Iron Saturation of Serum Ferritin in Patients with Adult Onset Still's Disease

JOOP ten KATE, JOOST P.H. DRENTH, MARCEL-FRANCIS KAHN, and CEES van DEURSEN

ABSTRACT. ***Objective.*** Patients with Still's disease show a prominent acute phase reaction. Our hypothesis is that under these circumstances the iron uptake of ferritin will not keep pace with its synthesis, and is therefore not a valid reflection of the iron status in these patients.

Methods. Previously we developed a method to measure the iron content of ferritin; we investigated the usefulness of this method to establish the iron status of patients with anemia of inflammation.

Results. In 9 patients with adult onset Still's disease (AOSD) we observed high ferritin concentrations and measured the iron saturation of ferritin. The mean value of saturation was 9.1%, while saturation in the healthy control group was 17.8%, a statistically significant difference ($p < 0.005$). Soluble transferrin receptor concentrations indicated a functional iron deficiency.

Conclusion. We conclude that the acute phase ferritin in patients with AOSD contains less iron in comparison to ferritin in healthy controls. We suggest that soluble transferrin receptor is the method of choice in estimating the iron status of patients with an acute phase reaction. (J Rheumatol 2001;28:2213–5)

Key Indexing Terms:
ADULT ONSET STILL'S DISEASE FERRITIN IRON METABOLISM ANEMIA

Adult onset Still's disease (AOSD) was first described by Bywaters[1] as a disorder reminiscent of Still's variant of juvenile rheumatoid arthritis in children. It is a systemic inflammatory disease with high spiking fever and a salmon-pink evanescent rash. Almost all patients will have arthritis at one point in the disease course, which can be complicated by deforming ankylosis in up to one-third of the patients[2]. Other clinical manifestations include intense myalgias, lymphadenopathy, hepato/splenomegaly, and serositis. In a few patients AOSD is complicated by amyloidosis. Although nonsteroidal antiinflammatory drugs are the mainstay of treatment, many patients will need corticosteroids or disease modifying antiinflammatory drugs such as methotrexate during one stage of the disease. Laboratory investigations point to a marked acute phase response, and neutrophilic leukocytosis and an accelerated erythrocyte sedimentation rate are invariably present. Two-thirds of patients present with a normocytic normochromic anemia, which reverts to normal during remission, and elevated hepatic enzyme levels occur in a similar proportion of patients with AOSD. Recent evidence shows attacks in AOSD are accompanied by grossly elevated serum ferritin concentrations[2]. Ferritin along with C-reactive protein (CRP) and serum amyloid A (SAA) are positive acute phase proteins, probably produced by the liver as part of the generalized inflammatory response. In patients with active AOSD ferritin levels exceed those observed in other inflammatory disorders, so that it may serve for diagnostic purposes[3]. The increase in ferritin is most probably cytokine driven[4]. Another possibility is suggested by the work of Coffernils, *et al*[5], who indicate that the very high ferritin levels encountered in AOSD reflect the presence of histiocytic hyperactivity that may lead to a hemophagocytic syndrome.

Ferritin stores intracellular iron. In clinical practice ferritin concentrations are most commonly used to estimate the iron status. During the acute phase response, however, elevated ferritin levels do not accurately reflect the amount of stored iron[6,7]. This greatly hampers assessment of iron deficiency in patients with an inflammatory process[7]. We developed a method to measure the iron saturation of ferritin[8]. In a preliminary study with a very diverse group of patients with an acute phase reaction we observed a significant decrease of iron saturation of serum ferritin compared to healthy controls[8]. AOSD may serve as a useful model for an acute phase reaction since extreme elevations of serum ferritin are commonly observed. We hypothesized that during attacks of AOSD ferritin loses its "iron storage" abilities, resulting in a lower ferritin-iron saturation. Serum transferrin receptor quantification was included in this study because it is considered a reliable marker of iron status, particularly when iron deficiency is associated with chronic disorders such as inflammation, infection, or malignancy[9].

From the Department of Clinical Chemistry and Department of Internal Medicine, Atrium Medical Center Heerlen/Brunssum, Heerlen; Department of Medicine, Division of Gastroenterology, University Medical Center St. Radboud, Nijmegen, The Netherlands; and Department of Rheumatology, Bichat Hospital, Paris, France.

J. ten Kate, PhD, Clinical Chemist, Department of Clinical Chemistry, Atrium Medical Center; J.P.H. Drenth, MD, PhD, Internist, Department of Medicine, Division of Gastroenterology, University Medical Center St. Radboud; M-F. Kahn, Professor, Department of Rheumatology, Bichat Hospital; C. van Deursen, MD, PhD, Internist, Department of Internal Medicine, Atrium Medical Center.

Address reprint requests to Dr. J. ten Kate, Department of Clinical Chemistry, Maasland Hospital, PO Box 5500, 6130MB Sittard, The Netherlands. E-mail: j.tenkate@orbisconcern.nl

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MATERIALS AND METHODS

Patients. Nine French patients, 7 women and 2 men, with active AOSD were invited to participate in the study. One patient donated samples on 2 separate occasions during an acute phase. All patients fulfilled the Reginato[3] as well as the Yamaguchi[10] criteria for AOSD. These patients were part of a study investigating the glycosylation pattern of ferritin[3]. The patients exhibited a low normal hemoglobin concentration. Disease activity was defined as spiking fever, arthritis, rash, and leukocytosis.

A cohort of volunteers served as healthy controls. This group consisted of 10 healthy male laboratory technicians aged 35 to 45 years who displayed a normal hematological profile.

Methods. Blood was drawn from an antecubital vein in sitting position. Serum iron concentrations were measured automatically on a Perkin-Elmer AS40 (Perkin-Elmer, Norwalk, CT, USA). Transferrin concentrations were determined by immunoturbidimetric assay on the Cobas Integra (Roche Diagnostics, Basel, Switzerland). Serum ferritin was determined with the Wallac 1235 Autodelphia automatic immunoassay system (Wallac, Breda, The Netherlands). The iron content of ferritin was determined as described using purification by immunochemical precipitation with beads coated with anti-ferritin antibodies (Biorad, Veenendaal, The Netherlands) and flameless atomic absorption spectrophotometry[8]. Soluble transferrin receptor concentration was determined using an indirect enzyme-immunometric assay from Imphos (Amersfoort, The Netherlands).

Statistical analysis. The paired nonparametric Wilcoxon test was used for comparison of statistical values between controls and patients. Probability values were calculated on the basis of 2 tailed tests. A correlation coefficient was calculated with Pearson's correlation test. A p value < 0.05 was accepted as the lowest level of significance. Results are expressed as means and standard deviation unless indicated otherwise.

RESULTS

Serum concentrations of ferritin, iron saturation of ferritin, and transferrin receptors. As shown in Table 1, serum ferritin concentrations were strongly elevated in the acute phase in patients with AOSD. The median value in our patients was 8835 ng/ml. This contrasted greatly with healthy controls (Table 2) in whom we found a median value of only 131 ng/ml ($p = 0.0002$).

In healthy controls the mean iron saturation of ferritin was 17.8% (SD 3.7%). In patients during an attack of AOSD we found significantly lower iron saturation of ferritin, a mean value of 9.1% (SD 2.2%). This difference was highly statistically significant — $p = 0.0051$. To establish whether our patients had functional iron deficiency we measured serum transferrin receptor concentration. In controls we found values of 3.73 mg/l (SD 8.4 mg/l), while patients had values of 7.7 mg/l (SD 3.1 mg/l), a significant difference ($p = 0.0077$). In spite of this increase in the soluble transferrin saturation the total amount of circulating iron was increased (Table 1).

Table 1. Iron saturation percentage of ferritin in 9 patients with AOSD.

Patient	Ferritin, ng/ml	Iron Saturation of Ferritin, %	Total Serum Ferritin Iron, nmol/ml	Soluble Transferrin Receptor, mg/l
1	5130	9.7	5.0	>11.0
2	9270	10.6	9.8	4.75
3	13230	7.5	10.0	7.35
4	8400	8.6	7.3	4.19
5	5980	11.9	7.1	3.77
6	1667	8.6	1.5	9.29
7	1510	13.5	2.0	—
8	11390	6.5	7.5	—
9	17050	6.8	11.6	>11.0
9*	11180	7.8	8.7	10.6

*Different episode.

Table 2. Iron saturation percentage of ferritin in 10 healthy controls.

Control	Ferritin, ng/ml	Iron Saturation of Ferritin, %	Total Serum Ferritin Iron, nmol/ml	Soluble Transferrin Receptor, mg/l
1	130	13.8	0.2	3.27
2	168	17.1	0.3	4.89
3	142	17.7	0.3	2.35
4	118	18.2	0.2	4.62
5	118	19.7	0.2	3.69
6	129	15.3	0.2	3.71
7	102	15.8	0.2	3.64
8	170	15.4	0.3	—
9	150	27.2	0.4	—
10	132	17.4	0.2	—

The total amount of serum ferritin iron for the 2 groups is shown in Tables 1 and 2, and patients have significantly ($p = 4.7 \times 10^{-6}$) higher levels of ferritin iron (mean 7.1 ng/ml, SD 3.3) in the circulation than the controls (mean 0.3 ng/ml, SD 0.1).

Statistical correlations. In patients, there was a highly significant negative correlation between ferritin and its iron saturation ($r = -0.71$, $p = 0.02$). In contrast such a correlation was absent in controls with normal ferritin values ($r = 0.13$, $p = 0.72$). Further, we detected a trend toward a significant negative correlation between the iron saturation of ferritin and transferrin receptor ($r = -0.62$, $p = 0.1$). Again, this trend was not discernible in the control population ($r = 0.17$, $p = 0.71$).

DISCUSSION

We investigated the iron saturation of ferritin in samples from patients with active AOSD in comparison with healthy controls. Ferritin correlates with disease activity of AOSD and can even be used as a diagnostic marker because levels in these patients greatly exceed those in other inflammatory disorders. Ferritin is clinically used as an indicator of iron stores, but being also an acute phase reactant, the interpretation is hampered in patients with an acute phase response[6,7]. In earlier studies we developed and applied a novel method to quantify the iron saturation of ferritin, and hypothesized that this percentage decreases during an acute phase response[8,11]. We found that the amount of iron bound to ferritin is much higher than expected on the basis of studies by Worwood, *et al*[12], while the study of Herbert, *et al*[13] was in line with our findings.

We employed samples from patients with active AOSD as a model of an acute phase reaction because of the known hyperferritinemia. The serum ferritin levels in active AOSD can even be higher than those reported in other conditions with acute phase reaction. The reason for these very high levels is not clear.

Suggestions for the mechanism causing this increase are: (1) release from hepatic cells[3] (but not all patients have increased amounts of transaminases, so liver damage is not present in all of them); (2) induction by cytokines, irrespective of the body iron stores, but perhaps influenced by the intracellular labile iron pool[14]; (3) a reflection of histiocytic hyperactivity that may sometimes lead to the so-called hemophagocytic syndrome[5].

It is beyond the scope of this retrospective study to discuss the value of these suggestions. But AOSD would be a good clinical model for prospective studies of cytokines in temporal relationship with ferritin synthesis, contribution of isoferritins, iron content of ferritin, H- and L-forms of ferritin in active and inactive periods of disease, taking into account the hematological state and determinants of the iron status such as serum iron, transferrin, transferrin saturation, serum transferrin receptor and bone marrow iron. Interesting questions remain concerning the mechanism of the hyperferritinemia and the origin of the iron in ferritin.

We detected highly significant decreased levels of iron saturation of ferritin in all samples, compared to samples from healthy controls, and in comparison to patients with hemochromatosis[11]. Although the iron saturation of ferritin is significantly lower in AOSD, the total amount of iron in the circulation (Table 1) is much higher than in controls. This is due to the extreme increase of ferritin during attacks of Still's disease. In spite of the increased total amount of iron, the soluble transferrin receptor concentrations in the sera were high, indicating functional iron deficiency[15]. This suggests that while AOSD is associated with a high total amount of iron, it is not beneficial because it probably resides in the "wrong" pool. The role of the ferritin receptor[16] in the uptake of ferritin iron in erythroid and hepatic cells is not clear.

Other studies on ferritin in AOSD have focused on the changes of its microheterogeneity — the glycosylated fraction is low during attacks[3] and remains so during remissions[17]. The link between these 2 phenomena is unclear. The described changes in glycosylation of ferritin in patients with AOSD may play a role in the changed iron loading of ferritin or may have consequences for the binding of ferritin to its receptor[16].

We observed a negative correlation between the iron saturation of ferritin and the concentration of ferritin in AOSD. This suggests that in active AOSD the rapid synthesis of ferritin exceeds the rate of iron incorporation in ferritin. This is in support of our working hypothesis. Further investigations are needed to prove the mechanism.

We suggest that for clinical practice serum transferrin receptor determination is to be preferred for estimation of the iron stores in acute phase conditions. Determination of the iron saturation of ferritin may be of value for scientific purposes, but this method is not advocated in clinical practice.

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