Beca Investigación '2010 / Informe final 6-5-13 / Dr. Pascual

Clinical Biochemistry 45 (2012) 1455–1458

Contents lists available at SciVerse ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

Soluble TNFα receptor type I and hepcidin as determinants of development of anemia in the long-term follow-up of heart failure patients

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ARTICLE INFO

Article history:
Received 20 January 2012
Received in revised form 2 April 2012
Accepted 6 May 2012
Available online 17 May 2012

Keywords:
Anemia
Heart failure
Inflammation
Iron metabolism

ABSTRACT

Background: Anemia is common in patients with chronic heart failure (CHF) and is associated with a worse prognosis. This study aims to identify the biological mechanisms which reflect evolutionary changes in the hemoglobin concentrations in heart failure patients who are still not anaemic.

Methods: Fifty-nine patients (54 ± 14 years, 83% males) with CHF (LVEF 28 ± 10%), who did not have anemia, and had not received any previous transfusions, were included. The parameters studied were: iron metabolism (ferritin, iron, transferrin, soluble transferrin receptor (sTfR), hepcidin); inflammation (C-reactive protein, soluble TNFα receptor I (sTNFRI), interleukin 6); and myocardial stress (NT-proBNP, high sensitivity TnT, growth differentiation factor 15). All parameters were measured on inclusion and 1 year after inclusion.

Results: Baseline hemoglobin (g/dL) was 14.7 ± 1.5 and at 1 year of follow-up it showed a significant decrease of −0.4 (RIC: −0.7 to −0.06) ($p = 0.02$). At baseline, only the sTNFRI was a predictor of a decrease in hemoglobin 1 year later ($p = 0.007$). During follow-up, the increase in sTNFRI ($p = 0.002$, $r = -0.39$) and hepcidin ($p = 0.006$, $r = -0.35$) were both associated with a decrease in hemoglobin. Similarly, the patients who became anemic (13%) had higher levels of hepcidin ($p = 0.001$) and sTNFRI ($p = 0.008$). The remaining parameters did not show any relationship with the evolution in the hemoglobin.

Conclusions: In CHF patients without anemia, the increase in the inflammatory state (sTNFRI) and the following deterioration in the iron metabolism (hepcidin) were the main determinants of a decrease in hemoglobin and the appearance of anemia in the long term follow-up period.

Introduction

Chronic heart failure (CHF) is a cardiovascular disease with a high rate of mortality. Anemia is a common comorbility, with a prevalence of between 15 and 70% depending on the definition and the population under investigation [1,2]. In patients with CHF, either the appearance of anemia and/or a decrease in hemoglobin concentration (Hb) during follow-up are associated with a higher rate of morbidity and mortality and worse prognosis [3]. However, the physiopathology of anemia in these patients is still not very well defined and is considered to be a complex and multifactorial process that could include: the reduction in intestinal iron absorption; an increase in inflammatory cytokines which causes bone marrow depression; the activation of the renin-angiotensin-aldosterone system which causes water and sodium retention, leading to dilutional anemia; and the kidney failure that comes with a reduction in erythropoietin (EPO) production [4,5].

Recently, several clinical trials have been set up to assess the benefit of correcting anemia through the use of iron and erythropoietin, given the poor prognosis associated with anemia in these patients [2,6]. By now, only iron treatment seems to be associated with an improvement in symptoms although an effect on prognosis has yet to be observed [7]. However, the preventive strategies aimed at avoiding the onset of anemia might have a greater value than therapeutic strategies designed to treat the presence of anemia. Thus, understanding the mechanisms which are most closely linked to the de novo appearance of anemia would be a fundamental step for enabling its prevention.

The objective of this study was to evaluate the biological mechanisms which best reflect evolutionary changes in hemoglobin concentration in CHF patients and who have not yet developed anemia. To this end, the biochemical parameters of iron metabolism, inflammation and myocardial stress were studied.

Abbreviations: sTNFRI, soluble TNFα receptor I; CHF, chronic heart failure; NT proBNP, N-terminal fragment of B-type natriuretic peptide; Hs TnT, high sensitivity troponin T; GDF15, growth differentiation factor 15; CRP, C-reactive protein; IL6, interleukin-6; sTfR, soluble transferrin receptor.

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doi:10.1016/j.clinbiochem.2012.05.011

Methods

Population and study design

A prospective study was designed which included 59 ambulatory patients (54 ± 14 years, 83% males) with an established diagnosis of CHF [8]. The following inclusion criteria were used for the study population: ambulatory patients with a stable clinical condition and NYHA II functional class (New York Heart Association); no hospitalizations in the previous 6 months; optimal medical treatment without having received anticoagulants or antiplatelets; no current or previous anemia (defined according to the World Health Organization Criteria (WHO): hemoglobin <13 g/dL in males and <12 g/dL in females) [9]; no previous blood transfusions; no advanced renal insufficiency (stage 4 or 5); no previous or current iron supplements. All patients were receiving an optimized therapy, according with contemporary guidelines, which included betablockers (100%), angiotensin inhibitors (100%), loop diuretics (100%), digoxine (55%) and aldosterone antagonists (52%). All patients had left ventricular systolic dysfunction (left ventricular ejection fraction 28 ± 10%) and the established etiologies were: idiopathic dilated cardiomyopathy (52%), hypertension (33%), hypertrophic myocardiopathy (15%).

Blood samples were obtained on inclusion and at the end of the 12-month follow-up, in accordance with the follow-up protocol of the National Network on Heart Failure Research ('REDINSCOR': Grant RD06/0003/0013, Instituto de Salud Carlos III, Ministry of Health, Madrid, Spain). The sample collection protocol was approved by the local ethics committee and written informed consent was obtained from each patient. Moreover, at inclusion time, clinical variables were registered and an echocardiogram was carried out on all the patients.

Laboratory

For sample collection, a venous extraction was carried out on all patients first thing in the morning, after a fasting period of more than 8 h. The samples were collected in EDTA anticoagulant tubes and did not contain any additives. After being spun at a speed of 3500 rpm for 10 min, both plasma and serum were extracted, respectively. The samples were frozen at −80 °C for subsequent processing. The hematological and biochemical parameters were determined at study inclusion and after 1 year of monitoring.

The following laboratory parameters were determined: (1) myocardial stress: N-terminal fragment of B-type natriuretic peptide (NT proBNP), high sensitivity troponin T (Hs TnT) and growth differentiation factor 15 (GDF15); (2) inflammation: C-reactive protein (CRP), soluble TNFα receptor I (sTNFRI) and interleukin-6 (IL6); (3) iron metabolism: ferritin, iron, transferrin, soluble transferrin receptor (sTfR) and hepcidin. Kidney function was determined from the estimated glomerular filtration rate (GFR mL/min/1.73 m^2) using the abbreviated MDRD formula [10]. GDF15 and TNFRI were determined using enzyme immunoassay (BioVendor, Brno, Czech Republic) and hepcidin was determined using another kind of enzyme immunoassay (Bachem, United Kingdom). The remaining biochemical determinations were calculated using a Cobas 6000 analyzer (Roche Diagnostics, Manheim, Germany). The hematological parameters were also studied (hemoglobin, red cell distribution width and mean corpuscular volume) using the Sysmex 5000 analyzer (Roche Diagnostics, Manheim, Germany). Absolute iron deficiency was defined as ferritin <30 μg/dL, whereas functional iron deficiency was defined as ferritin <100 μg/dL or 100–300 μg/dL with a transferrin saturation rate <20%.

Statistical analysis

The normal distribution of the variables was confirmed using the Kolmogorov–Smirnov test. In order to carry out the descriptive analysis of the population, both the mean and standard deviation were used for normally distributed variables, whereas both the median and the interquartile range (IQR) were used for those with a non-normal distribution. Parametric tests were applied to the variables with a normal distribution, and non parametric tests to the rest. For the analysis of correlations between the variables, Pearson's and Sperman's coefficient were obtained, respectively. To study the changes at baseline and at 1 year, Student's paired sample *t*-test and Wilcoxon's non-parametric test were used, respectively. We evaluated which factors were independently associated with the change on hemoglobin level at 1 year, by entering the significant correlates in a multiple linear regression analysis. In order to compare the patients with and without anemia the Kruskal–Wallis *H*-test was applied. Results with two-sided $p<0.05$ values, were considered to be significant. The statistical analysis was carried out using SPSS v18.0 statistical software (Chicago, Illinois, USA).

Results

A total of 59 patients (54 ± 14 years, 83% male) with CHF (left ventricular ejection fraction of 28 ± 10%) were studied using the aforementioned inclusion criteria. The hemoglobin concentrations were 14.7 ± 1.5 g/dL at inclusion, and at the end of 1-year of follow-up they had decreased to 14.3 ± 1.5 g/dL, which meant a significant median reduction of −0.4 g/dL (interquartile range: −0.70 to −0.06) ($p=0.02$). During the follow-up period, 8 out of the 59 patients (13%) developed anemia according to the WHO criteria. The concentrations of biological parameters at inclusion and the corresponding change on concentrations at 1 year are described in Table 1.

Biological parameters and hemoglobin concentration at inclusion

Taking into account only values at inclusion, no correlation was observed between hemoglobin concentrations and the measured biological parameters. We only observed that hemoglobin concentration was lower in the presence of functional iron deficiency ($n=27$, 46%) (15.1 ± 1.7 vs. 14.3 ± 1.1, $p<0.001$), but not in the presence of absolute iron deficiency, which only occurred in a few patients ($n=6$, 10%, $p=0.783$).

We studied the interrelationship between parameters of inflammation, myocardial stress and iron metabolism at inclusion. We found that sTNFRI levels correlated positively with the levels of NT proBNP ($p=0.013$, $r=0.32$), GDF15 ($p=0.047$, $r=0.26$), and ferritin ($p=0.026$, $r=0.026$), whereas hepcidin levels correlated positively

Table 1
Correlations between biological parameters and hemoglobin concentrations.

Variable	Baseline	Δ 12 months
Hemoglobin (g/dL)	14.7 [13.9–15.5]	−0.4 [−1.3 to 0.5]
CRP (mg/dL)	0.2 [0.1–0.4]*	+0 [0–0.2]
sTNFRI (ng/mL)	1.6 [1.1–2.3]**	+0.4 [−0.2 to 0.9]##
IL6 (pg/mL)	3.2 [2.2–5.6]	+0.2 [−0.8 to 1.9]
Fe (mcg/dL)	91 [73–105]	+5 [−19 to 33]
Ferritin (ng/dL)	132 [74–212]	+25 [−48 to 114]
Transferrin (mg/dL)	259 [208–304]	+6 [−46 to 56]
Transferrin saturation (%)	26.6 [22–32]	−10 [−7.2 to 6]
sTfR (mg/dL)	0.3 [0.2–0.4]	+0 [−0 to 0]
Hepcidin (ng/mL)	14.7 [3.5–63]	+7 [−15 to 29.4]##
GDF15 (pg/mL)	3391 [1634–4715]	+255 [−1254 to 1377]
NT proBNP (pg/mL)	587 [127–1313]	−40 [−294 to 49]
Hs TnT (ng/mL)	0.01 [0–0.03]	+0 [−0 to 0]
GFR (mL/min/1.73 m^2)	64.3 [51–78]	+12 [1.3–22]

*$p<0.05$, **$p<0.01$ indicates correlations between studied parameters at baseline and hemoglobin change (Δ). #$p<0.05$, ##$p<0.01$ indicates correlations between changes on studied parameters and hemoglobin change (Δ).
Data are expressed as median [interquartile range percentile25–percentile75].
C-reactive protein (CRP); soluble TNFα receptor I (sTNFRI); interleukin-6 (IL6); soluble transferrin receptor (sTfR); N-terminal fragment of B-type natriuretic peptide (NT proBNP); high sensitivity troponin T (Hs TnT); growth differentiation factor 15 (GDF15).

with ferritin ($p=0.001$, $r=0.41$) and negatively with iron levels ($p=0.039$, $r=-0.270$). No other correlations were found between the studied biological parameters.

Biological parameters and hemoglobin evolution at 1 year

Taking into consideration the initial values, as indicated in Table 1, we found that the inflammatory parameters sTNFRI ($p=0.002$, $r=-0.390$) and CRP ($p=0.025$, $r=-0.376$) were the only ones that correlated with the change of hemoglobin at 1 year.

In addition, when changes on these parameters were considered, the increase in sTNFRI concentrations ($p=0.002$, $r=-0.39$, Fig. 1) and the increase in hepcidin ($p=0.006$, $r=-0.35$, Fig. 2) were the only ones that correlate with the decrease in hemoglobin levels during the year of follow-up. No other correlations were found (Table 1).

In the multiple linear regression analysis, a higher concentration of sTNFRI at baseline ($p=0.007$, $\beta=-0.314$) and an increase on hepcidin levels over time ($p=0.013$, $\beta=-0.325$) were both independently associated with a decrease on hemoglobin concentration at 1-year of follow-up.

We studied the interrelationship between the changes on these parameters during the year of follow-up. The increase on sTNFRI correlated with the increase on GDF15 ($p=0.006$, $r=0.35$) but did not correlate with parameters of iron metabolism. Hepcidin levels correlated with ferritin levels ($p=0.005$, $r=0.360$), just as at baseline. Meanwhile, transferrin levels and sTfR correlated positively with both CRP ($p=0.007$, $r=0.34$ and $p=0.006$, $r=0.35$, respectively) and IL6 ($p=0.005$, $r=0.36$ and $p=0.003$ $r=0.37$, respectively). With respect to the change on transferrin saturation (%), it showed a negative correlation with the NT proBNP change ($p=0.026$, $r=-0.29$).

On comparing anemic ($n=8$) and non-anemic patients ($n=51$), it was seen that the appearance of anemia was accompanied by an increase in the inflammatory state shown by the increase in the sTNFRI and hepcidin levels, together with an increase in ferritin and GDF15 levels (Table 2).

Discussion

CHF is associated with a progressive decline in hemoglobin levels. It was also confirmed in our study, after 1 year of follow-up and evaluating a population without prior anemia, which reflects an early stage in the biological processes leading to the development of anemia.

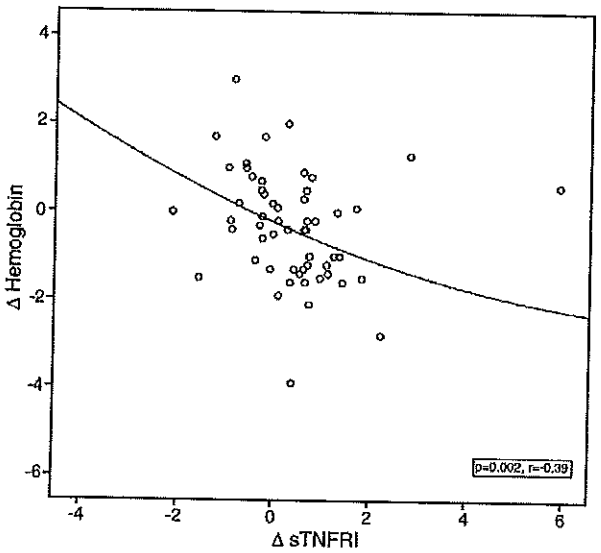

Fig. 1. Correlation of the change in hemoglobin with sTNFRI ($p=0.002$, $r=-0.39$).

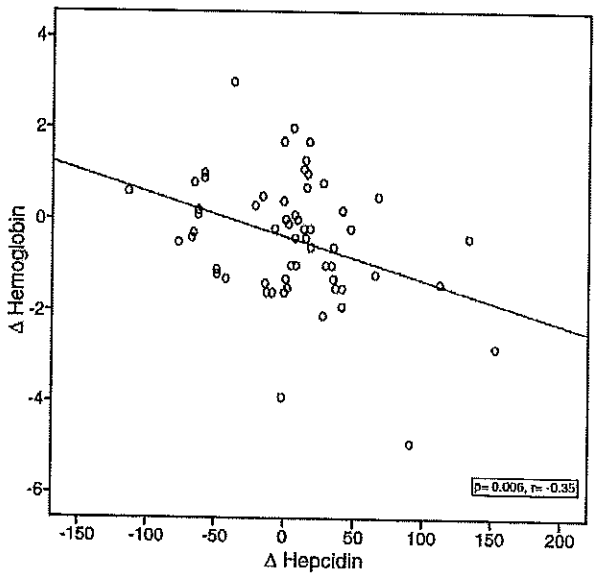

Fig. 2. Correlation of the change in hemoglobin with hepcidin ($p=0.006$, $r=-0.35$).

Regarding the baseline parameters that best reflect the decrease in hemoglobin levels, our study suggests that inflammation plays a crucial role and sTNFRI is the biological parameter that best reflect it. Nowadays, the exact etiology of anemia in CHF is not known, and there are many proposed causes such as hemodilution, malnutrition related to cachexia, kidney failure or antiplatelet and anticoagulant treatment [11]. In the present study, in spite of patients included were stables, with a slight functional deterioration (NYHA class II), having conserved body mass index and kidney function and not receiving antiplatelets or anticoagulants; a significant decrease in hemoglobin levels was observed. The etiology of anemia in CHF, as in other chronic diseases, could be related to the release of cytokines which are able of causing erythropoiesis inhibition. Several studies corroborate that the increase in levels of pro-inflammatory cytokines and CRP is associated with CHF severity [12]. Tumor necrosis factor α (TNF-α) and IL6 inhibit EPO production which in turn inhibits the proliferation of erythroid progenitor cells from the bone marrow. Therefore, one of the mechanisms proposed for anemia in CHF is inflammation (or anemia of chronic diseases).

As we mentioned earlier, our results show evidence of an increase in inflammation at baseline, especially in terms of sTNFRI, as the main predictor of a decrease on hemoglobin concentrations. Only a few studies have investigated the role of sTNFRI in CHF anemia. Pop et al. [13] studied the correlation between sTNFRI and other negative prognostic factors in 54 patients with CHF. They concluded that the increase in sTNFRI is an expression of chronic inflammation in CHF patients. This increase was found to be statistically significant on comparing patients both with and without anemia.

Table 2
Biological parameters associated with the onset of anemia at 1 year.

Variable	No anemia	Anemia	p
	$n=51$	$n=8$	
Hemoglobin (g/dL)	14.6 ± 1.2	12.2 ± 0.6	**<0.001**
Δ Hepcidin (ng/mL)	−3.1 ± 43.6	59.2 ± 59.5	**0.001**
Δ Ferritin (ng/dL)	11.1 ± 229.6	128.6 ± 212.8	**0.034**
Δ sTNFRI (ng/dL)	0.2 [−0.3 to 0.7]	0.9 [0.6–2.1]	**0.008**
Δ GDF15 (pg/mL)	236.4 ± 245.6	1359.3 ± 3416.8	**0.034**

Δ refers to the change from baseline to 1 year.
Data are expressed as mean ± SD and median [interquartile range].

During the follow-up, the changes on biological parameters associated with the decrease in hemoglobin over time were sTNFRI and hepcidin, both being negatively correlated with hemoglobin. Hepcidin is a marker which has been increasingly investigated in recent years due to its effect on anemia in chronic processes [14,15]. Until now only three studies have investigated the role of hepcidin in CHF anemia. Van der Putten et al. [16] compared levels of hepcidin, IL6, ferritin and CRP in a group of patients with CHF, kidney failure and anemia comparing them with a healthy population. They found that hepcidin levels were significantly higher in patients with anemia and correlated inversely with hemoglobin and positively with ferritin but not with IL6 or CRP. Our results also showed the same inverse correlation between hepcidin and hemoglobin but no correlation was found between hepcidin and IL6. By contrast, Divakaran et al. [17] did not find any differences on hepcidin levels in patients with and without anemia, and consequently they suggest that hepcidin does not have an effect on CHF anemia. Finally, Matsumoto et al. [18] found that concentrations of hepcidin were lower in patients with CHF and anemia. In our study we observed that a higher concentration of hepcidin was associated with a decline on hemoglobin and the development of anemia. This could be explained by the fact that in the presence of inflammatory processes, as occurred in CHF, the release of proinflammatory cytokines promotes hepcidin synthesis. Hepcidin could reduce the intestinal absorption of iron and, together with the lack of mobilization of the iron reserve from macrophages due to the increase in the proinflammatory cytokines, there will be inadequate erythropoiesis and a decrease in the mean erythrocyte life [19]. However, it still remains to be established what is the role of hepcidin in CHF anemia and if this marker may have future therapeutic applications for its correction.

About cardiac stress markers, no correlation was found between the change on these and the reduction on hemoglobin concentration over follow-up. This finding suggests that the progression of the cardiac disease on itself is not closely related to hemoglobin evolution. Nevertheless, the study found a relationship between cardiac stress parameters and measurements of inflammation and iron metabolism. In fact, sTNFRI and transferring saturation were related with NT-proBNP concentrations, the main biomarker reflecting cardiac stress among CHF patients. In addition the increase on GDF15 during the follow-up, a cardiac marker which is closely linked to inflammatory processes and worse prognosis in CHF [20], was a predictor of anemia development. Moreover Lakhal et al. [21] observed that GDF15 is increased in patients with iron deficiency, however we did not find an association between this marker and hepcidin, with is considered as the main iron metabolism regulator. Nevertheless, all above suggest that inflammation is the link between heart failure progression, as reflected by cardiac stress biomarkers, and anemia development.

The main limitation of this study was the relatively small population size which could have led to a bias in the observations, although this weakness was overcome because of the inclusion of a uniform population of stable patients, without anemia and over a prolonged followed-up time; which made it possible to obtain a long term view before the anemia appeared at more advanced stages. Further studies are still required to clarify the role of sTNFRI and hepcidin, as well as other markers such as GDF15; however, the present work add new knowledge about which are the earlier mechanism involved on the hemoglobin reduction over time among non-anemic CHF patients. The identification of these mechanisms could be used in the design of studies focused on therapeutic interventions aimed to correcting these and prevent the development of anemia before it is established.

In conclusion, in CHF patients without anemia, the increase in the inflammatory state (sTNFRI) and the following deterioration in the iron metabolism (hepcidin) were the main determinants of a decrease in hemoglobin and the appearance of anemia in the long term follow-up period.

Conflict of interest

No conflict of interest exist.

Acknowledgments

This study was supported by a grant from the Spanish Society of Cardiology, Heart Failure and Transplant Working Group, 2010.

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