

# Time-course analysis of serum hepcidin, iron and cytokines in a C282Y homozygous patient with Schnitzler's syndrome treated with IL-1 receptor antagonist

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## ABSTRACT

It is currently unknown if the increase of the hepatic iron regulatory hormone hepcidin during inflammation in man depends on an intact HFE-protein. Here we describe the temporal relationship of serum hepcidin, serum iron and cytokines in a patient with HFE-related (C282Y homozygous) hereditary hemochromatosis who was treated for an auto-inflammatory condition, i.e. variant Schnitzler's syndrome, with the potent anti-inflammatory cytokine interleukin-1 receptor antagonist (IL-1ra, anakinra). The patient had bouts of fever with peaking serum IL-6 concentrations followed by peaking serum hepcidin levels, while serum iron was low. Upon treatment, these peaks disappeared and hepcidin levels became non-detectable, consistent with HFE deficiency. In conclusion, this *in vivo* human model: i) supports the importance of an HFE-independent

IL-6-hepcidin axis in the development of hypoferrremia and anemia of inflammation; and ii) suggests that chronic inflammation protects patients with HFE-related hereditary hemochromatosis from iron accumulation.

Key words: hepcidin, iron, hemochromatosis, inflammation, interleukin-1.

Citation: van Deuren M, Kroot JJC, and Swinkels DW. Time-course analysis of serum hepcidin, iron and cytokines in a C282Y homozygous patient with Schnitzler's syndrome treated with IL-1 receptor antagonist. *Haematologica* 2009;94:1297-1300. doi: 10.3324/haematol.2009.005975

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## Introduction

One of the critical host defense strategies after infection is to withhold iron from the invading pathogen by redistribution of iron out of the circulation into macrophages and decrease of iron absorption by enterocytes.<sup>1,2</sup> This inflammation-associated reaction is mediated primarily by the hepatocellular peptide hormone hepcidin.<sup>3,4</sup> Hepatic hepcidin production is under the control of various physiological stimuli, i.e. it is down-regulated by low body iron status and erythropoietic activity and increased by pro-inflammation cytokines, particularly interleukin -6 (IL-6).<sup>5,6</sup>

Patients with *HFE*-gene related (C282Y-homozygous) hereditary hemochromatosis (HH) have an impaired hepcidin gene expression with decreased, although not uniformly low, serum hepcidin levels.<sup>7</sup> Experiments in HFE-knock out (KO) mice show that, in spite of this positive regulatory action of HFE-protein on basal hepcidin levels, HFE-protein is not required for hepcidin upregulation during inflammation.<sup>5,8,9</sup> One study, however, showed a blunted hepcidin response to inflammatory stimuli in the KO-mice.<sup>10</sup> Thus, with respect to hepcidin reg-

ulation at least two distinguishable pathways are operational: one which is HFE-protein dependent and one engaged after pro-inflammatory stimuli. To the best of our knowledge, these mouse data have not yet been confirmed by observations in humans. Here we describe the temporal relationships of serum hepcidin, serum iron and inflammatory cytokines, in a C282Y homozygous HH patient who was treated for an auto-inflammatory condition, i.e. variant Schnitzler's syndrome,<sup>11</sup> with the potent anti-inflammatory cytokine interleukin-1 receptor antagonist (anakinra).<sup>12</sup> We demonstrate the existence of an HFE-independent cytokine-hepcidin axis in the development of hypoferrremia and anemia of inflammation. Our observations further suggest that chronic inflammation countermands iron accumulation in C282Y-homozygotes.

## Case description

The patient, a 53-year old man, started having complaints in 1991, at the age of 36 years. From that time he experienced periodically malaise, myalgia, spiking fever, arthralgia, painful swelling and redness of eye lids. At first examination his spleen was slightly enlarged and laboratory analysis showed a

*Acknowledgments: we thank the patient for his consent, J. van der Ven-Jongekrijg for the cytokine assays and the team of the www.hepcidinanalysis.com initiative for fruitful discussions.*

*Manuscript received on January 14, 2009. Revised version arrived on March 6, 2009. Manuscript accepted on March 24, 2009.*

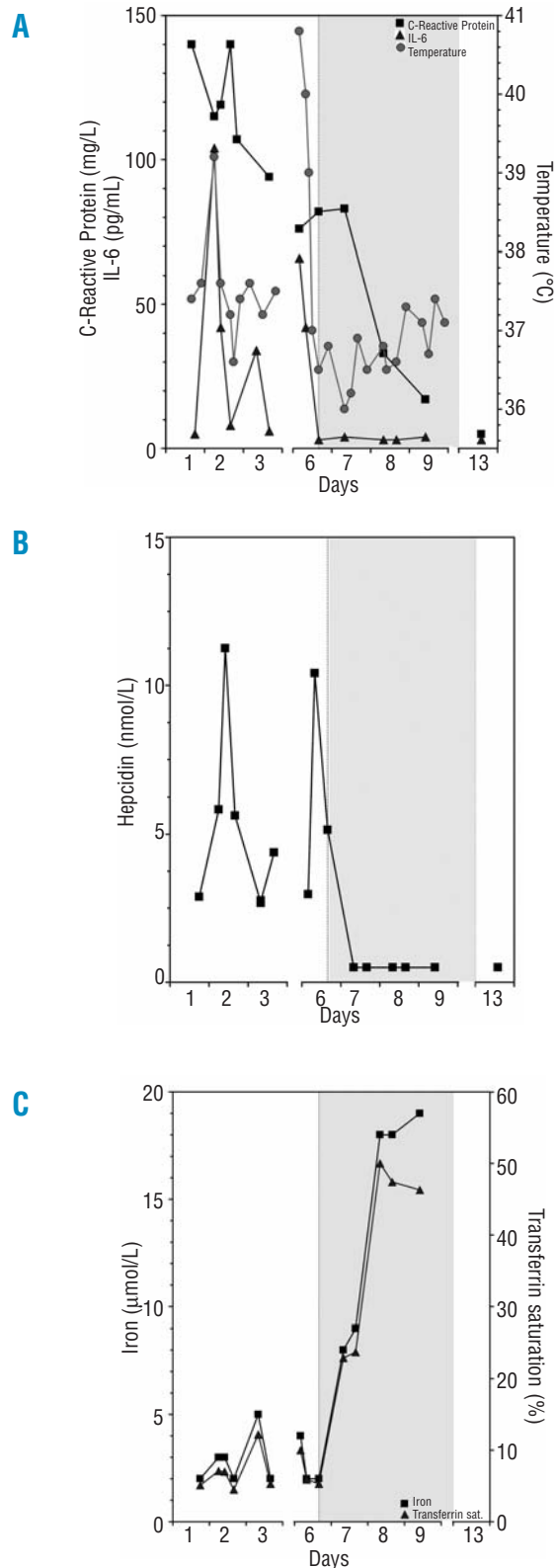
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marked IgG type- $\kappa$  monoclonal gammopathy of 21 g/L. Under the suspicion of malignant lymphoma, a splenectomy was performed with biopsy of liver and intra-abdominal lymph nodes. No malignancy could be detected. Unexpectedly, on histology spleen and liver showed signs of hemosiderosis. More specifically, in the absence of fibrosis and cirrhosis, Perls' stain was generalized and strongly positive in hepatocytes and also present in some foci of Kupffer cells. At that time ferritin was 1540  $\mu\text{g/L}$  (normal  $<280 \mu\text{g/L}$ ) and the transferrin saturation (TS) 45%. In the late 1990s, *HFE*-gene analysis disclosed homozygosity for the C282Y mutation in the patient and his brother. This confirmed the diagnosis of HFE-related HH.

Over the following year, the patient was treated with 30 phlebotomies of 500 mL each to a final ferritin level of 24  $\mu\text{g/L}$  (TS of 9.8%). Because the original physical complaints of arthralgia and periodic fever persisted, prednisone was started in a maintenance dose of 15 mg/day. Nevertheless, there were various short-lasting bouts of fever, arthralgia, red swollen eyes and auricles. In the late 1990s progressive deafness developed. The monoclonal gammopathy remained stable. From 1993 to 2007, the patient received a further 15 phlebotomies of 500 mL each. Serum ferritin levels, TS and CRP in this time interval, varied between 36 and 540  $\mu\text{g/L}$ , 11.4 and 106%, and 26 and 81 mg/L, respectively. Although these data appeared to be too incomplete to draw conclusions about a temporal relationship between inflammation episodes and iron parameters, it is clear that the patient was not always in an inflammatory state.

In 2007, at the age of 52 years, the physical complaints became progressively worse, and consisted of malaise, arthralgia, chills, fever, and short lasting generalized urticae. Clinical re-evaluation in 2008 showed, in spite of the invariably high (20-25 g/L) monoclonal gammopathy and high number (10-15%) of bone-marrow plasma cells, no other signs of malignant multiple myeloma. The bouts of fever were accompanied by IL-6 peaks followed by increased hepcidin-concentrations (Figure 1, panels A and B).

Based on the occurrence of chills with spiking fever and urticae in the presence of IgG- $\kappa$  gammopathy, the diagnosis of variant Schnitzler's syndrome was considered and it was decided to treat the patient with interleukin-1 receptor antagonist (IL-1ra; anakinra; 1<sup>st</sup> dose 300 mg i.v., followed by six days 100 mg s.c.).<sup>11,12</sup> Serum ferritin levels were around 200  $\mu\text{g/L}$  in the days before treatment, stayed between 200 and 250  $\mu\text{g/L}$  during treatment and decreased thereafter to 140, 80 and 22  $\mu\text{g/L}$ , at days 13, 17 and 36, respectively. Immediately after the start of IL-1ra all complaints disappeared. The treatment abrogated c.q. prevented the pattern of peaking fever in the following days and IL-6 and CRP normalized (Figure 1, panel A). Interestingly, serum hepcidin, clearly detectable in spite of the *HFE*-gene defect during the febrile inflammatory episode, decreased rapidly after start of IL-1ra whereas serum iron increased (Figure 1, panels B and C). IL-1 $\beta$  levels remained below 20 pg/mL before and after IL-1ra treatment (*data not shown*). The moderately increased IL-18 levels were not clearly affected by IL-1ra treatment (*data not shown*).



**Figure 1.** Laboratory measurements before and after treatment. Body temperature, CRP and IL-6 levels (panel A), serum hepcidin (panel B), iron and transferrin saturation (panel C) were measured in an iron deplete C282Y homozygous patient with an auto-inflammatory condition, i.e. variant Schnitzler's syndrome, before, during and after treatment with the potent anti-inflammatory cytokine interleukin-1 receptor antagonist (IL-1ra, anakinra). The period of IL-1ra treatment is indicated in grey.

## Design and Methods

Serum iron parameters and CRP were determined by standard laboratory analysis. Cytokines and hepcidin were assayed in one run in serum stored at  $-80^{\circ}\text{C}$ . Cytokines were measured using a multiplex Luminex assay.<sup>15</sup> Heparin was measured as described previously.<sup>14</sup> Lower limit of detection was 0.5 nmol/L. Heparin range in healthy volunteers (n=24) 0.5-13.9 nmol/L, median 4.2 nmol/L; in untreated C282Y homozygotes (n=22) <0.5-4.8 nmol/L, median 1.9 nmol/L; in C282Y homozygotes (n=4) in maintenance phase of phlebotomy (15 samples) <0.5-2.5 nmol/L.<sup>7</sup> The study was carried out in accordance with applicable Dutch regulations concerning IRB review and informed consent.

## Results and Discussion

We describe the first human *in vivo* observation of serum iron parameters, including hepcidin, in a patient with an aberrant HFE-protein and inflammation, before and after anti-inflammatory treatment. The patient presented with a combination of Schnitzler's syndrome and C282Y homozygosity of the *HFE*-gene under a regime of maintenance phlebotomies, and was subsequently treated with anti-inflammatory anti-IL-1ra.

Schnitzler's syndrome is a rare disabling auto-inflammatory disease. It is characterized by varying degrees of recurrent bouts of unexplained fever, arthralgia or arthritis, bone pain, lymphadenopathy, hepatosplenomegaly, leukocytosis, and elevated erythrocyte sedimentation rate accompanied by an urticarial rash in the presence of a monoclonal IgG or IgM gammopathy. The complaints respond well to treatment with IL-1ra.<sup>12</sup>

A biochemically penetrating C282Y homozygous patient is typically characterized by elevations of both serum ferritin and serum iron levels.<sup>15</sup> In the patient presented herein the ongoing and periodically exacerbating inflammation increased serum ferritin levels and lowered TS, at times leading to a pattern of serum iron indices less pathognomonic for HH. Thus, the iron status in the patient might be understood from a combination of HH treated with phlebotomies, and both sequestering of iron in the reticulo-endothelial (RE) macrophages and reduced intestinal iron uptake due to inflammation induced elevated hepcidin levels.

The bouts of fever and IL-6 peaks in the patient were followed by peaks of serum hepcidin. Heparin serum concentrations before treatment in our patient ranged from 2.9 to 11.2 nmol/L, high for an C282Y homozygote in the maintenance phase of phlebotomy.<sup>7</sup> Normalization of IL-6, triggered by IL-1ra treatment, led to undetectable hepcidin and higher serum iron levels. These findings confirm the (temporal) relations,

observed by us in an *in vivo* model of LPS induced inflammation,<sup>6</sup> and by Kawabata *et al.* in an IL-6 over-producing patient with multicentric Castleman's disease treated with IL-6 receptor antibodies.<sup>16</sup> In addition to this, IL-1ra may directly inhibit IL-1 dependent hepcidin production through the blockade of the IL-1 receptor.<sup>5</sup>

At first glance, our data seem to contrast with the observation that in C282Y homozygotes the total body iron does not correlate with the momentary IL-6 and CRP levels.<sup>17</sup> The latter report however can be criticized because of the different kinetics of IL-6, CRP and body iron. On the other hand, our observations of a patient with a defect of the HFE protein that can still respond to inflammatory stimuli by increasing hepcidin levels are in agreement to those described for patients with deficiency of the TfR2 protein.<sup>18</sup> Interestingly, HFE and TfR2 proteins have been found to interact in a common signal transduction pathway to hepcidin.<sup>19</sup>

From our observation it is clear that C282Y homozygosity and/or iron deficient anemia do not abrogate cytokine induced hepcidin production. However, in the absence of appropriate controls for this natural biological model as provided by the described patient, no conclusions can be made as to whether the level achieved in the patient is of the same level seen in humans without HFE-related HH. Nevertheless, the retained ability of C282Y homozygotes to increase hepcidin production upon inflammatory stimuli, and thus, according to the hepcidin mode of action,<sup>20</sup> to redistribute iron out of the bloodstream into RE macrophages, might be advantageous in the setting of infection by extracellular pathogens, but on the other hand might also increase hepatic damage through iron-induced mesenchymal activation.<sup>21</sup>

In conclusion, our *in vivo* human model of a C282Y homozygous patient with an auto-inflammatory disease treated with IL-1ra supports the importance of an HFE-independent cytokine-hepcidin axis in the development of hypoferrremia and anemia of inflammation. These findings on the cross-talk between pathways regulating hepcidin provides indirect information on the iron availability to certain pathogens relevant in critical host defense strategies.<sup>2,22-24</sup> It furthermore suggests that chronic inflammation may reduce iron stores in C282Y homozygotes, contributing to the variable penetrance of the C282Y mutation.

## Authorship and Disclosures

MvD and DWS designed research, collected and interpreted data and wrote the paper. MvD contributed patient samples. DWS and JJCK contributed laboratory tools. JJCK performed hepcidin measurements, collected and interpreted data.

The authors reported no potential conflicts of interest.

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