serum ferritin at the time of diagnosis and during follow up. No correlation was observed between the percentage of glycosylated ferritin and total serum ferritin (r = −0.19, NS).

of the last examination the mean percentage of glycosylated ferritin was 16 (16)% (range 2–49, median 9%) and did not differ from the initial percentage. The percentage of glycosylated ferritin had increased in four patients, decreased in two, and remained low in eight. In all patients the percentage of glycosylated ferritin was lower than 20% either initially, during follow up, or both. During further flares, total serum ferritin increased again, while the percentage of glycosylated ferritin remained low in the five patients tested. No correlation between the percentage of glycosylation and levels of total serum ferritin was seen either at the time of diagnosis or during follow up (r = −0.19, NS) (fig 1). Persistence of a low percentage of glycosylated ferritin was not associated either with the duration of follow up or with any treatment of AOSD.

Discussion

In our retrospective study we have investigated the levels of total serum ferritin and the percentage of the glycosylated form in AOSD. Increased concentrations of total serum ferritin were found in all but two patients at the time of diagnosis. These findings confirm previous reports.[9–10] For many authors, total serum ferritin is correlated with the activity of AOSD[11–12] and might be considered as an additional diagnostic criterion.[9] In contrast, normal levels of total serum ferritin are not an exclusion criterion for diagnosis of AOSD. Remissions of AOSD are characterised by normalisation of total serum ferritin.[12] Van Reeth *et al* compared total serum ferritin in AOSD and in different diseases such as systemic lupus erythematosus, rheumatoid arthritis, and dermatopolymyositis.[4] Most of their patients with inflammatory diseases (23/27) were in remission when tested. Total serum ferritin levels were normal, but they were very high in active AOSD. The authors showed that low percentage levels of glycosylated ferritin were seen in active phases of AOSD in comparison with other inflammatory disorders.[4] These results were confirmed by Higashi *et al* during the active phase of AOSD.[13] We obtained similar results during the flares of AOSD. Moreover, the percentage of glycosylated ferritin remained low in remission whereas total serum ferritin levels were normalised. The duration of follow up was not correlated with the persistence of low levels of glycosylated ferritin. In our study no correlation was found between the percentage of glycosylation and the level of total serum ferritin, whereas Higashi *et al* found the proportion of glycosylated ferritin was significantly lower when the serum ferritin level exceeded 1000 ng/ml.[13]

Precise mechanisms leading to an increase in total serum ferritin and a decrease in the percentage of glycosylated ferritin remain unclear in active AOSD as there is no iron overload. Ferritin in serum samples from patients with massive hepatic necrosis—that is, non-glycosylated, did not bind to Con A.[5] Abnormal liver function tests were reported in 75% of 228 patients with AOSD.[14] But, in AOSD, hepatic cell damage with the potential for releasing ferritin into the plasma is rarely severe. Another mechanism may be responsible, in part, for these biological abnormalities—enhanced ferritin production suggesting that ferritin may enter the circulation by secretion rather than by release from damaged cells or erythrophagocytosis, or both. However, this possible cause, which is sometimes is a complication of AOSD, is currently associated with cytopenia and not with neutrophilic leucocytosis, which is a major criterion in the diagnosis of AOSD.

In addition, in active AOSD, Van Reeth *et al* showed that isoferritins were partially or completely desialylated owing to a defect in sialylation during synthesis or to a decreased number of the hepatic membrane galactose specific receptors for asialoglycoprotein during transport and catabolism.[15] Another protein of iron metabolism, such as transferrin, may be modified during the active phase of AOSD, with a decrease in sialylation.[16] In other inflammatory diseases the sialylation of ferritin has been said to be moderately decreased (20–40%) but was not extensively studied.[4] In Sjögren's syndrome the glycosylation of serum IgG and IgA, two glycoproteins, is abnormal. The proportion of asialyted IgG is markedly high, whereas subclasses IgA1 and IgA2 seem to be oversialylated.[17] To date, glycosylation of immunoglobulins has not been studied in AOSD.

We conclude that total serum ferritin is a marker of the active phase of AOSD. However, the percentage of glycosylated ferritin is low both at the time of diagnosis—that is, when AOSD is active, and during follow up—that is, when the disease goes into remission. Further studies are needed to confirm these data and to determine their specificity for AOSD before considering any possible use of a low percentage of glycosylated ferritin as a diagnosis tool in suspected AOSD, especially when the disease is incomplete, atypical, or has been previously treated.

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