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Hepcidin, interleukin-6 and hematological iron markers in males before and after heart surgery☆

Michael Hoppe[a,*], Bo Lönnerdal[b], Bakhtiar Hossain[b], Sigvard Olsson[c], Folke Nilsson[d], Per-Arne Lundberg[e], Stig Rödjer[f], Lena Hulthén[a]

[a]Department of Clinical Nutrition, Sahlgrenska Academy at Göteborg University, S-405 30 Gothenburg, Sweden
[b]Department of Nutrition and Program of International Nutrition, University of California, Davis, CA, USA
[c]Department of Medicine, Sahlgrenska University Hospital, Gothenburg, Sweden
[d]Department of Transplantation and Liver Surgery, Sahlgrenska University Hospital, Gothenburg, Sweden
[e]Clinical Chemistry Laboratory, Sahlgrenska University Hospital, Gothenburg, Sweden
[f]Department of Hematology and Coagulation, Sahlgrenska University Hospital, Gothenburg, Sweden

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Abstract

Anemia of inflammation in patients with acute or chronic acute-phase activation is a common clinical problem. Hepcidin is a peptide shown to be the principal regulator of the absorption and systemic distribution of iron. Main inducers of hepcidin are iron overload, hypoxia and inflammation, where the latter has been linked to hepcidin via increased interleukin-6 (IL-6).

This article addresses the impact and time course of postoperative acute-phase reaction in humans following heart surgery on prohepcidin, hepcidin, hematological markers and IL-6 concentrations.

Serum concentrations of prohepcidin, hepcidin, IL-6 and hematological iron parameters were studied in five male patients without infection before and after heart surgery.

This study, which is the first to report the impact on serum hepcidin and serum prohepcidin concentrations in patients following surgery, clearly demonstrates the induction of hypoferremia due to the postoperative acute-phase reaction. Significant changes were seen for serum iron concentration, transferrin saturation, total iron binding capacity and hemoglobin concentration. A significant increase in ferritin concentration was seen 96–144 h postoperatively. Additionally, there were significant alterations in both serum hepcidin after 96–144 h and serum prohepcidin after 48 h compared with preoperative values. Serum prohepcidin decreased, whereas serum hepcidin increased.

In conclusion, changes in serum prohepcidin were followed by an increase in serum hepcidin. This speaks in favor of a chain of action where proteolytic trimming of serum prohepcidin results in increased serum hepcidin. However, hypoferremia appeared prior to the changes in serum prohepcidin and serum hepcidin.

Keywords: Hepcidin; Interleukin-6; Anemia of inflammation; Surgery

1. Introduction

The so-called anemia of inflammation (or anemia of chronic disease) in patients with acute or chronic acute-phase activation has been the subject of thorough investigation for more than 60 years [1]. Although knowledge of inflammation–hypoferremia pathophysiology has expanded over the years, further increased understanding of this common clinical problem is needed [2].

Hepcidin is a recently discovered antimicrobial peptide synthesized in the liver and is proposed to be the key

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* Corresponding author. Tel.: +46 31 786 36 34; fax: +46 31 82 94 75.
E-mail address: michael.hoppe@nutrition.gu.se (M. Hoppe).

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mediator of iron metabolism and systemic distribution. It is synthesized by hepatocytes in response to both inflammatory stimuli and iron overload [3–5] and acts by down-regulating both iron absorption and iron release by enterocytes and macrophages [6,7]. In healthy subjects, hepatic hepcidin production is regulated in a feedback loop manner by circulating iron. Since its discovery, research on hepcidin has increased considerably; however, to date, much of our knowledge comes from animal and cell models and there are limited data on hepcidin in human subjects. Additionally, enhancement of our understanding concerning the chronological order of the inflammation–hepcidin–hypoferremia axis is needed — especially when it comes to the interplay between prohepcidin and its metabolites.

Surgical procedures induce an inflammatory reaction commonly known as the postoperative acute-phase reaction [8], which in turn affects a number of acute-phase proteins, such as ferritin [9] and the pleiotropic cytokine interleukin-6 (IL-6) [10]. Since IL-6 has been proposed as a major inducer of hepcidin [11,12], via STAT3 (signal transducers and activators of transcription protein 3) [13], this work addressed questions regarding the impact of major surgery on prohepcidin, hepcidin, hematological iron markers and IL-6 in humans.

2. Materials and methods

2.1. Patients

Serum specimens were collected from five male patients without infection [IL-6, <5 ng/L; C-reactive protein (CRP), <5 mg/L] and aged between 70 and 79 years. These patients were chosen due to their planned operation in a cardiopulmonary bypass pump. Coronary artery bypass grafting was performed in three patients, whereas aortic valve replacement with biological prosthesis was performed in two. Due to autotransfusion, none of the subjects experienced net blood loss during their operation. One patient (Patient 2) received exogenous blood transfusion after the procedure. The study protocol was approved by the regional ethics review board in Gothenburg (Registration No. 651-06).

2.2. Blood samples

Blood samples were collected at three time points: (I) preoperatively (10 days before in Patient 1 and 1 day before in the other four patients); (II) 2 days postoperatively; and (III) 4–6 days postoperatively. For each patient, blood samples for determination of prohepcidin, hepcidin, IL-6, CRP, serum iron, serum ferritin, hemoglobin, total iron binding capacity (TIBC) and transferrin saturation were collected preoperatively and 2 days postoperatively (Fig. 1). In addition, blood samples for determination of these same parameters, except for CRP, were collected on days 4–6 (Fig. 1).

2.3. Assays

Serum IL-6 was determined using enzyme-linked immunosorbent assay (ELISA) (Human IL-6 ELISA Ready-SET-Go! Kit, eBioscience, San Diego, CA, USA).

The propeptide of hepcidin in serum was assayed by using a commercial ELISA kit by DRG Instruments (Marburg, Germany).

Hepcidin in serum was determined using ELISA. Human hepcidin peptide (DTNFPICLFCCKCCKNSSCGLCCIT) was synthesized with an additional cysteine residue at the C terminus for conjugation to keyhole limpet hemocyanin (Genemed Synthesis, San Francisco, CA, USA). The sequence of the peptide was verified by amino acid sequencing and mass spectroscopy. The purity of the peptide was >95%. Keyhole limpet hemocyanin-conjugated peptide was injected into New Zealand white rabbits (1 mg of peptide per rabbit) to produce the primary antibody (Genemed Synthesis). Ninety-six-well plates (MaxiSorp surface, Nalge Nunc International, Rochester, NY, USA) were coated with the peptide (100 ng/well) overnight at 4°C. The coating buffer was 50 mM $NaHCO_3$, pH 9.6. On the following morning, plates were washed four times with 0.1% phosphate-buffered saline (PBS)–Tween 20 and blocked with 1% bovine serum albumin (Sigma, St. Louis, MO, USA) in PBS–Tween 20 (100 μl/well) for 1 h at room temperature. A standard curve was produced by adding 0–500 ng of peptide per well with 1000× diluted primary antibody in each plate. Serum samples from human subjects were diluted 50× with 1000× diluted primary antibody (in PBS–Tween 20) and added to the plate (100 μl/well). After 1-h incubation, plates were washed four times with PBS–Tween 20. Horseradish peroxidase-conjugated secondary antibody (1000×; Amersham Biosciences, Piscataway, NJ, USA) was added to each well (100 μl/well) and incubated for 1 h at room temperature. Plates were washed four times with PBS–Tween 20, and 100 μl of TMB (3,3′,5,5′-tetramethylbenzidine, Sigma) substrate was added to each well. After 6 min, the reaction was stopped with 100 μl of 2N H_2SO_4 and the plates were read at 450 nm. Samples were analyzed in duplicate. The concentration of hepcidin in serum was calculated from the standard curve.

CRP, serum iron, serum ferritin, hemoglobin, TIBC and transferrin saturation analyses were conducted at an accredited reference laboratory (Clinical Chemistry Laboratory, Sahlgrenska University Hospital, Gothenburg, Sweden).

2.4. Statistics

The values provided in Section 3 represent means and standard deviations.

Normality test was done using a Shapiro–Wilk test. One-way repeated-measures analysis of variance and Student's paired t test were used when comparing means. Correlations were tested using the Pearson correlation test. All P values are two tailed. The statistical

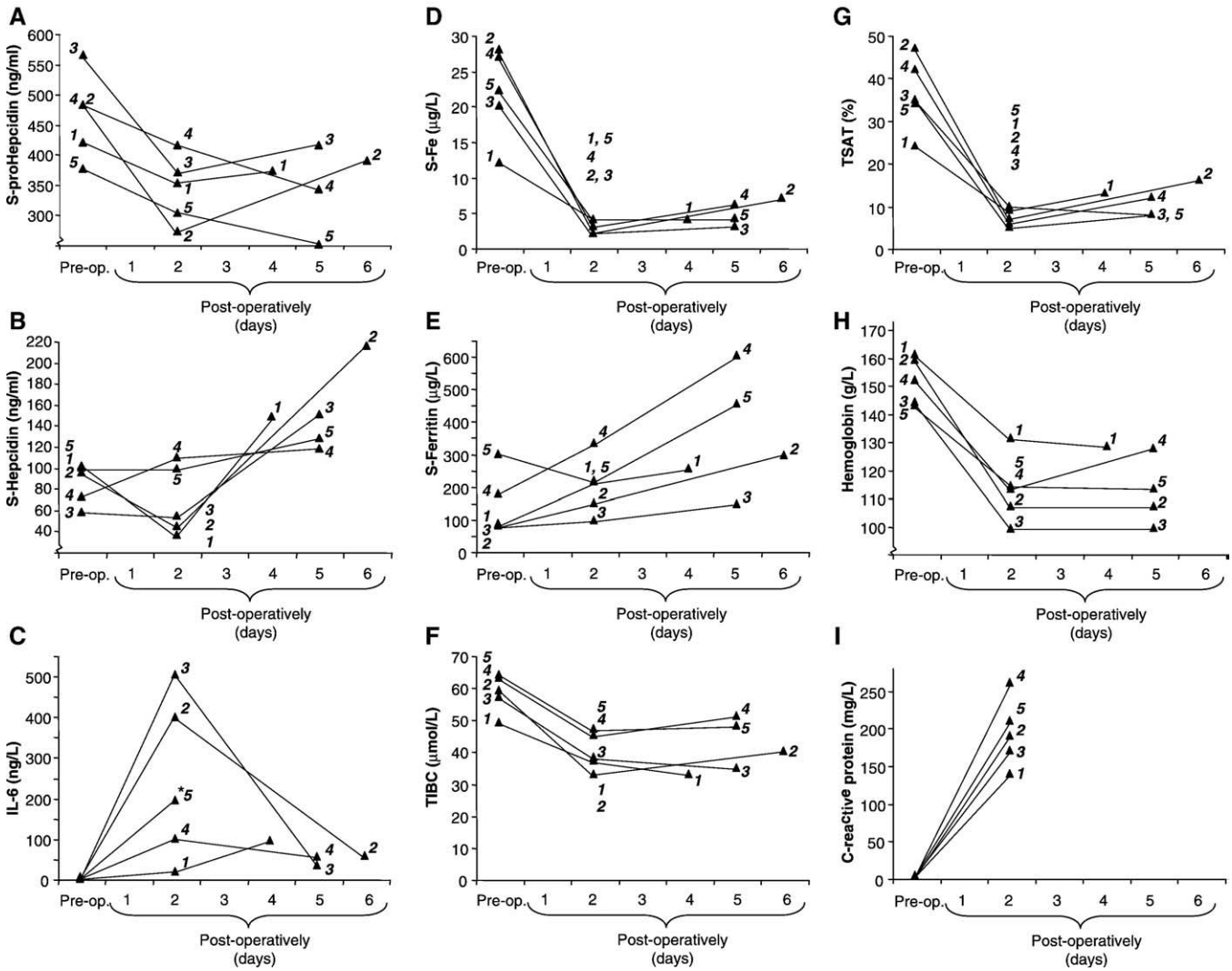

Fig. 1. Laboratory measurements. Blood samples for determination of (A) prohepcidin, (B) hepcidin, (C) IL-6, (D) serum iron, (E) serum ferritin, (F) TIBC, (G) transferrin saturation and (H) hemoglobin were drawn preoperatively (10 days before in one patient and 1 day before in the other four patients) and 2 days and 4–6 days postoperatively. Blood samples for determination of (I) CRP were drawn preoperatively (10 days before in one patient and 1 day before in the other four patients) and 2 days postoperatively. The asterisk indicates that, unfortunately, an error occurred at the laboratory analysis of one of the IL-6 samples, resulting in a missing value for the 5-day postoperative occasion in one patient. The values (1–5) represent each of the five patients.

program used was SPSS for Windows, version 11.5.1 (SPSS, Chicago, IL, USA).

3. Results

3.1. Serum prohepcidin

The serum prohepcidin concentration decreased significantly 2 days postoperatively (344±56 ng/ml) ($P<.021$) compared with the value before the surgical procedure (468±74 ng/ml). At the next time point (i.e., 4–6 days postoperatively) (356±63 ng/ml), there was no concordance concerning further changes in prohepcidin. The concentrations in two patients were still on a decrease, whereas those in the other three turned upward again. However, none of the patients showed a postoperative return of serum prohepcidin to preoperative baseline concentrations (Fig. 1). Combining samples for days 4–6 postoperation of the third blood sampling occasion, serum prohepcidin concentrations were still significantly lower compared with preoperative values ($P<.005$).

3.2. Serum hepcidin

From preoperation to 2 days postoperation, serum hepcidin rose in one subject, whereas it descended in two patients and remained practically unchanged in the other two (mean change=not significant) (Fig. 1). Combining samples for days 4–6 postoperation of the third blood sampling occasion, there was a significant rise in serum hepcidin at the third blood sampling occasion (150±38 ng/ml) compared with both preoperation (84±20 ng/ml) and 2 days postoperation (67±33 ng/ml) ($P<.023$ and $P<.047$, respectively).

3.3. IL-6 and CRP

From preoperation to 2 days postoperation, CRP rose in all patients (<5 to 194±45 mg/L, $P<.001$). Postoperatively, IL-6 increased in all patients (2±1 to 243±203 ng/L). However, even though the minimum rise in IL-6 was 17 ng/L, it was not statistically significant ($P<.058$). Four to six days postoperatively (60±25 ng/L, final data point missing for one patient), compared with preoperatively, there was a significant increase in IL-6 ($P<.019$).

3.4. Serum iron, transferrin saturation and TIBC

Two days and 4–6 days postoperatively, there were significant decreases in serum iron concentration (3±1 μg/L, $P<.004$, and 5±2 μg/L, $P<.002$, respectively), transferrin saturation (7±2%, $P<.003$, and 11±3%, $P<.002$, respectively) and TIBC (40±6 μg/L, $P<.001$, and 41±8 μg/L, $P<.001$, respectively) compared with preoperative values (serum iron=22±6 μg/L, transferrin saturation=36±9% and TIBC=58±6 μg/L). There was no significant difference in values between 2 days and 4–6 days postoperatively.

3.5. Serum ferritin

Serum ferritin concentration increased (nonsignificantly at $P<.257$) 48 h postoperatively in four of the five patients. Four to six days postoperatively (351±180 μg/L), there was a significant increase in ferritin compared with preoperative (145±95 μg/L) and day 2 postoperative (202±89 μg/L) values ($P<.028$ and $P<.035$, respectively).

3.6. Hemoglobin concentration

From preoperation (152±8 g/L) to 2 days (113±12 g/L) and 4–6 days (115±12 g/L) postoperation, hemoglobin concentrations decreased significantly ($P<.001$ and $P<.002$, respectively).

3.7. Correlations

The only significant correlations observed were on day 2 postoperation between serum hepcidin and CRP ($r^2=.81$, $P<.036$) as well as between serum hepcidin and TIBC ($r^2=.83$, $P<.031$).

No pattern could be established between the specific type of heart surgery or blood transfusion and hematology, IL-6, serum prohepcidin or serum hepcidin.

4. Discussion

This work reports the impact on both serum hepcidin and serum prohepcidin concentrations in patients following a major surgery. To the best of our knowledge, this is the first time the effect from an acute-phase reaction on hepcidin and prohepcidin has been studied during a longer period (up to 4–6 days postoperation). In this article, we clearly demonstrate the induction of the well-known postoperative acute-phase reaction. Additionally, there were significant alterations in both serum hepcidin and serum prohepcidin concentrations postoperatively compared with preoperatively.

Possible limitations of this study include the small sample size, variability in the causes of the surgeries and possibility of hemolysis impacting the study findings. When using the cardiopulmonary bypass pump in surgical procedures taking several hours, there is a degree of mechanical hemolysis that can affect hematological biomarkers. However, in the patients described in this article, the bypass pump was used for less than 1 h and the degree of mechanical hemolysis was minimal, thus not affecting the findings. Furthermore, since the two surgical procedures used are equal when it comes to inducing the postoperative acute-phase reaction, which was the primary concern of this study, and since hemolysis was minimal, the causes of the surgeries most likely do not affect the study findings.

In the clinic, there are a variety of conditions in which iron metabolism is negatively altered as a response to inflammation [14–17]. A prolonged effect of this inflammation-induced hypoferremia is the so-called anemia of inflammation, which is characterized by decreased serum iron, TIBC, transferrin saturation and hemoglobin [18] as well as elevated ferritin [19]. Studies have shown that this can be difficult to correct [20].

CRP, IL-6 and ferritin are all referred to as acute-phase reactants. However, the time scales of their responses differ widely, with ferritin considered as the slowest of the three. Observations in patients have shown that, despite significant alterations in CRP and IL-6, changes in ferritin kinetics were delayed [21,22]. In this study, we found that ferritin concentrations increased 48 h postoperatively in all patients except for one patient who had a rather high initial ferritin concentration (298 μg/L).

Our observations seem to be in agreement with those of earlier studies showing that CRP starts to increase within the first 24 h following an acute-phase reaction and peaks on the second postoperative day [23,24]. Unlike ferritin, which can be increased for weeks following an acute-phase reaction [19,25], the decline back to baseline is faster for CRP [23,24].

All patients in this study had baseline concentrations of IL-6 below what is considered the upper limit of normal (i.e., 5 ng/L) [26–28]. At both 48 and 96–144 h postoperation, all patients had increased IL-6 concentrations compared with their preoperative values. However, even though mean IL-6 was substantially higher, the 48-h postoperative mean increase was not statistically significant compared with the considerably lower mean IL-6 96–144 h postoperatively. Nevertheless, since the normal IL-6 value is <5 ng/L and since the minimum rise in IL-6 was 17 ng/L, there was a clear clinically relevant change. Earlier observations have shown that IL-6 reaches a maximum 4–6 h postoperatively and returns toward preoperative concentrations after 48–120 h [23,29]. Consequently, IL-6 had most likely already peaked and was on a decline when the first postoperative blood samples were drawn in this study.

Following heart surgery, serum prohepcidin concentrations decreased significantly after 48 h and remained low for the 4- to 6-day observation period (Fig. 1). In a recent experimental lipopolysaccharide model, the investigators did not observe any change in serum prohepcidin during a shorter time frame of 22 h, although there was a tendency toward a decrease at the final observation [21]. Accordingly, this indicates that serum prohepcidin decreases following an acute-phase reaction but that it is not to be expected until after >22 h, and at 48 h at most.

With regard to time course changes in serum hepcidin following an acute-phase reaction, there is, to our best knowledge, no such previous information available. It is known that serum hepcidin does not appear to correlate as well with clinical diagnosis using urinary hepcidin [30]. It has been proposed that serum hepcidin might have a conformation different from that of urinary hepcidin [31]. Our serum hepcidin data show a different behavior compared with earlier observed urinary hepcidin data, which have shown a rapid increase at the onset of an acute phase.

The relationship between prohepcidin and hepcidin is of interest with regard to regulation of iron homeostasis. The possibility that serum prohepcidin is a biological nonfunctional precursor of the active hepcidin has previously been raised [32,33]. It has been demonstrated in mice that it is the truncated 25-amino acid form of hepcidin that exercises the hypoferremia effect [34]. Furthermore, a previous study that assessed the relation between iron absorption and prohepcidin concentrations in healthy women did not see any correlation [35]. Thus, a plausible interpretation of the present serum prohepcidin data showing a decrease following surgery is that serum prohepcidin (as insulin and proteases) is synthesized as an inactive precursor that, in "times of need," is proteolytically trimmed to be activated. Common characteristics for proteins that are synthesized as propeptides are that they are rapidly required for maintaining homeostasis and that their synthesis rate is inadequate, necessitating a pool of available precursors. Considering the rapid action executed by hepcidin, this could theoretically also be the case for hepcidin and iron metabolism regulation. However, the present results, in combination with those of Kemna et al. [21], indicate that changes in serum prohepcidin concentrations take place >18 h later compared with urinary hepcidin as studied by other groups. Consequently, the current chain of action where serum prohepcidin is converted into active hepcidin present in urine following an induced acute-phase reaction seems not to be the case. Serum hepcidin, on the other hand, could be an object of this mechanism.

Since there are rather large time spans from presurgery to 2 days and 4–6 days postsurgery, potential events in-between these observation points would have been left undetected. This opens up for the possibility that serum prohepcidin is proteolytically trimmed shortly before the 48-h observation point, thus displaying an initial decrease at just this observation point. Similarly, it might be possible that serum hepcidin concentrations start to increase shortly after the 48-h observation point. If this is in fact what took place, it speaks in favor of a chain of action where proteolytic trimming of serum prohepcidin results in increased serum hepcidin. However, since the hematology was altered at the 48-h observation point (i.e., at latest shortly before 48 h), it means that hypoferremia occurred prior to the changes in serum prohepcidin and serum hepcidin, making these observations enigmatic. Perhaps some clues can be found in the renal tubule synthesis of hepcidin [36].

In summary, the hematology of the patients undergoing heart surgery shows a clear and distinct alteration in response to the postoperative acute-phase reaction. Serum ferritin increased, whereas serum iron, TIBC, transferrin saturation and hemoglobin all decreased, resulting in the well-known anemia of infection. However, even though there were significant alterations in both hepcidin and prohepcidin serum concentrations, a relationship with the hematological alterations that had occurred 2 days postsurgery still needs to be established. Also, although based on a small sample, these results provide valuable insight into how to design future studies, including more frequent sampling and thorough studies of the chronological order of the markers involved in the inflammation–hepcidin–hypoferremia axis.

References

[1] Cartwright GE, Lauritsen MA, Jones PJ, Merrill IM, Wintrobe MM. The anemia of infection: I. Hypoferremia, hypercupremia, and alterations in porphyrin metabolism in patients. J Clin Invest 1946; 25(1):65–80.
[2] Weiss G, Goodnough LT. Anemia of chronic disease. N Engl J Med 2005;352(10):1011–23.
[3] Krause A, Neitz S, Magert HJ, Schulz A, Forssmann WG, Schulz-Knappe P, et al. LEAP-1, a novel highly disulfide-bonded human peptide, exhibits antimicrobial activity. FEBS Lett 2000;480(2–3): 147–50.
[4] Park CH, Valore EV, Waring AJ, Ganz T. Hepcidin, a urinary antimicrobial peptide synthesized in the liver. J Biol Chem 2001;276 (11):7806–10.
[5] Pigeon C, Ilyin G, Courselaud B, Leroyer P, Turlin B, Brissot P, et al. A new mouse liver-specific gene, encoding a protein homologous to human antimicrobial peptide hepcidin, is overexpressed during iron overload. J Biol Chem 2001;276(11):7811–9.
[6] Ganz T. Hepcidin, a key regulator of iron metabolism and mediator of anemia of inflammation. Blood 2003;102(3):783–8.
[7] Nemeth E, Tuttle MS, Powelson J, Vaughn MB, Donovan A, Ward DM, et al. Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. Science 2004;306(5704): 2090–3.
[8] Pien F, Ho PW, Fergusson DJ. Fever and infection after cardiac operation. Ann Thorac Surg 1982;33(4):382–4.
[9] Rubin C, Wood PJ, Archer T, Rowe DJ. Changes in serum ferritin and other 'acute phase' proteins following major surgery. Ann Clin Biochem 1984;21(Pt 4):290–4.
[10] Roth-Isigkeit A, Borstel TV, Seyfarth M, Schmucker P. Perioperative serum levels of tumour-necrosis-factor alpha (TNF-alpha), IL-1 beta, IL-6, IL-10 and soluble IL-2 receptor in patients undergoing cardiac surgery with cardiopulmonary bypass without and with correction for haemodilution. Clin Exp Immunol 1999;118(2):242–6.
[11] Nemeth E, Valore EV, Territo M, Schiller G, Lichtenstein A, Ganz T. Hepcidin, a putative mediator of anemia of inflammation, is a type II acute-phase protein. Blood 2003;101(7):2461–3.

[12] Nemeth E, Rivera S, Gabayan V, Keller C, Taudorf S, Pedersen BK, et al. IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. J Clin Invest 2004; 113(9):1271–6.
[13] Wrighting DM, Andrews NC. Interleukin-6 induces hepcidin expression through STAT3. Blood 2006;108:3204–9.
[14] Chant C, Wilson G, Friedrich JO. Anemia, transfusion and phlebotomy practices in critically ill patients with prolonged ICU length-of-stay: a cohort study. Crit Care 2006;10(5).
[15] Jurado RL. Iron, infections, and anemia of inflammation. Clin Infect Dis 1997;25(4):888–95.
[16] Corwin HL, Krantz SB. Anemia of the critically ill: "acute" anemia of chronic disease. Crit Care Med 2000;28(8):3098–9.
[17] Solis JV, Portero JL, Diaz J, Garcia R, Ligero JM, Vazquez E, et al. Iron deficiency in the acute-phase reaction after open aortic surgery. Vasc Endovascular Surg 2006;40(5):392–8.
[18] Weinberg ED. Iron and infection. Microbiol Rev 1978;42(1):45–66.
[19] Hulthen L, Lindstedt G, Lundberg PA, Hallberg L. Effect of a mild infection on serum ferritin concentration — clinical and epidemiological implications. Eur J Clin Nutr 1998;52(5):376–9.
[20] Madi-Jebara SN, Sleilaty GS, Achouh PE, Yazigi AG, Haddad FA, Hayek GM, et al. Postoperative intravenous iron used alone or in combination with low-dose erythropoietin is not effective for correction of anemia after cardiac surgery. J Cardiothorac Vasc Anesth 2004;18(1):59–63.
[21] Kemna EH, Pickkers P, Nemeth E, van der Hoeven H, Swinkels D. Time-course analysis of hepcidin, serum iron, and plasma cytokine levels in humans injected with LPS. Blood 2005;106(5): 1864–6.
[22] Feelders RA, Vreugdenhil G, Eggermont AM, Kuiper-Kramer PA, van Eijk HG, Swaak AJ. Regulation of iron metabolism in the acute-phase response: interferon gamma and tumour necrosis factor alpha induce hypoferraemia, ferritin production and a decrease in circulating transferrin receptors in cancer patients. Eur J Clin Invest 1998;28(7): 520–7.
[23] Bruins P, te Velthuis H, Yazdanbakhsh AP, Jansen PG, van Hardevelt FW, de Beaumont EM, et al. Activation of the complement system during and after cardiopulmonary bypass surgery: postsurgery activation involves C-reactive protein and is associated with postoperative arrhythmia. Circulation 1997;96(10):3542–8.
[24] Berger D, Bolke E, Seidelmann M, Beger HG. Time-scale of interleukin-6, myeloid related proteins (MRP), C reactive protein (CRP), and endotoxin plasma levels during the postoperative acute phase reaction. Shock 1997;7(6):422–6.
[25] Eskeland B, Baerheim A, Ulvik R, Hunskaar S. Influence of mild infections on iron status parameters in women of reproductive age. Scand J Prim Health Care 2002;20(1):50–6.
[26] Walsh BW, Cox DA, Sashegyi A, Dean RA, Tracy RP, Anderson PW. Role of tumor necrosis factor-alpha and interleukin-6 in the effects of hormone replacement therapy and raloxifene on C-reactive protein in postmenopausal women. Am J Cardiol 2001;88(7):825–8.
[27] Brambilla F, Monti D, Franceschi C. Plasma concentrations of interleukin-1-beta, interleukin-6 and tumor necrosis factor-alpha, and of their soluble receptors and receptor antagonist in anorexia nervosa. Psychiatry Res 2001;103(2–3):107–14.
[28] Blade J, Filella X, Montoto S, Bosch F, Rosinol L, Coca F, et al. Interleukin 6 and tumour necrosis factor alpha serum levels in monoclonal gammopathy of undetermined significance. Br J Haematol 2002;117(2):387–9.
[29] Hewitt PM, Ip SM, Kwok SP, Somers SS, Li K, Leung KL, et al. Laparoscopic-assisted vs. open surgery for colorectal cancer: comparative study of immune effects. Dis Colon Rectum 1998;41(7): 901–9.
[30] Dallalio G, Fleury T, Means RT. Serum hepcidin in clinical specimens. Br J Haematol 2003;122(6):996-1000.
[31] Kemna E, Tjalsma H, Wetzels JF, Swinkels DW. Measuring serum hepcidin concentrations. Hepatol 2005;2(4).
[32] Taes YE, Wuyts B, Boelaert JR, De Vriese AS, Delanghe JR. Prohepcidin accumulates in renal insufficiency. Clin Chem Lab Med 2004;42(4):387–9.
[33] Brookes MJ, Sharma NK, Tselepis C, Iqbal TH. Serum pro-hepcidin: measuring active hepcidin or a non-functional precursor? Gut 2005;54 (1):169–70.
[34] Rivera S, Nemeth E, Gabayan V, Lopez MA, Farshidi D, Ganz T. Synthetic hepcidin causes rapid dose-dependent hypoferremia and is concentrated in ferroportin-containing organs. Blood 2005;106(6): 2196–9.
[35] Hadley KB, Johnson LK, Hunt JR. Iron absorption by healthy women is not associated with either serum or urinary prohepcidin. Am J Clin Nutr 2006;84(1):150–5.
[36] Kulaksiz H, Theilig F, Bachmann S, Gehrke SG, Rost D, Janetzko A, et al. The iron-regulatory peptide hormone hepcidin: expression and cellular localization in the mammalian kidney. J Endocrinol 2005;184 (2):361–70.