The Association for Clinical Biochemistry & Laboratory Medicine
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Annals of Clinical Biochemistry
2019, Vol. 56(1) 179–182


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DOI: 10.1177/0004563218793166
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Falsely decreased ferritin concentrations in two patients with haemophagocytic lymphohistiocytosis: A case report

Geert Roozen[1], Ingrid Revet[1], Reinier Raymakers[2] and Hans Kemperman[1]

Abstract

The high-dose hook effect, or prozone effect, can lead to negative or falsely lowered plasma ferritin results. Here, cases of a 16-year-old boy and a 70-year-old woman with haemophagocytic lymphohystiocytosis with extremely high concentrations of plasma ferritin (387,000 μg/L and 138,000 μg/L, respectively) are presented. In both cases, falsely lowered ferritin results were reported without any analyser flag. This article emphasizes the importance of recognition of the high-dose hook effect, since a watertight solution is lacking.

Keywords

High-dose hook, prozone, ferritin, haemophagocytic lymphohistiocytosis

Accepted: 11th July 2018

Case presentation

A 16-year-old boy was admitted to our hospital to receive an allogeneic stem cell transplant (SCT) for a systemic T-cell lymphoproliferative disease of childhood. Myeloablative conditioning with multiple cytostatics was initiated in preparation of the transplant.

Within a few days after admission, he developed fever which did not react to treatment with broad-spectrum antibiotics and antifungals. An extensive diagnostic work-up revealed no evidence of an infection, but an ultrasound of the abdomen showed hepatosplenomegaly. During this period, ferritin concentrations had risen from 17,400 μg/L (25–250 μg/L) at the moment of admission to 105,000 μg/L. Under the suspicion of haemophagocytic lymphohistiocytosis (HLH), soluble IL-2 receptor alpha (sIL-2) was measured and found to be 37,000 pg/mL (0–3000 pg/mL). A bone marrow biopsy was performed showing active haemophagocytosis.

Despite T-cell depletive therapy, there were still T-cells detected in the peripheral blood. Hence, high doses of dexamethasone, antithymocyte globulin and etoposide were given in the days prior to the allogeneic SCT targeting both the lymphoproliferative disease and HLH.

The day after receiving the SCT, ferritin concentrations dropped dramatically to 1310 μg/L.

Shortly thereafter, an unexpected rapid decrease of ferritin concentrations was seen in a 70-year-old woman with HLH. Because of her limited cutaneous

[1]Department of Clinical Chemistry and Haematology, University Medical Center Utrecht, Utrecht, The Netherlands
[2]Department of Haematology, University Medical Center Utrecht, Utrecht, The Netherlands

Corresponding author:
Geert Roozen, Department of Clinical Chemistry and Haematology, University Medical Center Utrecht, Heidelberglaan, Utrecht 3584 CX, The Netherlands.
Email: G.V.T.Roozen-2@umcutrecht.nl

Table 1. Ferritin concentrations during admission of both patients.

Case 1. Measured concentrations of ferritin during hospital admission of the 16-year-old boy									
Number of days in hospital	1	4	7	10	12	14	16	18	20
Plasma ferritin concentrations (μg/L)	17,400	13,600	17,900	21,400	16,800	8950	8160	8200	13,300
Number of days in hospital	22	25	26[a]	27	29	32	34	36	39
Plasma ferritin concentrations (μg/L)	>7500[b]	105,000	88,700	1310[c] 87,000[d]	68,000	67,100	448[c] 274,000[d]	260[c] 387,000[d]	308[c] 348,000[d]

Case 2. Measured concentrations of ferritin during hospital admission of the 70-year-old woman							
Number of days in hospital	4	5	6	7[e]	8	9	10
Plasma ferritin concentrations (μg/L)	12,800	24,600	35,200	87,700	1500[c] 121,000[d]	982[c] 138,000[d]	940[c] 130,000[d]

SCT: stem cell transplant; ICU: intensive care unit.
[a]Day of SCT.
[b]No enough plasma for dilution.
[c]Due to high-dose hook effect.
[d]After manual dilution.
[e]Transfer to ICU.

systemic sclerosis and non-specific interstitial pneumonia, she used immune suppressants (prednisone and mycofenolatemofetil). She was admitted to the hospital with fever, dyspnoea and disorientation. She was found to have a progressive pancytopenia and a high viral load for the herpes simplex virus, which was thought to be the trigger for HLH. A bone marrow biopsy showed active haemophagocytosis and sIL-2 was 7470 pg/mL. Ferritin on the day of admission was 12,800 μg/L.

She was treated with acyclovir, dexamethasone, etoposide and intravenous immunoglobulins. She was transferred to the intensive care unit (ICU) because of progressive liver failure, acute kidney failure and hepatic encephalopathy. At that time, her ferritin had risen to 87,700 μg/L. On the first day in the ICU, ferritin concentrations had dropped to 1500 μg/L.

Considering the *in vivo* half-life of ferritin of circa 5 to 50 h, depending on the level of glycosylation, the rapid decrease in ferritin concentrations (from 88,700 μg/L to 1310 μg/L in one day) in case 1 was highly unlikely, as was the decrease in case 2 (from 87,700 μg/L to 1500 μg/L overnight). Such sudden drops in concentrations should always be treated with the befitting suspicion and laboratories should have a validation check to recognize these results. In our laboratory, the falsely decreased ferritin results of both patients were automatically placed on a clinical validation list based on violation of a delta check rule. This resulted in manual dilution of the samples and ferritin concentrations of 87,000 μg/L and 121,000 μg/L, respectively. The falsely decreased results turned out to be caused by the high-dose hook effect. Table 1 shows ferritin concentrations in time during admission of both patients. In case of the hook effect, both falsely decreased reported concentrations and concentrations obtained after manual dilution are shown.

Both patients did not survive. The lymphoproliferative disease of the 16-year-old boy relapsed and progressed rapidly after the transplant. He developed kidney and liver failure and encephalopathy and died a couple of days later. Despite maximum supportive care, the 70-year-old woman did not recover and passed away after four days in the ICU.

Discussion

HLH is a rare but life-threatening immune disorder characterized by extreme inflammation, haemophagocytosis and the development of cytopenias, hepatitis and central nerve system dysfunction. The hyperinflammation is caused by a cytokine storm of various pro-inflammatory mediators produced by persistently activated macrophages, cytotoxic lymphocytes and natural killer cells. There is a genetic form, but the disease can also be triggered by malignancies, autoimmune diseases or infections. Diagnosis is made when five out of eight criteria are met: fever, splenomegaly, cytopenias (affecting at least two out of three lineages in the peripheral blood), hypertriglyceridaemia and/or hypofibrinogenaemia, haemophagocytosis (in either bone marrow, spleen, lymph nodes or liver), low or absent natural killer cell activity, elevated ferritin (≥500 μg/L) and elevated sIL-2 concentrations.

Therapy consists of dexamethasone, etoposide and cyclosporine A, to suppress the hyperinflammation.[1]

Although hyperferritinaemia is associated with a wide variety of conditions, a concentration above 10,000 μg/L has a sensitivity of 90% and a specificity of 96% for HLH.[2] Therefore, falsely decreased ferritin concentrations can lead to a delay in or missing of the diagnosis of HLH, potentially leading to suboptimal or delayed therapy.

The high-dose hook effect, or prozone effect, is a laboratory phenomenon known to cause false negative or decreased results in immunoassays caused by a surplus of analyte. In heterogeneous immunoassays, antigen excess results in solely binding of each antigen to either a soluble detection antibody or a solid-phase coupled antibody. The reduction or absence of antigens sandwiched between the solid phase and detection antibodies causes the detection antibodies bound to free antigens to be washed away instead of adhering to the solid-phase, leading to an erroneously low result. In homogeneous immunoassays, the antigen excess prevents formation of large antigen–antibody complexes since every antibody is saturated with two separate antigen molecules.[3]

Analytes with broad plasma ranges in physiologic or pathologic conditions, like tumour markers and several hormones, are especially susceptible for the high-dose hook effect. Therefore, these assays are made to function properly in these broad ranges. Depending on which immune analyser is used for determining ferritin concentrations, the hook effect occurs at concentrations ranging from >40,000 μg/L to >250,000 μg/L.[4] Product specifications of the Beckman Unicel DxI, the single step immunoassay used in our cases, guarantee that the analyser is not susceptible to this phenomenon for concentrations up to 40,000 μg/L. In case a sample exceeds a concentration of 1500 μg/L, an automatic five-fold dilution is performed by the analyser. If, after this dilution, the concentration still exceeds the highest standard of the calibration curve, a result of '>7,500 μg/L' is reported and a manual dilution will be performed. This normally does not lead to any errors since in most pathological conditions ferritin concentrations do not exceed this value. However, ferritin concentrations in our first patient peaked at 387,000 μg/L and in our second patient at 138,000 μg/L and lead to falsely decreased results without any error notification.

To show the hook characteristics of the ferritin assay, plasmas of both patients were diluted. Results are shown in Figure 1. In case 1, at an expected concentration of circa 370,000 μg/L, the immunoassay 'hooked' to a reported concentration of circa 270 μg/L. The high-dose hook effect disappeared after a manual eight-fold dilution. In case 2, at an expected concentration of circa 140,000 μg/L, it 'hooked' to circa 1200 μg/L. In this case, the effect disappeared after a two-fold dilution. These dilutions show that the effect occurs at concentrations of approximately 80,000 μg/L. However, there seems to be no clear sharp cut-off value, above which reported results always are falsely decreased. This is depicted by day 25 of case 1 where

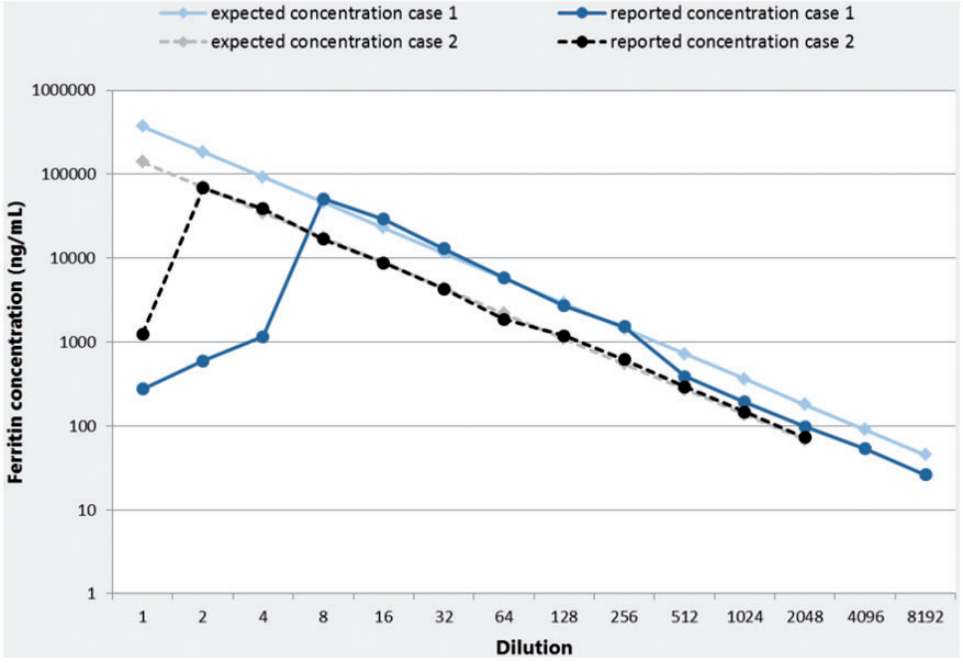

Figure 1. Ferritin concentrations vs. dilution rate.

the phenomenon did not occur at 105,000 μg/L. Because the high-dose hook effect often leads to realistic results, not being nil, recognition of falsely decreased results can be challenging.

Fortunately, in these particular cases, the falsely decreased ferritin concentrations were instantly questionable during clinical validation of patient results. Both falsely decreased results were reported on a clinical validation list because of violation of a delta check rule showing an unrealistic drop in ferritin concentrations and were therefore reanalysed after manual dilution of the samples. However, if these patients would not have had previous extremely high ferritin results, the falsely decreased results would not have been selected based on a delta check rule violation.

Several solutions for the hook effect have been proposed, like a cut-off above which samples are auto-diluted, as is the case for our assay.[4] However, if concentrations are much higher than the dilution cut-off concentration, results can be falsely decreased without any analyser flag. Another suggestion is the use of a two-step immunoassay with an extra wash before adding the detection antibody.[5] This would prevent the high-dose hook effect from occurring; in the presence of analyte excess the measurement signal will show a plateau rather than a decrease. In that case, a dilution will still be necessary to get the correct result. However, such adjustments in assay protocols can only be done by the manufacturers of the assay. Implementation of a predilution was shown to be effective in reducing the possibility of the hook effect.[6] The downside of this approach is that the lower limit of detection is increased. Doing all measurements in two different dilutions could solve this problem but will double the reagent costs of all ferritin results.

Another option is the possibility to order a diluted ferritin when extremely high concentrations are expected.[7] This, however, would not fully solve the problem since it is not always possible for clinicians to foresee high ferritin concentrations and clinicians should always be aware of this option when ordering a ferritin. Therefore, we urge manufacturers to offer a two-step immunoassay for ferritin. As long as a watertight solution to recognize falsely decreased results has not been implemented, it is important for physicians and laboratory staff to be aware of the existence of the high-dose hook effect.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Ethical approval

No written consent has been obtained since both patients did not survive the consequences of their disease.

Guarantor

GR.

Contributorship

GR wrote the initial draft of the manuscript. RR participated in patient care. HK ran manual dilutions of the samples. All authors reviewed and edited the manuscript and approved the final version of the manuscript.

ORCID iD

Geert Roozen http://orcid.org/0000-0002-3979-4616

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