Hepcidin as a Predictor of Response to Epoetin Therapy in Anemic Cancer Patients

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BACKGROUND: Hepcidin is thought to be the central regulator of iron metabolism. Iron deficiency is associated with low hepcidin concentrations, and anemia in patients with cancer is associated with high concentrations of hepcidin.

STUDY OBJECTIVES: Our main objective was to assess the potential role of hepcidin for predicting response to epoetin therapy in anemic cancer patients. We also aimed to identify a cutoff value for hepcidin as a potential predictive marker for response to epoetin therapy.

METHODS: Using data from 525 anemic cancer patients enrolled in 5 studies, we assessed serum hepcidin concentrations in 408 of these patients at baseline and analyzed pooled data from the 408 patients. The analysis population was separated into 2 categories using a threshold hepcidin concentration of 13 nmol/L: low hepcidin (<13 nmol/L) and high hepcidin (≥13 nmol/L).

RESULTS: A significantly higher percentage of responders (defined as hemoglobin increase ≥10 g/L or ≥20 g/L from baseline) was observed in the low hepcidin group compared with the high hepcidin group (P = 0.04 for ≥10 g/L increase and P = 0.009 for ≥20 g/L from baseline). There was also a statistically significant difference between the 2 groups for hematopoietic response (hemoglobin rise at least once ≥20 g/L from baseline or at least once ≥120 g/L) to epoetin therapy (P = 0.0004).

CONCLUSIONS: The results of this analysis suggest a potential role of hepcidin serum concentrations in predicting the response to epoetin therapy.

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anemia is characterized by decreased erythrocyte survival, impairment of iron efflux from macrophages and enterocytes, and hyporesponsiveness of erythroid precursors to erythropoietin (5–7).

Preclinical and clinical evidence suggests an association between epoetin concentrations and hepcidin as a regulator of the erythroid demand for iron in producing erythrocytes, e.g., injection of epoetin was found to decrease liver hepcidin expression, thus facilitating intestinal iron absorption (8, 9). This association suggests that hypoxia due to anemia acts both on erythropoiesis induction and hepcidin gene down-regulation through erythropoietin. The decrease in hepcidin could explain the increased iron release from reticuloendothelial cells and the increased intestinal iron absorption during hypoxia, which increases iron supply for erythropoiesis (9, 10).

About 35%–40% of anemic cancer patients undergoing chemotherapy and treated with epoetins do not respond with an increase in Hb (11). A large part of this hyporesponsiveness is thought to be caused by the decreased availability of iron for the synthesis of hemoglobin caused by inflammatory processes often observed in anemic cancer patients.

Based on a pooled analysis of hepcidin serum concentrations from 5 clinical trials, we aimed to contribute to the understanding of the phenomenon of hyporesponsiveness to epoetin therapy in anemic iron-replete cancer patients.

Our main objective was to assess a potential predictive role of baseline hepcidin concentrations for response to epoetin treatment in anemic cancer patients receiving chemotherapy. This included the identification of an optimized hepcidin cutoff value as a predictive marker for response to epoetin therapy. We sought to analyze the response to epoetin therapy and changes of hepcidin concentrations from baseline to the end of treatment phase in the subgroup of patients with baseline and follow-up hepcidin concentrations.

### Materials and Methods

#### Patient Population

Data from 572 patients who had been enrolled in 5 different studies were pooled for our analysis (12–14) (Y.K. Eid, unpublished report, July 2007; M. Nowrousian, A. Scherhag, and H-U Burger, unpublished report, August 2007). Serum hepcidin concentration at baseline was available in 439 patients. From these patients, only those 408 who were at least 5 weeks in the study have been taken into consideration for analyzing response to epoetin treatment. All five studies were conducted in compliance with the Helsinki Declaration (15). Enrolled patients were anemic cancer patients [hemoglobin (Hb) ≤110 g/L at baseline] who received epoetins for correction of anemia and chemotherapy for the treatment of cancer disease for approximately 12 weeks and stayed at least 5 weeks in the study (Table 1). In addition, major inclusion criteria for enrollment of patients in those studies were as follows: male and female adult patients with confirmed diagnosis of cancer, screening transferrin saturation >20%, WHO performance grade 0–2, and life expectancy >6 months. Major exclusion criteria were relevant acute or chronic bleeding, grade 3/4 thrombocytopenia, any erythropoiesis-stimulating therapy within past 3 months, acute infection, or inflammatory disease. In a subgroup of 70 patients from 2 studies, hepcidin concentrations were also measured at study end (end of 12 weeks of therapy) (Table 1).

#### Assays

We assessed hepcidin using an isotope dilution micro-HPLC–tandem MS (MS/MS) method that allows the quantification of hepcidin-25 present at less than nmol/L concentrations (Roche Diagnostics) (16). We measured C-reactive protein (CRP) using the conventional immunoturbidimetric assay method (detection limit ≥3 mg/L).

#### Table 1. Overview of studies and baseline hepcidin concentrations.

<table>
<thead>
<tr>
<th>Study</th>
<th>Patients with baseline hepcidin, N</th>
<th>Tumor type</th>
<th>Mean baseline hepcidin level, nmol/L (SD)</th>
<th>Hepcidin assessed at end of treatment (12 weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NA17101[12]</td>
<td>155</td>
<td>Non–small cell lung cancer</td>
<td>8.5 (6.8)</td>
<td>No</td>
</tr>
<tr>
<td>BA16558[13]</td>
<td>63</td>
<td>Multiple myeloma</td>
<td>26.8 (25.7)</td>
<td>No</td>
</tr>
<tr>
<td>BA16728[14]</td>
<td>88</td>
<td>Non-Hodgkin lymphoma</td>
<td>20.1 (15.6)</td>
<td>No</td>
</tr>
<tr>
<td>NH19960*</td>
<td>63</td>
<td>Non–small cell lung cancer</td>
<td>8.2 (7.6)</td>
<td>Yes</td>
</tr>
<tr>
<td>BH20198*</td>
<td>39</td>
<td>Mixed solid tumors</td>
<td>12.0 (12.8)</td>
<td>Yes</td>
</tr>
</tbody>
</table>

STATISTICAL ANALYSES

The results of this study are derived from the post hoc exploratory analysis of the pooled data set. All analyses were conducted following a predefined statistical plan. The analysis population has been defined as all patients who were in the study for at least 5 weeks and for whom hepcidin concentrations were assessed at baseline. All values, if not indicated otherwise, are reported as mean (SD).

In an attempt to identify an optimal cutoff value of hepcidin, the median value of the analysis population (9.58 nmol/L rounded to 10 nmol/L) was used to separate the patients into low hepcidin and high hepcidin groups. Hb response (defined by 6 criteria, see below) were compared between these 2 groups. Because the results seemed suboptimal with this approach, ROC curves were produced to test various other cutoff hepcidin values. A cutoff of 13 nmol/L turned out to be a better threshold for differentiation and identification of responders and nonresponders in this clinical setting. With the 13 nmol/L cutoff, the ratio of true forecasted responders among the actual responders over the proportion of false forecasted responders among the actual nonresponders was higher than using the median hepcidin value as a cutoff.

Consequently, the analysis population (n = 408) was separated into 2 categories, those individuals whose hepcidin values were above and below 13 nmol/L. We analyzed the effect on several established hemoglobin response criteria. The following 6 response criteria were used for the assessment of response to epoetin therapy:

- Hb rise $\geq 10$ g/L from baseline at least once during 12 weeks of treatment period;
- Hb rise $\geq 20$ g/L from baseline at least once during 12 weeks of treatment period;
- Hb rise $\geq 10$ g/L at least once from baseline to week 4;
- Hb $\geq 110$ g/L at least once during 12-week treatment period;
- Hb $\geq 120$ g/L at least once during 12-week treatment period;
- Hematopoietic response, defined as Hb increase $\geq 20$ g/L from baseline at least once or Hb $\geq 120$ g/L at least once.

In individuals who received blood transfusions, the hemoglobin values in the 28 days after the transfusion were replaced by the pretransfusion value. We compared response to epoetin therapy between the 2 groups using the $\chi^2$ test and evaluated the association of baseline hepcidin concentrations and time to response using Kaplan–Meier estimates and log-rank tests.

### Results

#### Baseline Characteristics

Mean (SD) hepcidin concentration at baseline was 5.58 (3.68) nmol/L in the low hepcidin group and 27.9 (17.6) nmol/L in the high hepcidin group (Table 2). The demographic characteristics of patients in the 2 groups, at baseline with respect to age, height, and weight, were well matched. All kinds of epoetin treatments (Aranesp®, NeoRecormon®, Mircera®) were evenly distributed between the 2 hepcidin concentration groups (Table 2). Mean baseline hepcidin concentrations were lower in patients with lung cancer or solid
tumors and higher in patients with hematological tumors (Table 1).

The hemoglobin concentrations at baseline were comparable between the 2 groups. Minor differences between the 2 study groups at baseline were seen with respect to concentrations of CRP: more patients with hepcidin \(\geq 13\) nmol/L had CRP concentrations \(\geq 10\) mg/L (51% vs 41%) and more patients with hepcidin \(\geq 13\) nmol/L had CRP concentrations \(>30\) mg/L (30% vs 19%) (Table 2). With respect to the stage of underlying disease at baseline, more patients with advanced lung cancer had hepcidin \(\leq 13\) nmol/L, whereas more patients with multiple myeloma stage 2 and 3 and non-Hodgkin lymphoma stage 3 and 4 had hepcidin \(\geq 13\) nmol/L (Table 2).

**RESPONSE TO EPOETIN THERAPY BY BASELINE HEPcidIN CONCENTRATION**

We observed a significantly higher percentage of responders (where response was defined as hemoglobin increase by \(\geq 10\) g/L or \(\geq 20\) g/L from baseline) in the low hepcidin group and a higher number of non-responders in the high hepcidin group \((P = 0.04\) and \(P = 0.009\) for the \(\geq 10\) g/L and \(\geq 20\) g/L response criteria, respectively). This difference was also statistically highly significant when the response to epoetin treatment was defined as reaching Hb concentration \(\geq 110\) g/L and \(\geq 120\) g/L \((P < 0.0001\) and \(P < 0.0001\))

A statistically significant difference between the 2 groups was also demonstrated when a hematopoietic response was used as a definition for response to erythropoietic therapy \((P = 0.0004)\) (Table 3). The positive predictive value of a baseline hepcidin value \(\leq 13\) nmol/L to predict an increase of Hb by \(\geq 10\) g/L was 77%. No statistical difference was observed when response to epoetin therapy was defined as Hb increase \(\geq 10\) g/L in \(<4\) weeks from baseline \((P = 0.6486)\) (Table 3).

Taking into consideration criteria of 10 or 20 g/L of Hb increase from baseline, more responders were observed in study BA16558 (multiple myeloma), where patients had relatively high hepcidin values at baseline, than in study NA17101 (lung cancer), where patients had relatively low values of hepcidin at baseline (Tables 1 and 4).

| Table 3. Summary of responders (%) by baseline hepcidin level.\(^a\) |
|-----------------------------------------------|-------------------|-----------------|-----------------|-----------------|
| **Responders** | **Nonresponders** | | | |
| **Low hepcidin** | **High hepcidin** | **Low hepcidin** | **High hepcidin** | **p** |
| Hb increase \(\geq 10\) g/L | 77.0 | 67.9 | 23.0 | 32.1 | 0.0444 |
| Hb increase \(\geq 20\) g/L | 54.4 | 41.0 | 45.6 | 59.0 | 0.0088 |
| Hb increase \(\geq 10\) g/L during 26 days after first dose | 32.9 | 30.8 | 67.1 | 69.2 | 0.6486 |
| Hb value \(\geq 110\) g/L | 79.8 | 61.5 | 20.2 | 38.5 | \(<0.0001\) |
| Hb value \(\geq 120\) g/L | 61.5 | 41.7 | 38.5 | 58.3 | \(<0.0001\) |
| Hematopoietic response | 66.3 | 48.7 | 33.7 | 51.3 | 0.0004 |

\(^a\) Percentages are based on 252 patients with baseline hepcidin \(<13\) nmol/L (low hepcidin group) and 156 with baseline hepcidin \(\geq 13\) nmol/L (high hepcidin group).

| Table 4. Summary of responders (%) by study. |
|-----------------------------------------------|-------------------|-----------------|-----------------|-----------------|
| **Definition of response** | **NA17101, Non-small cell lung cancer** | **BA16558, Multiple myeloma** | **BA16728, Non-Hodgkin lymphoma** | **NH19960, Non-small cell lung cancer** | **BH20198, mixed solid tumors** |
| Hb increase \(\geq 10\) g/L | 66.5 | 85.7 | 70.5 | 81.0 | 76.9 |
| Hb increase \(\geq 20\) g/L | 44.5 | 63.5 | 45.5 | 55.6 | 43.6 |
| Hb increase \(\geq 10\) g/L after 3 weeks | 21.9 | 50.8 | 35.2 | 30.2 | 38.5 |
| Hb value \(\geq 110\) g/L | 73.5 | 76.2 | 67.0 | 77.8 | 69.2 |
| Hb value \(\geq 120\) g/L | 51.0 | 61.9 | 52.3 | 58.7 | 48.7 |
| Hematopoietic response | 54.8 | 69.8 | 55.7 | 68.3 | 56.4 |
We compared Kaplan–Meier curves of the time to achieve a response to epoetin therapy (using response criteria of Hb $\geq 110$ g/L, Hb $\geq 120$ g/L, and hematopoietic response) between the low hepcidin and high hepcidin groups. The differences in time to response were highly significant in favor of the low hepcidin concentration groups ($P = 0.0001$, $P = 0.0003$, $P = 0.0030$, respectively, log rank test) (Fig. 1).

In the subgroup of 70 patients with solid tumors from 2 studies in which baseline and follow-up hepcidin concentrations were measured, we compared baseline hepcidin concentrations and changes from baseline to the end of the treatment phase in the 59 responders (defined as an increase of Hb by 10 g/L from baseline) vs the nonresponders. Mean (SD) hepcidin concentrations at baseline in responders and nonresponders were 8.26 (8.91) and 12.73 (14.44) nmol/L, respectively (Table 5). The mean hepcidin concentrations in the group of responders did not change significantly during the treatment phase but did increase in the group of nonresponders by 6.54 nmol/L at the end of week 12 (Table 5).

**Discussion**

The data presented in this study provide the first analysis of the potential influence of baseline hepcidin concentrations on the response to epoetin therapy and document the changes of hepcidin concentrations over time in anemic cancer patients receiving epoetins for the correction of anemia. Because hyporesponsiveness to epoetins therapy is poorly understood, it would be advantageous to have a biomarker able to identify patients who are not going to respond adequately to epoetin therapy. Inflammation is considered to be one of the most frequent causes of hyporesponsiveness to epoetins therapy.

### Table 5. Summary of hepcidin changes (nmol/L) by responder category in a subset of patients with solid tumors.a

<table>
<thead>
<tr>
<th>Category</th>
<th>Mean (SD)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Responders (n = 59)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>8.3 (8.9)</td>
<td></td>
</tr>
<tr>
<td>Week 12</td>
<td>8.7 (11.3)</td>
<td></td>
</tr>
<tr>
<td>Change from baseline</td>
<td>0.4 (11.5)</td>
<td></td>
</tr>
<tr>
<td>Nonresponders (n = 11)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>12.7 (14.4)</td>
<td></td>
</tr>
<tr>
<td>Week 12</td>
<td>19.3 (23.7)</td>
<td></td>
</tr>
<tr>
<td>Change from baseline</td>
<td>6.5 (14.8)</td>
<td></td>
</tr>
</tbody>
</table>

* Responders exhibited increases in Hb concentration $>10$ g/L from baseline; nonresponders exhibited increases in Hb concentration $\leq 10$ g/L from baseline.
epoetin therapy (17–20). A large part of this hyporesponsiveness to epoetin therapy in cancer patients is thought to be caused by the decreased availability of iron for the synthesis of hemoglobin during inflammatory stages, because the use of endogenous iron and the iron absorption is blocked by upregulated hepcidin (9, 10).

With a goal of predicting patients who will respond or not respond adequately to epoetin therapy, we tried to identify a useful hepcidin cutoff for this purpose. As a standard approach when using a continuous dataset, we initially used the median hepcidin value at baseline (9.58 nmol/L rounded to 10 nmol/L) to separate 2 patient groups and then investigated and compared the response to epoetin therapy between these groups. Because results seemed suboptimal with this approach, we produced ROC curves using other cutoff values of hepcidin and found that a threshold of 13 nmol/L turned out to be the best cutoff value for differentiation and identification of responders and nonresponders in this clinical setting.

In our study, patients with low hepcidin concentrations at baseline responded better to epoetin therapy. This was demonstrated by a significantly higher number of responders in the group of patients with low baseline hepcidin concentrations (<13 nmol/L) when using 5 of 6 Hb response criteria frequently applied to assess efficacy of epoetins. The positive predictive value of baseline hepcidin value below the proposed cutoff value of 13 nmol/L was 77%. Conversely, in the high hepcidin group, a higher number of nonresponders and a low number of responders were observed (Table 3).

A possible explanation of our findings is the upregulation of circulating IL-6 concentrations and consequent upregulation of hepcidin associated with inflammatory conditions typically observed in many patients with metastatic cancer. Increased IL-6 concentrations have been shown to be a predictor of poor outcome in several solid tumor types (21). An important effect of IL-6 is the activation of hepcidin expression, thereby inhibiting intestinal iron uptake and preventing iron release from macrophages (locking iron) (9, 10). Hepcidin is the key protein involved in the regulation of iron absorption and iron release (9, 10). Furthermore, administration of epoetins may decrease hepcidin concentrations through downregulation of the hepcidin gene (9).

In the context of hepcidin as a marker of inflammation, it should be noted that there was an association between CRP concentrations and baseline hepcidin concentrations in patients with CRP concentrations <10 mg/L at baseline (Table 2). Patients with CRP concentrations ≥30 mg/L were equally distributed in the low and high hepcidin groups (46 each) (Table 2). More analyses are needed to elucidate the correlation between CRP as a marker of inflammation and hepcidin concentrations.

From a clinical perspective, our results suggest that baseline hepcidin concentrations may play a potential role as a predictive marker to identify patients who either are going to respond adequately to epoetin therapy or need to be excluded from anemia correction with epoetins due to a low chance of responding. Commercially available assays for measurement of hepcidin concentrations will enable further research in this area (16, 22, 23).

In the subgroup of 70 patients with baseline and follow-up hepcidin concentrations, only a slight increase of hepcidin from baseline to the end of therapy was observed in patients who responded to epoetin therapy. Conversely, in patients who did not respond to epoetin therapy, an increase of hepcidin by 6.54 nmol/L was seen (Table 5). In our view, this finding supports the concept that hepcidin concentrations may also be regulated via metabolic needs for endogenous iron (in our case, e.g., an increased need for iron for hemoglobin synthesis due to epoetin-stimulated erythropoiesis) aiming to increase or decrease iron availability.

LIMITATIONS

The results of our study have several limitations. First, our findings are based on a retrospective analysis of a pooled dataset of patients who were enrolled into 5 different studies that had a primary objective to investigate the response to epoetin therapy and correction of anemia and for whom serum samples for hepcidin measurements were available. The patients enrolled in these studies were admittedly heterogeneous in terms of tumor types and stages, and hence, the hepcidin concentrations would be expected to vary.

Another important limitation of our analyses is that very often cancer progression is associated with inflammatory effects that can upregulate hepcidin concentrations through IL-6 stimulation. It must also be noted that of the 572 patients enrolled in the 5 studies, 185 patients had received at least 1 blood transfusion, a fact that might have confounded the results of analyses of response to epoetin therapy.

Some patients enrolled in those studies received iron orally, intramuscularly, or intravenously. Moreover, due to the design of the included studies, neither a placebo nor a control arm could be included in any of our analyses. Finally, our data were derived from a population of cancer patients receiving chemotherapy, and the chosen cutoff of 13 nmol/L for baseline hepcidin concentrations to predict response to epoetin therapy may not be appropriate for other patient populations in other clinical settings.
Conclusions

This is the first analysis using a hepcidin test to investigate the potential role of baseline serum hepcidin concentrations for predicting the response to epoetin therapy in anemic cancer patients. Patients with lower baseline hepcidin concentrations had a better response to epoetin therapy. Because of the retrospective nature of this study, which was conducted on a pooled dataset of anemic cancer patients, our results should be interpreted with the appropriate caution. Future clinical studies on the predictive role of hepcidin are needed to further explore the clinical potential of this new marker.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

References