Opinion Paper

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Hepcidin levels in chronic hemodialysis patients: a critical evaluation

Abstract: Altered systemic iron metabolism is a key element of uremia, and functional iron deficiency mainly related to subclinical inflammation makes it difficult to maintain proper control of anemia in chronic hemodialysis patients (CHD). In the last decade, the hepatic hormone hepcidin has been progressively recognized as the master regulator of circulating iron levels through the modulation of cellular iron fluxes in response to iron stores, as well as to erythroid and inflammatory stimuli. Hepcidin is cleared by the kidney and progression of renal disease has been associated to increased serum hepcidin levels. This, in turn, reduces iron availability for erythropoiesis, suggesting anti-hepcidin strategies for improving anemia control. Moreover, hepcidin has been recently implicated in the pathogenesis of long-term complications of dialysis, like accelerated atherosclerosis. Initial studies almost invariably reported a sustained increase of serum hepcidin in chronic hemodialysis patients. Noteworthy, such studies included relatively few patients and controls that were poorly matched for major determinants of serum hepcidin at population level, i.e., age and gender. More recent data based on accurately matched larger series challenge the view that hepcidin is intrinsically increased in hemodialysis patients, showing a marked inter- and intra-individual variability of hormone levels. Here we take a critical look to the data published so far on hepcidin levels in CHD, analyze the reasons underlying the discrepancies in available studies and the hepcidin variability in CHD, and point out the need for further studies in large series of well-characterized CHD patients and controls.

Keywords: anemia; chronic hemodialysis; chronic kidney disease; hepcidin; iron.

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List of abbreviations

CBC, complete blood count; CHD, chronic hemodialysis; CKD, chronic kidney disease; CRP, C-reactive protein; Epo, erythropoietin; ESAs, erythropoiesis stimulating agents; FT, ferritin; HFE, hemochromatosis gene; MS, mass spectrometry; TMPRSS6, Matriptase-2 gene; TS, transferrin saturation %; VBS, Val Borbera study.

Anemia and iron status in chronic hemodialysis patients

Anemia is observed in nearly all chronic hemodialysis (CHD) patients, and is associated with increased morbidity and mortality. The pathogenesis is multifactorial [1], but a deficiency of erythropoietin (Epo) due to kidney injury is thought to play a major role, concomitant with reduced red blood cells life span, bleeding diathesis due to platelet dysfunction, and deficiency of iron, folate, and cobalamin. However, the persistence of anemia despite Epo, iron and vitamin supplementation suggests a concomitant bone marrow hypo-responsiveness in these patients. Indeed, another major determinant of the anemia in CHD is the chronic inflammatory state, in which cytokines further decrease Epo production, induce apoptosis of erythroid precursors, and also reduce iron absorption and availability for erythropoiesis [2]. CHD is typically associated with alterations of circulating markers of iron metabolism, namely decreased transferrin saturation and increased serum ferritin levels, mostly due to iron retention in monocytes/macrophages [3]. The consequence is "functional" iron deficiency, which results in Epo resistance in 10%–20% of cases, even in the presence

of apparently increased body iron stores, poorly available to the erythron. Routine monitoring of biochemical iron parameters and indices of adequate hemoglobinization of erythrocytes (e.g., transferrin saturation, mean corpuscular volume of erythrocytes) is therefore an essential component of the management of CHD patients, attempting to provide adequate supplementation, when needed.

Hepcidin as a player in the anemia of CHD

In the last decade, it has been progressively clarified that hepcidin, a small peptide hormone mainly produced by the liver, represents the principal regulator of systemic iron metabolism (for a review, see [4]), via its ability to bind the cellular iron exporter ferroportin-1, blocking its iron transport activity and increasing its degradation [5]. In enterocytes, hepcidin-induced ferroportin-1 internalization from the basolateral surface causes the retention of iron with subsequent loss by cell desquamation and inhibition of absorption, while the same process in macrophages blocks the release of iron recycled from erythrophagocytosis [5]. Since the latter represents the primary source of iron in plasma [6], the final effect of hepcidin is reduction of plasma iron availability. Noteworthy, hepcidin is upregulated by both increased iron stores and inflammation, whereas it is reduced in response to iron deprivation, hypoxia and enhanced erythropoiesis [7]. In addition, hepcidin is cleared by the kidneys, and progression of chronic renal disease has been associated with a parallel rise in serum hepcidin levels [8–10]. This increase is partly counteracted by active clearance of this small hormone peptide during the dialytic procedure [11–13]. The clearance of hepcidin is likely to be substantially influenced by the type of hemodialysis technique [12, 13], but this remains an area of further active research. Nevertheless, the majority of studies available until the beginning of this year have reported increased pre-dialytic serum hepcidin levels in CHD patients as compared to controls [10–12, 14–20]. It has therefore been hypothesized that in CHD patients increased hepcidin could aggravate functional iron deficiency by decreasing macrophage iron release and intestinal iron absorption, paving the way to the possible utilization of anti-hepcidin drugs for optimizing the management of anemia [9, 21]. In regards to this point, it is of interest to note that administration of erythropoiesis stimulating agents (ESAs), by itself, has been reported to reduce hepcidin in patients with chronic kidney disease [15, 22]. This is likely due to hypoxia-inducible factor via Epo-induced erythropoiesis [23], and suggests that the beneficial effect of Epo in CHD may be partly mediated by normalization of iron delivery.

Is hyperhepcidinemia a constant and distinct feature of CHD? Novel data confuting the early reports

Available studies that reported circulating hepcidin levels in CHD patients as compared to healthy controls, including some previous ones from our group, are presented in Table 1. They nearly all invariably reported increased hepcidin levels in CHD. In most of these studies, hepcidin was measured by mass spectrometry-based assays, which are currently considered among the most accurate ones for measuring hepcidin-25, the iron bioactive isoform of the hormone [25, 26]. Other well-performing immunoassays [26, 27], while generally showing a high correlation with validated mass spectrometry-based assays, are characterized by variable degree of cross-reactivity with minor circulating hepcidin isoforms like hepcidin-20 [28]. The biological meaning of these isoforms, lacking amino acids at the N-terminus essential for proper functional interaction with ferroportin [29], is still uncertain. Nevertheless, they also have been reported unusually elevated in renal patients [12, 26]. Of note, nearly all the early studies on CHD were characterized by the evaluation of a limited number of patients (<100), and even smaller number of controls, which in the majority of cases were not adequately matched for demographic features (Table 1). Similarly, these controls were not screened for the presence of other potential confounders, such as the presence of subclinical iron deficiency and/or common genetic variants influencing iron metabolism, i.e., mutation in the HFE gene (Table 1). Our most recent results [24], using a widely validated mass spectrometry-based assay [30] in one of the largest CHD cohort (n=155) reported so far, did not detect a clear and generalized increase of hepcidin-25 levels as compared to healthy controls (n=188) from the Val Borbera Study [31], who were meticulously matched for age, gender, and genetic background. The discrepancy as compared to apparently uniform data from previous reports may be explained by at least two orders of reasons. The first deals with the above-mentioned relatively low number of subjects included, and, in particular, with the poor matching between CHD patients and controls as regards to age and gender. Indeed, while renal patients are usually elderly, controls were generally enrolled

among young people (Table 1). In most of the studies, the mean age of controls was around 35 years, while that of CHD patients was ≥60 years. Two recently completed large population surveys, the Val Borbera Study [31] and the Nijmegen Biomedical Study [32], including 1657 and 2998 subjects, respectively, have concordantly demonstrated that age and gender are major determinants of serum hepcidin levels. This is particularly important for females, as it has been pointed out that post-menopausal women have hepcidin levels about three-fold higher than pre-menopausal women [31, 32]. Due to this new awareness, in our most recent study [24] we devoted particular accuracy in selecting an appropriate number of controls (the largest reported so far) from the Val Borbera dataset, to allow proper matching with CHD patients for age and gender.

The second reason for discrepancy with earlier reports is related to the iron status of both CHD patients and controls. All previous studies concordantly suggested that the regulation of hepcidin by iron stores is preserved in CHD patients, as illustrated by the fact that serum ferritin levels were invariably among the strongest predictors of hepcidin levels in CHD (Table 1), as observed in the general population [31, 32]. Of note, the CHD population investigated in our last study [24] was enrolled in a center characterized by a clinical policy favoring relatively low iron supplementation to avoid iron toxicity. Indeed, the median ferritin level in this CHD population was "only" 265 μg/L (interquartile range 155–411 μg/L), while, e.g., in the previous study by Ashby and colleagues [15] the mean ferritin level in 94 CHD patients was 550 μg/L (95% CI 291 and 1014 μg/L, respectively). Moreover, again in the effort to select the most appropriate controls, we included only control subjects with biochemically documented normal iron status. To this purpose, we considered as exclusion criteria ferritin levels lower than 30 μg/L and 40 μg/L in females and males, respectively, TS lower than 16%, and decreased levels of hemoglobin. This enabled us to

Table 1 Available studies comparing serum hepcidin levels between adult CHD patients and healthy controls.

Reference	CHD patients: n of subjects, age (years)	Healthy controls: n of subjects, age (years)	Age and sex matching (CHD vs. controls)	Method for hepcidin quantification	Hepcidin results[a]	Hepcidin determinants
Tomosugi [10]	40 Age n.a.	16 Age n.a.	n.a.	MS-based (semiquantitative)	Higher in CHD	FT, IL-6
Zaritsky [11]	33 60.3±20.7	24 28.4±6.6	Sex matched; Age unmatched	Competitive ELISA	Higher in CHD (total hepcidin)	FT, TS, CRP
Campostrini [12]	54 67±14	57 35±15	Sex matched; Age unmatched	MS-based (quantitative)	Higher in CHD	FT
Valenti [14]	65 65.5±12	57 35±15	Sex matched; Age unmatched	MS-based (quantitative)	Higher in CHD	FT, CRP, HFE mutations
Ashby [15]	94 64.6 (39.2–83.0)[b]	64 Age n.a.	Sex and age unmatched	Radioimmunoassay (RIA)	Higher in CHD (total hepcidin)	Hb, Epo dose
Peters [16]	48 61±15	24 39±12	Sex and age unmatched	MS-based (quantitative)	Higher in CHD	FT, serum iron
Tessitore [17]	56 67±14	57 35±15	Sex matched; Age unmatched	MS-based (quantitative)	Higher in CHD	FT, sTfR, hypochromic red cells
Ghoti [18]	21 63.4±12.9	63 63.4±12.9	Sex and age matched	MS-based (quantitative)	Higher in CHD	n.a.
Costa [19]	33 59.5±17.6	17 Age n.a.	n.a.[c]	MS-based (quantitative)	Higher in CHD	CRP
Kurugano [20]	198 58±11	33 34±8	Sex and age unmatched	MS-based (quantitative)	Higher in CHD	FT, transferrin, IL-6[d]
Pelusi [24]	155 64.3±14	188 60±17	Sex and age matched	MS-based (quantitative)	Similar in CHD and controls	FT, CRP, genetic factors (HFE and TMPRSS6)

[a]refers to "hepcidin-25 isoform" where not otherwise specified; [b] 95% confidence interval; [c] matched as far as possible for sex and age; [d] IL-6 determinant of hepcidin only in subjects with CRP >0.3 mg/dL. CBC, complete blood count; CHD, chronic hemodialysis; CKD, chronic kidney disease; CRP, C-reactive protein; FT, ferritin; sTfR, soluble transferrin receptor; IL-6, interleukin-6; MS, mass spectrometry; TS, transferrin saturation %.

avoid the common pitfall of enrolling controls subjects with subclinical low iron status, and ensuing lower than normal hepcidin levels.

Finally, blood samples from all CHD patients were taken after an inter-dialytic period of 3 days [33], thus excluding any possible clearance effect of recent dialysis procedures. For all the reasons outlined above, we are confident that the lack of difference in mean hepcidin levels between CHD patients and controls [24] was likely the result of a balance between flawless controls matched for age/gender and without even a subclinical iron deficiency, and, also of CHD patients characterized on average by lower iron stores than those usually observed in most of the previously published cohorts. Further support to this balanced view arises from our direct experience. During the same period in which we performed the last large study on CHD [24], we also analyzed in our laboratory CHD samples from another dialysis center using a different policy as regards to intravenous iron supplementation. In this particular setting, including selected CHD patients with MR-ascertained iron overload and serum ferritin levels >1000 μg/L [18], we observed serum hepcidin levels substantially higher than controls even after careful matching using the same criteria outlined above for the patients enrolled in Milan [24]. In agreement with the view that iron status rather than uremia per se appears as the major determinant of hepcidin in CHD, serum ferritin was the major predictor of hormone level in nearly all the studies published so far, which also included CRP as covariate (Table 1). Similarly, when we excluded from the analysis of CHD patients enrolled in Milan those with possible "functional" iron deficiency (i.e., with ferritin levels <200 μg/L or TS <30%), we showed that hepcidin levels were significantly higher in CHD patients than in controls (Supplemental Data, Figure 1, which accompanies the article at http://www.degruyter.com/view/j/cclm.2014.52.issue-4/issue-files/cclm.2014.52.issue-4.xml). Overall, our results suggest that the current "dogma" of hyperhepcidinemia as a constant feature of CHD patients need to be reconsidered by further studies based on careful control selection and matching.

Clinical correlates of hepcidin levels in CHD patients

An unpublished sub-analysis of our entire CHD cohort with complete clinical and genetic characterization further supports the concept of the high variability of hepcidin levels in CHD patients, as well as the lack of hepcidin upregulation in some of them. Supplemental data, Table 1 shows the main features of these patients stratified according to hepcidin-25 quartiles. As expected, the major determinant of hepcidin levels was represented by serum ferritin, with a lower contribution by inflammation. Most patients in the lowest hepcidin quartile (<0.55 nM) were likely iron deficient, as estimated by ferritin and TS levels. They also had significantly higher body mass index, although it was not possible to discriminate whether this could be explained by inadequate control of body fluids or by increased adiposity.

An adequate iron balance was likely present in most patients included in the second and third hepcidin quartiles (0.56–17.83 nM), showing ferritin levels mostly within recommended ranges for these patients and absence of inflammation. However, patients in the top quartile of hepcidin (>17.83 nM) had either subclinical inflammation (as detected by increased CRP levels) and/or increased iron stores, resulting in decreased iron availability and increased need for Epo. Interestingly, the wide variability of hepcidin levels among patients did not appear to be entirely explained by differences in iron stores and inflammation, as hepcidin/ferritin and hepcidin/CRP ratios increased with hepcidin quartiles. It is therefore likely that additional factors related to secretion, processing, and clearance affect hepcidin levels in CHD. The hepcidin/ferritin ratio observed in lowest hepcidin quartiles is lower than in controls [24], paradoxically indicating inappropriately low hepcidin levels for the level of iron stores and inflammation. This would suggest that hepcidin clearance by the hemodialysis procedure plays a role in maintaining low/normal serum hepcidin levels in CHD patients without increased iron stores [34]. In a previous pilot study, no significant differences in hepcidin clearance could be detected according to the dialysis technique [12], while hemodiafiltration, which would facilitate the clearance of small peptides such as hepcidin, has been recently reported superior to conventional hemodialysis [35]. However, the linear correlation between serum hepcidin and calcium levels (Supplemental data, Table 1) does not clearly support a predisposing effect of the failure to correct severe uremia on the development of high hepcidin levels [34]. It is currently unknown whether calcium levels or parathyroid hormone and vitamin D dependent signaling pathways may affect hepcidin release. Finally, we confirm the role of genetic factors regulating iron metabolism, namely the C282Y HFE mutation of hereditary hemochromatosis [14] and the TMPRSS6 A736V polymorphism [24] in modulating hepcidin production in CHD (Supplemental data, Table 1).

Potential clinical usefulness of hepcidin determination in CHD patients

Recent data based on MRI suggest that a substantial fraction of CHD patients receiving Epo and intravenous iron supplementation have hepatic iron overload [19, 36]. This illustrates the need for updating guidelines on the amount of iron infused in these patients which may promote not only organ damage, but also a vicious cycle because of increased hepcidin production and ensuing iron maldistribution, without improving anemia. Whether or not evaluation of hepcidin levels may help clinicians in driving iron supplementation therapy in CHD patients is matter of debate. To date, the relatively larger study (n=56) did not show hepcidin as a useful predictor of hemoglobin response to i.v. iron [17]. However, this study had some limitations like possible underpowering and lack of a control group, so that larger trials comparing hepcidin-driven to standard therapeutic approaches are eagerly awaited.

Another intriguing application of hepcidin in CHD has recently emerged, as regards to cardiovascular complications, which represent a major cause of morbidity and mortality in these patients. Experimental data suggest that hepcidin may be implicated in the pathogenesis of atherosclerosis though its ability to induce iron accumulation and oxidative stress within macrophages of the arterial walls [37]. Indeed, hepcidin levels have been associated with both reduced cholesterol efflux in macrophages [37], and release of the atherogenic chemokine macrophage chemoattractant protein-1 (MCP-1) [38]. In CHD patients, hepcidin levels have been recently shown to correlate with arterial stiffness [39], as well as to predict cardiovascular events independently of inflammation [40]. Thus, hepcidin appears to be a good candidate in explaining accelerated atherosclerosis in CHD patients, and its measurement may help to stratify individual patients risk. However, besides known difficulties in technical standardization [25, 26], another factor specific to CHD that may limit the use of hepcidin assay in this setting is the large inter- and intra-individual variability [33]. In this respect, future studies should be carefully standardized for any possible confounder such as, dialysis technique, distance to the last dialytic session and to last administration of iron/Epo, and subclinical infective episodes.

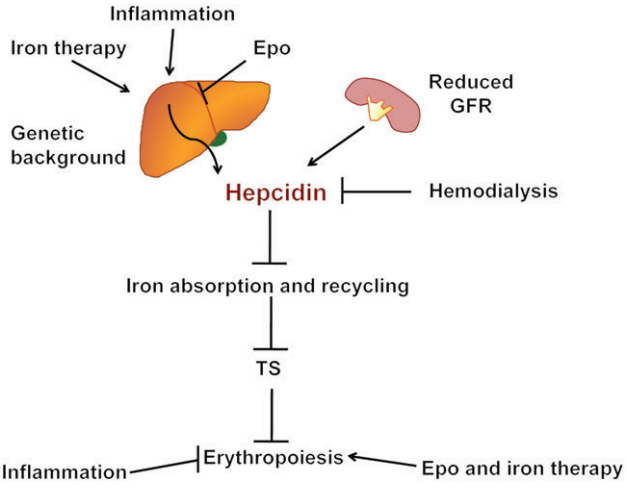

Figure 1 Schematic representation of the determinants of hepcidin levels in chronic hemodialysis patients.
Genetic background: presence of C282Y and H63D HFE and/or of the A736V TMPRSS6 polymorphisms; GFR: glomerular filtration rate; TS: transferrin saturation.

Conclusions

The relatively large number of studies published so far has generated the common idea that hepcidin is invariably high in CHD, with clinical implications lower than expected [33]. However, it is now clear that the quality of most of these studies was suboptimal in terms of numbers, control of confounders, and clinical heterogeneity (Table 1). More rigorous recent data are not consistent with hyperhepcidinemia as an intrinsic and constant feature of CHD patients. In this setting, undoubtedly, several factors drive an increase of serum hepcidin, including severe reduction in glomerular filtration rate, frequent iron supplementation, and inflammation. However, hepcidin is fairly well cleared during dialysis, and the regulation by iron appears to be relatively preserved in CHD patients, even during depletion. As a consequence, although hepcidin is frequently increased in CHD, the net result in the individual patient is highly variable depending on iron stores, inflammatory status, genetic factors, the dialysis technique, and other factors still unknown. Figure 1 recapitulates the many opposing stimuli determining hepcidin levels in CHD patients. The variability of hepcidin levels in CHD patients may have important clinical and therapeutic implications, even beyond the classical target of anemia correction. For example, increased serum hepcidin levels may herald a pathological iron overload inducing interruption of intravenous iron supplementation, as well as an increased risk of cardiovascular complications. CHD patients with sustained hyperhepcidinemia may be good candidates for the use of hepcidin antagonists [4] to

promote proper iron utilization for erythropoiesis. Available studies on hepcidin level as a guide to intravenous iron supplementation in CHD are limited to the observation of a lack of correlation with hemoglobin increase in relatively small and unselected populations [17]. However, the subgroup of CHD patients with repeatedly normal to low serum hepcidin levels may represent the ideal target for safe and effective iron supplementation.

In summary, we are still at the beginning of the hepcidin era in CHD, and there is urgent need of further studies in large series of well-characterized CHD patients and controls.

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Conflict of interest statement

Authors' conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article. Research funding played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.
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