

# Blunted erythropoietin production and defective iron supply for erythropoiesis as major causes of anaemia in patients with chronic heart failure

Cristina Opasich<sup>1\*</sup>, Mario Cazzola<sup>2</sup>, Laura Scelsi<sup>3</sup>, Stefania De Feo<sup>1</sup>, Enzo Bosimini<sup>4</sup>, Rocco Lagioia<sup>5</sup>, Oreste Febo<sup>6</sup>, Roberto Ferrari<sup>7</sup>, Alessandro Fucili<sup>7</sup>, Remigio Moratti<sup>8</sup>, Roberto Tramarin<sup>1</sup>, and Luigi Tavazzi<sup>3</sup>

<sup>1</sup>Department of Cardiology, Institute of Pavia, IRCCS Fondazione Salvatore Maugeri, Strada Ferrata 8, 27100 Pavia, Italy; <sup>2</sup>Division of Haematology, IRCCS Policlinico S. Matteo, University of Pavia, Pavia, Italy; <sup>3</sup>Division of Cardiology, IRCCS Policlinico S. Matteo, University of Pavia, Pavia, Italy; <sup>4</sup>Department of Cardiology, Institute of Veruno, IRCCS Fondazione Salvatore Maugeri, Veruno, Italy; <sup>5</sup>Department of Cardiology, Institute of Cassano Murge, IRCCS Fondazione Salvatore Maugeri, Cassano Murge, Italy; <sup>6</sup>Department of Cardiology, Institute of Montescano, IRCCS Fondazione Salvatore Maugeri, Montescano, Italy; <sup>7</sup>Department of Cardiology, University of Ferrara, Ferrara, Italy; and <sup>8</sup>Laboratory of Chemical Analysis, IRCCS Policlinico S. Matteo, University of Pavia, Pavia, Italy

Received 11 December 2004; revised 28 March 2005; accepted 2 June 2005; online publish-ahead-of-print 29 June 2005

See page 2213 for the editorial comment on this article (doi:10.1093/eurheartj/ehi509)

## KEYWORDS

Chronic heart failure;  
Anaemia;  
Cytokines

**Aims** Anaemia is often observed in patients with chronic heart failure (CHF), and it may be associated with a worse prognosis. Aim of this study was to identify the individual mechanisms of anaemia in CHF patients.

**Methods and results** One hundred and forty-eight consecutive patients with haemoglobin concentration < 13 g/dL (if males) or < 12 g/dL (if females) were enrolled. Factors responsible for anaemia were investigated by evaluating endogenous erythropoietin (Epo) production, serum cytokines levels, body iron status, and iron supply for erythropoiesis. Most patients (57%) presented anaemia of chronic disease and among them, 92% showed evidence of a defective endogenous Epo production. This was indicated by an observed/predicted log(serum Epo) ratio less than 0.8 and/or a defective iron supply for erythropoiesis diagnosed by low transferrin saturation and/or increased value of soluble transferrin receptor. According to regression analysis sex, renal failure, and serum Epo were correlated with anaemia.

**Conclusion** According to our study, about half of anaemic CHF patients showed anaemia of chronic disease with blunted endogenous Epo production and/or a defective iron supply for erythropoiesis. Determination of the individual mechanisms of anaemia in CHF could justify a rational therapeutic approach to anaemia.

## Introduction

Chronic heart failure (CHF) is a complex syndrome related to both dysfunctional regulatory systems and a heart performance that is inadequate to meet the tissues' demands.<sup>1</sup> Adequate tissue oxygen supply depends not only on cardiac output but also on arterial blood haemoglobin (Hb) concentration and oxygen saturation.<sup>2</sup> Each of these components has its own mechanisms to compensate for hypoxia. Sustained reductions in the tissue oxygen pressure (PO<sub>2</sub>) stimulate both red cell production, by means of erythropoietin, (Epo) and 2,3-diphosphoglycerate production to shift the Hb oxygen-dissociation curve for greater oxygen delivery.<sup>3</sup> Anaemia may significantly affect oxygen delivery. In the last few years, several studies have

shown that anaemia is frequent in patients with CHF,<sup>4</sup> has a prognostic power,<sup>5–13</sup> and is a target of therapy.<sup>14–17</sup> However, the heterogeneity of both aetiology and progression processes of the heart failure syndrome suggests that the anaemia's pathophysiology might be non-univocal in the universe of heart failure patients.<sup>18–20</sup>

In this study, we focus on some of the mechanisms of impaired red cell production in consecutive anaemic CHF patients.

## Methods

### Study design and patients

All patients evaluated in this study suffered from CHF due to either left ventricular systolic or diastolic dysfunction and were regularly attending heart failure clinics. The diagnostic criteria for CHF were those recommended by the European Society of Cardiology

\* Corresponding author. Tel: +39 0382 592604; fax: +39 0382 592900.  
E-mail address: copasich@fsm.it

guidelines.<sup>21</sup> Patients were included in the study only if they were in stable clinical conditions (absence of physical findings of congestion, significant changes in diuretics, and weight or blood pressure in the previous four weeks) and only if they presented at least once in the last 3 months an Hb concentration value <13 g/dL if males and <12 g/dL if females. Patients were not considered eligible for the study if they had any comorbid condition causing anaemia (i.e. malignancies, chronic inflammatory states, collagen vascular diseases, myelodysplastic or myelophthisic syndromes, immunosuppressant therapy, and chemo/radiation therapy). New cases of heart failure defined by a duration of symptoms <6 months were also excluded.

The patients were enrolled from January to October 2002 in three heart failure clinics.

In all patients, blood samples were collected to determine complete blood cell count, body iron status, iron supply for erythropoiesis, endogenous Epo production, and cytokines levels.

The procedures we followed were in accordance with the ethical standards of the institutional committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 1983.

After overnight fasting, an intravenous cannula was inserted. After at least a 20 min supine rest, blood samples were withdrawn. An assay to determine blood cell count, body iron status, and iron supply for erythropoiesis was performed using the hospital's routine analysis procedures. Serum samples for cytokines, cytokines receptor, and Epo levels were stored at  $-80^{\circ}$  until analysis.

### Haematological profile and iron status

Blood counts were determined with automatic counters. Body iron status was evaluated by measuring serum iron, serum ferritin, and soluble transferrin receptor (Dade Behring, Marburg, Germany). Creatinine clearance was estimated by the Cockcroft–Gault formula.<sup>22</sup>

### Serum Epo assay

Circulating Epo levels were measured by partly automated two-step enzyme immunoassay on the basis of two monoclonal antibodies used to recognize natural or recombinant Epo (EPO ELISA–Medac, Hamburg, Germany). The standard curve of the assay is linear up to 80 mIU/mL and it is substandardized against the Third International Standard for Epo. To define Epo levels as appropriate or inappropriate for a given degree of anaemia, an exponential regression of serum Epo vs. Hb was determined in 31 reference subjects (Epo  $14.1 \pm 2.8$  mIU/mL) and 95% confidence limits were defined.<sup>23</sup> For Hb values <13 g/dL, the regression equation was  $\log(\text{Epo}) = 4.746 - (0.275 \times \text{Hb})$ . This equation was employed to predict Epo on the basis of Hb concentration and to derive the observed/predicted log(Epo) ratio (O/P ratio) as following:  $\text{O/P ratio} = [\log(\text{observed Epo})]/[\log(\text{predicted Epo})]$ . Mean O/P ratio in reference subjects was  $1.01 \pm 0.11$  (95% CI 0.80–1.22).<sup>24–26</sup> A defective endogenous Epo production was defined as an O/P ratio less than 0.8.

### C-reactive protein assay

C-reactive protein was measured by high-sensitive C-reactive protein assay (Dade Behring) by means of particle-enhanced immunonephelometry using BN<sup>TM</sup> System, designed to measure C-reactive protein concentrations in the overall range of 0.0175–110 mg/dL. Our reference interval was up to 0.5 mg/dL.<sup>27</sup>

### Serum cytokines assay

Tumour necrosis factor-alpha (TNF- $\alpha$ ) and its two soluble receptors, sTNFR-I and sTNFR-II, and interleukin-6 (IL-6) and interleukin-1 receptor (IL-1Ra) levels were measured using ELISA commercial kits (TNF- $\alpha$ : Biosource, Europe SA Nivelles, Belgium; sTNFR-I and sTNFR-II, IL-6 and IL-1Ra: R&D System Inc., Minneapolis, MN, USA) as previously described.<sup>28</sup>

The sensitivity of the kits used for measurements of TNF- $\alpha$ , sTNFR-I and sTNFR-II, and IL-6 and IL-1Ra was <0.5 pg/mL, 3 pg/mL, 1 pg/mL, 0.7 pg/mL, and 1 pg/mL, respectively. All kits had a good linearity in the detected intervals. Coefficients of variation were 3.9, 5.5, 3.8, 3.5, and 4.4%, respectively.

For cytokines levels, the reference subjects were 24 healthy males and 16 females, mean age  $65 \pm 6$  years.<sup>28</sup>

### Statistical analysis

The association between clinical and haematological variables was assessed by Pearson's coefficient of correlation or by analysis of variance depending on whether the variables were continuous or categorical, respectively. All tests were two-sided. No formal sample size calculations were done. Data are reported as mean value  $\pm 1$  SD. All tests of hypothesis were carried out using 0.05 as the level of statistical significance.

### Results

One hundred and forty-eight patients were included in the study. Their clinical characteristics are reported in *Table 1*. The majority of patients were males, affected by a dilated ischaemic cardiomyopathy with prevalent systolic dysfunction, in New York Heart Association (NYHA) class II and III. Heart failure symptoms were longstanding and renal dysfunction (defined as plasma creatinine >1.5 mg/dL, range 1.53–3.9 mg/dL) was found in 30% of the patients.

**Table 1** Baseline characteristics of the study population

Characteristics	Patients
Age, years	64.5 $\pm$ 12.2
Males, n (%)	109 (74)
NYHA class II/III/IV, n (%)	58/69/21 (39, 47, 14)
CHF symptoms duration (months)	33.9 $\pm$ 36
Diabetes, n (%)	43 (29)
Smokers (past or present), n (%)	49 (33)
Patients with $\geq 1$ comorbidity, n (%)	104 (70)
Serum creatinine (mg/dL)	1.47 $\pm$ 0.6
Aetiology, n (%)	
Ischaemic cardiopathy	71 (48)
Hypertensive cardiopathy	7 (5)
Dilated cardiomyopathy	45 (30)
Valvular cardiopathy	14 (10)
Others	7 (5)
Not known	4 (2)
Echocardiographic data	
Left ventricular end-diastolic diameter (mm)	62.4 $\pm$ 10.7
Left ventricular end-diastolic volume (mL)	185.7 $\pm$ 85
Left ventricular ejection fraction (%)	31 $\pm$ 12.1
Therapy	
Digoxin, n (%)	60 (40)
ACE-inhibitors/ATII-inhibitors, n (%)	133 (90)
Captopril equivalent dose (mg)	67.3 $\pm$ 26.5
Beta-blockers, n (%)	82 (55)
Furosemide, n (%)	141 (95)
Other diuretics, n (%)	15 (10)
Nitrates, n (%)	67 (45)
Antiplatelet agents, n (%)	55 (37)
Warfarin, n (%)	83 (56)

Nearly all patients were receiving diuretics, ACE-inhibitors or ATII-inhibitors, antiplatelet drugs or warfarin; 55% of them were on beta-blockers.

Haematological data and parameters of body iron status are reported in *Table 2*. Distribution of the anaemia causing factors in our population is reported in *Figure 1*.

Although the major criterion of inclusion was an Hb concentration <13 g/dL (if males) or <12 g/dL (if females) on at least one occasion, at the time of enrolment three males and two females showed borderline Hb values (Hb 13.1, 13.2, and 13.5 g/dL and Hb 12.0 and 12.4 g/dL, respectively).

Thirty-one patients had microcytic anaemia, defined as mean corpuscular volume (MCV) value <80 fL. Eight of them had heterozygous beta-thalassaemia: a 5% frequency is comparable to that of thalassaemia carriers in the Italian population. Only two patients had microcytic iron deficiency anaemia (ferritin <15 ng/mL in males and <10 ng/mL in females).

Seven patients had macrocytic anaemia (MCV > 100 fL) associated with folate deficiency. One hundred and five patients were found to have normocytic anaemia. Six of them had iron deficiency according to their low ferritin values. In thirty-five cases, there was evidence of renal impairment (serum creatinine >1.5 mg/dL) indicating renal anaemia, with mean serum Epo  $18.2 \pm 24.8$  mU/mL; 80% of them showed a defective endogenous Epo production (indicated by O/P ratio less than 0.8). No patient displayed signs of haemolytic anaemia.

### Anaemia of chronic disease

Out of the entire study population, eighty-five patients had anaemia of chronic disease, as defined by the following parameters: reduced concentrations of serum iron, transferrin, and total iron binding capacity; normal or raised ferritin; normal or slightly increased soluble transferrin receptor. All patients had normal ferritin values; 50 of them had low serum iron (<60 µg/dL), 36 had low transferrin saturation (<15%), and 47 had increased levels of soluble transferrin receptor (>1.76 g/L). Overall, the

combination of high soluble transferrin receptor and/or low transferrin saturation, indicating iron deficiency for erythropoiesis, was found in 54 patients. Two persons had fasting iron saturation >60%, suggesting iron overload. A C-reactive protein level >0.50 mg/dL was found in 53 patients (62%), urea was >50 mg/dL in 60 patients (71%), and all but 14 individuals showed elevated (higher than mean + 2SD of the reference values) TNF-α levels (*Table 3*). In these patients with chronic disease anaemia, serum Epo levels correlated with cardiac disease severity (NYHA class II  $8.4 \pm 7.6$  mU/mL; NYHA class III  $29 \pm 37$  mU/mL; NYHA class IV  $53.4 \pm 74$  mU/mL;  $F = 6.7$ ;  $P = 0.001$ ), with C-reactive protein ( $r = -0.25$ ;  $P = 0.021$ ) and, strongly, with IL-6 ( $r = 0.57$ ;  $P = 0.0001$ ). According to the calculated O/P ratio, the vast majority of anaemic patients showed evidence of defective endogenous Epo production. In fact, using a cut-off value of 0.8 for the O/P ratio, 76% (65/85) of the investigated patients had a ratio below this value.

Out of the group classified as having anaemia of chronic disease, 78 patients (92%) showed iron deficiency for erythropoiesis (that is high soluble transferrin receptor and/or low transferrin saturation) and/or defective endogenous Epo production.

In these patients, Hb significantly correlated with gender (females  $10.4 \pm 0.9$  g/dL vs. males  $11.1 \pm 1.0$  g/dL;  $P = 0.0002$ ) and inversely related with serum creatinine ( $r = -0.42$ ;  $P < 0.001$ ), urea ( $r = -0.29$ ;  $P = 0.008$ ), sTNFR-I ( $r = -0.44$ ;  $P < 0.001$ ), IL-6 ( $r = -0.41$ ;  $P = 0.001$ ), and Epo levels ( $r = -0.21$ ;  $P = 0.04$ ).

### Discussion

Multiple factors contribute to anaemia in our CHF population (*Figure 1*). A reduced Hb concentration may be a consequence of the haemodilution, which is often due to the activation of rennin-angiotensin-aldosterone and vasopressin systems, leading to sodium and water retention. Pseudo-anaemia due to plasma volume seems to have a worse prognosis than 'true' anaemia.<sup>29</sup> In our study, the volume status was assessed only by physical examination, whereas

**Table 2** Haematological parameters of the study population

Parameter	Males (n = 109)	Females (n = 39)	Reference values
Hb (g/dL)	$11.1 \pm 1.04$	$10.5 \pm 0.98$	Males: 13–17 Females: 12–16
MCV (fL)	$86.2 \pm 9.8$	$84.5 \pm 10.4$	83–97
Reticulocytes ( $10^9/L$ )	$12.8 \pm 17.2$	$22 \pm 24.4$	20–100
Platelets ( $10^9/L$ )	$232 \pm 86.7$	$256 \pm 70$	150–450
Serum iron (µg/dL)	$64.3 \pm 31$	$57.6 \pm 28$	60–150
Transferrin saturation (%)	$22.4 \pm 13$	$16.8 \pm 9.4$	15–45
Serum ferritin (ng/mL)	$182 \pm 193$	$102 \pm 132$	Males: 15–250 Females 10–150
Soluble transferrin receptor (mg/L)	$2.2 \pm 0.3$	$2.5 \pm 1.58$	0.83–1.76
Serum erythropoietin (mU/mL)	$19.9 \pm 33$	$25.5 \pm 36.4$	5–25 in non-anaemic subjects <sup>26</sup>
EPO O/P ratio	$0.64 \pm 0.4$	$0.49 \pm 0.3$	0.80–1.20 <sup>26</sup>
Serum urea (mg/dL)	$76.1 \pm 36$	$82.7 \pm 48$	10–50
Creatinine clearance (mL/min)	$74 \pm 16$	$73.8 \pm 14$	90–150
Serum creatinine (mg/dL)	$1.5 \pm 0.57$	$1.4 \pm 0.6$	0.7–1.20
C-reactive protein (mg/dL)	$1.6 \pm 2.5$	$1.5 \pm 2.1$	<0.50

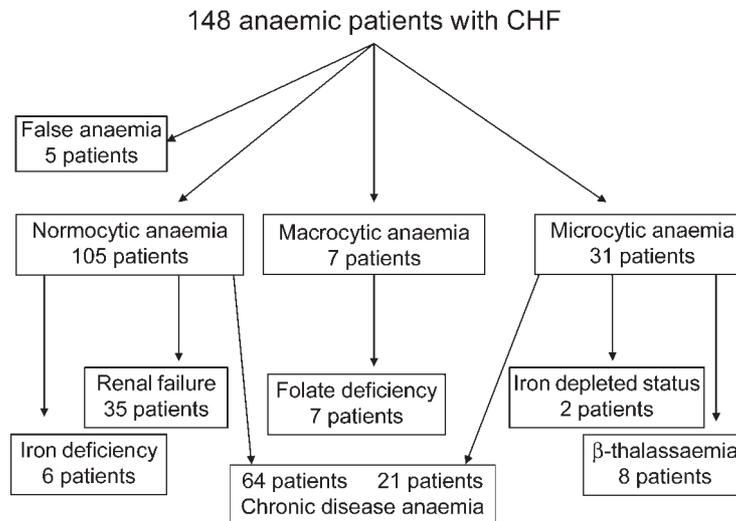


Figure 1 Causes of anaemia in our population of CHF patients.

haemodilution may occur even in some patients appearing as being euvoletic on clinical examination. All the patients we selected were in stable conditions and had at least two Hb determinations spaced of minimum 10 days (mean 49 days) from each others to limit the likelihood of a significant haemodilution.

Only few patients showed haematinic deficiencies (iron, folate, and/or vitamin B12), which are also reported as being a rare event in other series of CHF patients,<sup>18,30</sup> likely due to poor nutrition, gastrointestinal malabsorption, chronic use of aspirin, uremic gastritis, and other comorbidity.

Among the potential causes of normocytic anaemia, which affected 71% of our patients, renal dysfunction with consequent decreased renal Epo secretion was found in a third of our cases.<sup>18,31</sup>

Poor utilization of seemingly adequate iron stores, excessive cytokines activation, and inhibited synthesis of Epo were common, suggesting that these are probably the most important factors closely connected with anaemia of chronic disease. They were found in 57% of our population, a proportion similar to the one that is reported in the literature.<sup>9</sup> In the vast majority of these patients, we found evidence of inadequate endogenous Epo production and/or defective iron supply for erythropoiesis. These observations may have clinical implications, as in most instances, intravenous iron, combined, in selected cases, with subcutaneous administration of recombinant human Epo would represent a rational therapeutic approach to these anaemic CHF patients.<sup>14-17,32</sup>

A variety of causes were identified for defective endogenous Epo production. Epo is mostly produced by the kidney and hypoxia is the fundamental physiologic stimulus to the Epo renal production through an exponential increase in the number of Epo producing cells.<sup>32</sup> Defective Epo production is the major cause of anaemia associated with renal failure and likely of anaemia in CHF patients with concomitant renal damage.

Inflammatory cytokines may interfere with *Epo* gene expression.<sup>25,33</sup> Interleukins, TNF- $\alpha$ , and transforming growth factor-beta have been found to inhibit hypoxia-induced Epo production *in vitro*. At variance, IL-6 was shown to mimic hypoxia *in vitro*, and elevated levels in humans are

Table 3 Cytokine profile in anaemia of chronic disease patients

Variables (pg/mL)	Patients (n = 85)	Healthy subjects (reference values, n = 40) <sup>28</sup>
TNF- $\alpha$	40.6 $\pm$ 11.6	18.2 $\pm$ 4
sTNFR-I	3052 $\pm$ 1438	978 $\pm$ 775
sTNFR-II	4647 $\pm$ 2339	1947 $\pm$ 1403
IL-6	19.6 $\pm$ 24.9	4.3 $\pm$ 8
IL-1Ra	613 $\pm$ 623	224 $\pm$ 112

associated with increase endogenous Epo production.<sup>25</sup> By inhibiting erythroid progenitor cell proliferation, these cytokines also play a major role in the pathogenesis of anaemia of chronic disease, which was established as a distinct entity in 1962 and which is frequently associated with diabetes mellitus, connective tissue disease, chronic infections, and malignancy.<sup>34</sup> Anaemia of chronic disease is often normochromic normocytic, even if it may also show hypochromic microcytic indices similar to the effects of iron deficiency. It is characterized by reduced concentrations of serum iron, transferrin, and total iron binding capacity; normal or raised ferritin (instead of reduced as when iron stores are diminished); normal or slightly increased soluble transferrin receptor (which are raised in iron deficiency anaemia); and high erythrocyte sedimentation rate.<sup>34</sup> Moreover, elevated plasma levels of inflammatory cytokines, particularly TNF- $\alpha$ , are implicated in the Epo resistance process and have been shown to interfere with the peripheral actions of Epo.<sup>19</sup> Epo resistance could also be relevant to anaemia in CHF and could explain why elevated plasma Epo levels were associated with an impaired prognosis in van der Meer's study on 74 CHF patients.<sup>35</sup> In our study, IL-6 resulted strictly related with Epo endogenous production. In fact, nearly all the anaemic patients we studied showed evidence of cytokines activation, and it is very likely that this factor also contributed to the development of blunted endogenous Epo production.

Inflammatory cytokines not only interfere with *Epo* gene expression, but also have a major impact on iron metabolism, determining the so-called reticuloendothelial

iron block with defective iron supply to the erythropoiesis. The molecular mechanisms responsible for this effect have been recently elucidated.<sup>36</sup> Hepcidin is the key regulator for iron metabolism and mediator of anaemia during inflammation. This small peptide is induced by inflammatory cytokines and its overproduction results in macrophage iron retention and, consequently, less iron is available for erythropoiesis. This reticuloendothelial iron block cannot be overcome by oral iron administration, whereas intravenous iron may be effective for this purpose.<sup>37</sup> The best parameters to recognize impaired iron supply to the erythroid marrow are transferrin saturation and soluble transferrin receptor.<sup>38</sup> In an anaemic patient with CHF, a transferrin saturation <15% and an increased level of soluble transferrin receptor are together suggestive of iron deficient erythropoiesis.

Finally, several drugs may interfere with *Epo* gene expression. Angiotensin-converting enzyme-inhibitors may cause a reduction in the Hb levels by attenuating the production of *Epo* and may therefore contribute to anaemia in CHF patients.<sup>39</sup> The signal seems to be mediated via AT<sub>1</sub> receptors and a possible advantage of AT<sub>1</sub> antagonists over ACE-inhibitors with regard to *EPO* effect has not been elucidated yet.<sup>40</sup>

### Limits of the study

Patients here investigated had moderate heart failure; however, it cannot be ruled out, though it is unlikely, that in more symptomatic patients with a more severe systolic dysfunction, mechanism of anaemia might be somehow different.

Moreover, undernourishment was not routinely searched in our patients; however, only few of them showed haematinic deficiencies.

In the present study, chronic bleedings were not specifically investigated in our out-patients, which did not present clinical indicators of historical or present haemorrhages, although many of them were on antiplatelet drugs or warfarin. However, in our series, severe liver dysfunction, bleeding diathesis, and coagulopathy were considered as exclusion criteria.

Our study was multicentred and focused on anaemic patients. Unfortunately, a log book of all patients among whom the anaemics were found has not been held and the real prevalence of anaemic patients is not available. Anyway, we reviewed the number of patients visited at the heart failure clinics during the period of pertinent enrolment in the study and obtained a prevalence of 15.6%, similar to that reported in the literature.<sup>4,5-13</sup>

Finally, no healthy control subjects were included in the study. It was considered that being data on normal people largely available, the fact of asking subjects to provide their blood samples just to serve as controls might appear as non-ethical.

### Practical implications

According to the present study, it appears that many anaemic patients with CHF have chronic disease anaemia with both blunted endogenous *Epo* production and iron deficient erythropoiesis.

An observed/predicted log *Epo* ratio less than 0.80 in an anaemic patient could be taken as a reliable indicator of blunted *Epo* production.<sup>41</sup> These patients are very likely to

respond to renal doses of recombinant human *Epo* with an amelioration of anaemia, even when renal function is still normal.

### Acknowledgements

We are grateful to Dr Gloria Francolini (Laboratory of Chemical Analysis, Fondazione S. Maugeri, Gussago) and Dr Giulia Albonico (Laboratory of Chemical Analysis of the IRCCS Policlinico S. Matteo) for the technical support.

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