Anemia in Heart Failure: Pathophysiology, Pathogenesis, Treatment, and Incognitae

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Although anemia now occupies an important place in our present understanding of the pathogenesis of heart failure, the condition is surrounded in mystery. Anemia is highly prevalent in patients with heart failure and is of great clinical significance. However, the treatment targets for anemia in patients with heart failure have still not been accurately defined. The present article reviews of the clinical and pathophysiological characteristics of anemia in this context. Particular emphasis has been placed on cellular and molecular regulatory mechanisms, and their implications for treatment.

Key words: *Heart failure. Anemia. Chronic renal disease. Erythropoietin. Iron. Inflammation. Interleukins. Hepcidin.*

Anemia en la insuficiencia cardiaca: fisiopatología, patogenia, tratamiento e incógnitas

Aunque la anemia ha pasado a ocupar un plano relevante en la concepción patogénica actual de la insuficiencia cardiaca (IC), se trata aún de una entidad rodeada de incógnitas. La prevalencia de anemia y su importancia clínica en la población con IC son muy elevadas. Sin embargo, no se han establecido aún con certeza suficiente los objetivos de tratamiento de la anemia en la población con IC. El presente trabajo revisa aspectos clínicos y fisiopatológicos de esta forma particular de anemia, con especial atención a los mecanismos celulares y moleculares de regulación, y sus implicaciones en el tratamiento.

Palabras clave: *Insuficiencia cardiaca. Anemia. Enfermedad renal crónica. Eritropoyetina. Hierro. Inflamación. Interleucinas. Hepcidina.*

INTRODUCTION

Anemia is known to be associated with heart failure (HF), but it is not widely considered in clinical practice. Previous works[1] that pointed out the role of anemia as a risk factor in this complex condition were not received with much acceptance. This situation has recently taken a notable turn and anemia has come to occupy a more relevant position in the understanding of the pathogenesis of heart failure. In an illustrative example, while the clinical guidelines for the management of HF issued by the American College of Cardiology and the American Heart Association between 1999 and 2001[2] did not mention anemia, in those of 2005,[3] it is recognized as a frequent finding that is associated with the rates of morbidity and mortality. From that time on, the data has become increasingly extensive, and a recent review of new aspects of HF[4] acknowledges an even more relevant pathogenic role of anemia than that mentioned in the European guidelines for HF.[5] This recognition has generated a high level of expectation with respect to the possible beneficial role of the treatment of anemia in the natural history of HF. This expectation, however, has not been accompanied by the systematization of its study and treatment. In contrast, there has been a progressive increase in the application of therapeutic measures, not always sufficiently individualized and systematized. All in all, anemia in patients with heart failure is still enveloped in unknowns, especially with respect to its pathogenesis and the importance of its course in HF, constituting a terrain in which opinion still predominates over scientific evidence.

EPIDEMIOLOGY AND MAGNITUDE OF THE PROBLEM

The Prevalence Depends on the Population Being Studied and the Comorbidity

In published series, the percentage of patients in which HF is accompanied by anemia differs widely,[6,7] ranging

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between 9.9%[8] and over 50%.[9] This variability depends in part on the differences between the populations analyzed (comorbidity, New York Heart Association [NYHA] class), but, above all, on the cutoff point used to define anemia.

Patients with anemia and HF tend to be of more advanced age, in NYHA functional class III-IV, with more drug treatment and more comorbidity (diabetes mellitus, chronic renal disease [CRD], and hypertension), as well as longer and more frequent hospital stays,[6,10] but these individuals are not usually included in drug trials.[11] As an example, in an analysis of older patients, more than half had a hemoglobin level < 12 g/dL and, among this subgroup, 79.1% were in NYHA class IV.[12] A large body of data supports the concept that the prevalence of anemia increases with more severe HF, but they do not explain the mechanisms involved in this relationship.[12,13]

The Estimation of the Prevalence Depends on the Definition of Anemia

A major drawback when assessing population-based data is the fact that uniform cutoff points have not been employed to define anemia. At the present time, the situation remains unstable in terms of definition. The clearest example is that observed in renal patients. In individuals with CRD, the National Kidney Foundation (NKF), in its 2000 guidelines, defined anemia as a hemoglobin level < 12.0 g/dL in men and postmenopausal women.[14] In a new version of these guidelines (2006), the limits were raised to < 13.5 g/dL in men and remained at < 12 g/dL in women.[15] However, the publication of new works in the months following the appearance of these modified guidelines (see section on "Current perspectives") has led to their immediate revision, and a third version is now being drafted in which lower target hemoglobin values are again being proposed (Adeer Levin, personal communication, 2007). As a whole, the instability of this issue is a clear invitation to act with caution when establishing objectives in the anemia of HF.

According to a review on the prevalence of anemia in HF,[6] the most widely used cutoff point is hemoglobin < 12 g/dL. This is not a trivial detail since a change in the cutoff point of 1 g/dL for hemoglobin or of 1% for hematocrit produces a substantial change in the prevalence.[9] For example, in the Euro-Heart Failure Study,[16] the estimate of the prevalence of anemia increased by 33% with the cutoff point of 12 g/dL. Finally, the World Health Organization utilizes limits of hemoglobin < 12 g/dL in regularly menstruating women and < 13 g/dL in men and in postmenopausal women.

Along these lines of interpretation, a central idea is that, in the context of cardiovascular disease, asymptomatic anemia does not exist. That means that the classification of an individual as anemic and, thus, in need of treatment takes on unforeseen implications in terms of the possible administration of costly drug treatments.

PATHOPHYSIOLOGY AND PATHOGENESIS

Heart failure, like other chronic diseases, is practically nonexistent outside the human species. The adaptive mechanisms are changes in physiological responses, originally developed for other purposes. In nature, anemia depends nearly exclusively on hemorrhage, and induces the activation of mechanisms to maintain perfusion, and the oxygen supply to tissues, but also to preserve volume. Focusing on the hemorrhage, the organism sets in motion an integrated response with actions in different regions, which include vasoconstriction and thrombosis, fluid retention, stimulation of erythropoiesis, and vascular repair. It is interesting to observe that the system most competent in inducing sustained vasoconstriction, the renin-angiotensin-aldosterone system (RAAS), is also pivotal in a mechanism capable of activating many of the aforementioned functions, including vascular fibrous scar formation.

Pathogenic Sequence: Anemia as an Adverse Influence on Heart Failure

Figure 1 shows some of the possible causes and consequences of anemia in HF, the rest of which appear in Table 1. Anemia can cause tissue hypoxia, which is accompanied by lactic acidemia, vasodilatation, and a hyperdynamic circulatory state. In individuals with NYHA class III-IV HF, the major determinant of hypoxemia is low output, in which tissue hypoxia occurs even in the absence of significant anemia.[17] In HF, vasodilatation associated with anemia may not be present due to the predominance of the vasoconstrictor response over the low output.

Renal hypoxia would be an effective stimulus for the secretion of erythropoietin (EPO). However, the signals that mediate the hyperdynamic state are not fully understood, and the same can be said, above all, for the true magnitude and distribution of anemic hypoxia throughout the tissues. A solid line of interpretation relates the vasodilatation of anemia with the decrease in the inhibitory effect of nitric oxide caused by hemoglobin.[18] However, there are no available data on this mechanism in animal models or in HF patients. In turn, the decrease in the mean arterial blood pressure activates the sympathetic nervous system, which provokes systemic and renal vasoconstriction and activates the RAAS. This system acts synergistically with the sympathetic nervous system in the peripheral vasoconstriction and produces renal sodium retention (angiotensin II in proximal tubules, aldosterone in distal tubules) and favors vasopressin release, which, in turn, also has a synergistic pressor effect, and causes fluid retention. In the most severe cases,

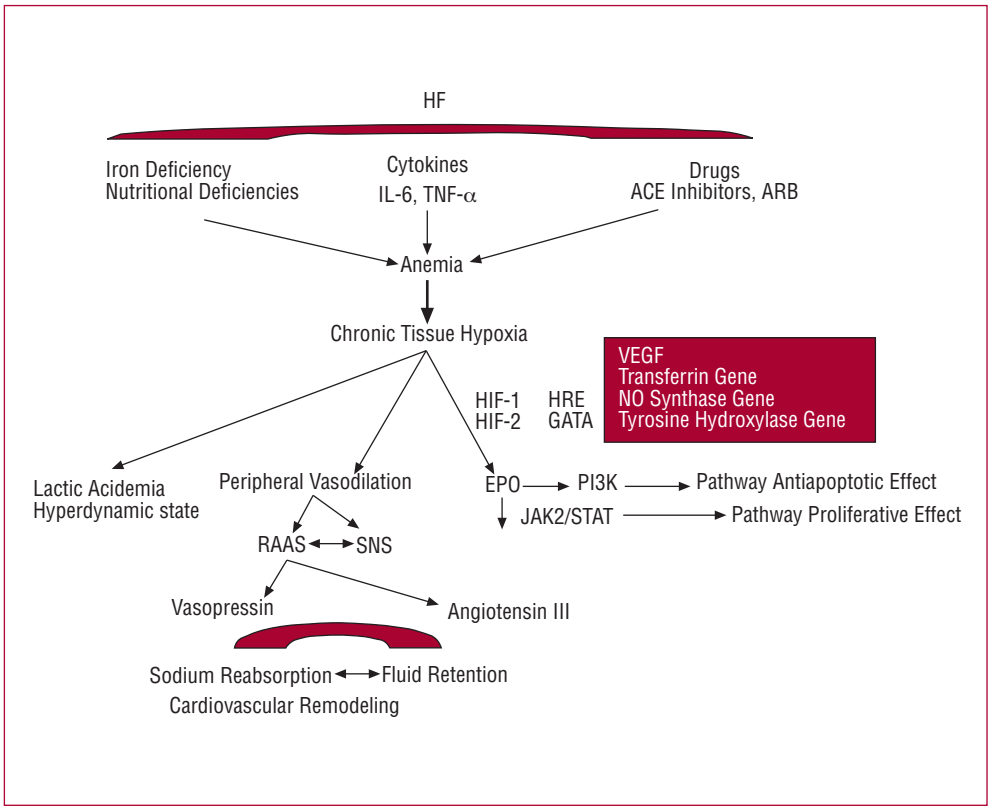

Figure 1. Diagram of the pathogenic mechanisms and the effects of anemia of heart failure (HF).
ACE indicates angiotensin-converting enzyme; ARB, angiotensin receptor blockers (angiotensin II receptor antagonists); EPO, erythropoietin; HIF, hypoxia-inducible factor; HRE, hypoxia responsive element; IL-6, interleukin 6; JAK2, Janus kinase 2; NO, nitric oxide; PI3K, phosphatidylinositol 3-kinase; RAAS, renin-angiotensin-aldosterone system; SNS, sympathetic nervous system; STAT, signal transducer and activator of transcription; TNF-α, tumor necrosis factor alpha; VEGF, vascular endothelial growth factor.

there is a decrease in renal flow and the glomerular filtration rate.

In studies involving renal transplant recipients,[19] it has been observed that individuals with hemodilution have a poorer prognosis, since this is associated with a more severe decompensation, related to an increased activation of the systems of fluid retention.[20] Hemodilution, a term that refers to a condition that remains to be clearly defined, reduces oxygen release into the tissue.[19]

In the presence of anemia, the heart undergoes remodeling, and both the sympathetic nervous system and the RAAS contribute to this phenomenon. In this respect, taking into account the recently discovered trophic role of EPO in the prevention of cardiomyocyte apoptosis,[21] as well as in myocardial revascularization, an EPO deficiency can result in important defects in remodeling. In other words, EPO may be necessary, or at least useful, in the maintenance of myocardial viability in the presence of anemia and under other circumstances. Myocardial failure itself, through the secretion of cytokines such as tumor necrosis factor alpha (TNF-α), can, in turn, worsen anemia, completing the vicious circle, with extremely negative results.[22] However, if we review the available scientific evidence, we discover that there is a lack of validated data, obtained with current techniques, concerning critical aspects of the sequence of events leading to anemia. Moreover, a considerable portion of the concepts employed is based on extrapolations of findings in normal physiology, or even intuitive ideas. An important reason for this lack is the result of the absence of studies on this subject in experimental models of HF.

TABLE 1. **Consequences of Anemia in Heart Failure***

Cardiovascular
Left ventricular hypertrophy
Precipitation of HF
Precipitation of CRF
Exacerbations of ischemic heart disease
Reduction
Aerobic capacity
Exercise tolerance
Subjective well-being: quality of life
Higher mental functions
Possible acceleration of the course of HF and RF

*CRF indicates cardiorenal failure; HF, heart failure; RF, renal failure.

PATHOGENIC FACTORS RELATED TO ANEMIA IN HEART FAILURE

Of the few attempts to establish the pathogenic classification of anemia in HF, most have corresponded to the pattern described for anemia associated with chronic diseases[23] (58%) and, less frequently, to iron deficiency (21%), nutritional deficiencies (8%), and other causes, including chronic bleeding in patients receiving

antiplatelet or anticoagulant therapy (13).[10] However, in recent series, on paper, a more important role is granted to iron deficiency, which is reported to be a major cause of anemia in nearly 80% of the cases.[24] In any case, it can be considered to be multifactorial and always requires a highly individualized study and treatment.

Chronic Renal Disease and Cardiorenal Failure

Two aspects of the new epidemiology of CRD have direct consequences in patients with HF: the marked increase in the number of patients with CRD, including its most severe forms, and the significant change in the causes, with a growing predominance of vascular diseases, within the arteriosclerosis-hypertension-diabetes complex.[25,26] Of maximum interest, the conditions that lead to renal failure in these individuals basically overlap those that favor HF and ischemic heart disease; thus, we observe a situation in which the 3 diseases, CRD, HF, and ischemic heart disease, can coincide.

It is essential to keep in mind that CRD in patients of this type is usually clinically silent, a circumstance that is supported by data that indicate that only 1 of every four subjects with a glomerular filtration rate of 15 to 59 mL/min is aware of the fact that he or she has CRD.[25] Moreover, CRD can remain occult because the glomerular filtration has not been assessed. In recent series, it has been demonstrated that 30% to 50% of the patients with HF have a creatinine clearance (CCr) < 60 mL/min,[27] even with plasma creatinine (Cr_P) < 2 than mg/dL, which conceals the true deterioration of renal function.[26,28] On this basis, the direct measurement or use of estimative equations for CCr in the protocols and clinical pathways for treating HF is becoming generalized,[27,28] a fact that has been confirmed by means of a survey recently performed by the HF sections of the Spanish Society of Cardiology and the Spanish Society of Internal Medicine (Gil et al, unpublished data).

The cardiorenal anemia syndrome[25,26,29] is based on the theoretical assumption that chronic HF and renal failure have a reciprocol negative influence, and that anemia is an aggravating factor. In other words, the coexistence of CRD gives rise to a new operative definition, cardiorenal failure (CRF), which entails substantial changes in the conventional therapeutic approach.[25,26,29] The degree of association among these 3 entities is such that anemia has been reported to be a marker of subclinical CRD in patients with HF.[6] However, in practice, doubts remain as to the extent to which anemia is a marker of more severe CRD or HF, or whether it, in itself, is a cardiovascular risk factor.[28]

Anemia is a multifactorial clinical problem inherent in CRD. To illustrate the magnitude of the dilemma, in the Third National Health and Nutrition Examination Surveys (NHANES III), carried out in 800 000 patients with CRD, the mean hemoglobin concentration was 11 g/dL, with a proportion of anemia that exhibited an inverse linear association with the glomerular filtration rate.[30,31] One datum with practical consequences for HF is the fact that anemia is an early complication of CRD; even with Cr_P values 2 mg/dL, 45% of the patients have a hematocrit level < 36%.[30,31]

Inflammation

Cytokines

Heart failure frequently coexists with a chronic inflammatory component, with the production of a repertoire of cytokines (TNF-α, interleukins [IL] 1, 6, and 10, interferon) that contribute to the pathogenesis of anemia through different mechanisms.[23] On the other hand, C-reactive protein (CRP) can act both as a biochemical marker and as a mediator of cardiovascular inflammation. The data that support an inverse relationship among the cytokines such as, for example, TNF-α and its soluble receptor, and the hemoglobin values are consistent.[23] The cytokines act on erythropoiesis in several ways: they inhibit EPO production at the transcriptional and transductional levels[32] and, above all, they interfere with the action of EPO on erythroid precursors. In studies carried out in the field of nephrology, high IL-6 and TNF-α levels correlated with greater needs for exogenous EPO in patients undergoing hemodialysis,[33] a fact that demonstrates that inflammation also affects the possible success of the treatment.

Hepcidin (Figure 2)

Hepcidin probably plays a relevant role in a high percentage of the anemia of HF. The discovery of hepcidin has added a new functional dimension to iron metabolism. While the traditional concepts for the evaluation of anemia, such as the balance between inputs and outputs or the existence of ferritin deposits and transport via transferrin, continue to be maintained, hepcidin and inflammatory mediators constitute a new and powerful source of information. Hepcidin, a small peptide that is synthesized in the liver, released into the plasma and excreted in the urine, plays a key role in orchestrating iron metabolism and the links between this metabolism, inflammation, and innate immunity.[34,35] At the present time, hepcidin is considered to be the homeostatic regulator of iron in its intestinal absorption, its recycling by macrophages and its mobilization from liver stores. Its transcription is markedly induced in inflammatory processes, especially by cytokines like IL-6, in which it coincides with CRP and the amyloid protein.[34,35]

The principal mechanism of hepcidin is its inhibition of cellular iron efflux, blocking the effect of the transport protein, ferroportin; under these conditions, macrophages, hepatocytes, and enterocytes retain iron, probably as a

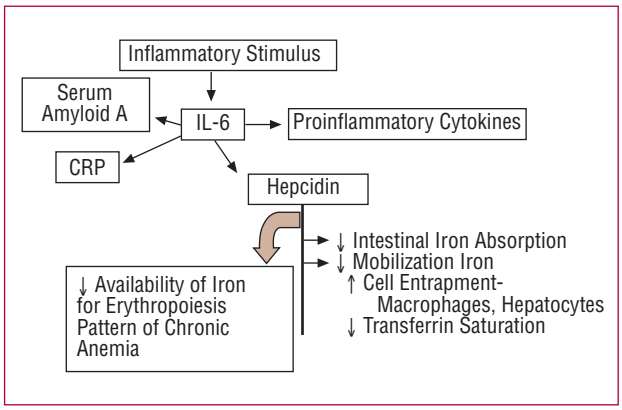

Figure 2. Inflammatory mechanisms in chronic anemia: critical role of interleukin 6 (IL-6) and hepcidin.
CRP indicates C-reactive protein.

defense against microorganisms. As iron is not released into the circulation, the availability to erythroid precursors is reduced.[34,35] High hepcidin concentrations cause cellular iron overload, as occurs in hemochromatosis, and contribute to the typical pattern of chronic anemia. To date, there is no data on the hepcidin levels in HF patients, but the recent introduction of commercially available methods for its measurement indicates that information will be made available in the near future. At the present time, there are no drug interventions applicable to hepcidin.

Drugs

The pathogenesis of anemia in HF also involves different groups of drugs. Among the most important ones, because of their widespread utilization, are the angiotensin-converting enzyme (ACE) inhibitors and the angiotensin II receptor antagonists (angiotensin receptor blockers [ARB]).

A number of studies have suggested that ACE inhibitors induce or worsen anemia. In the Studies of Left Ventricular Dysfunction (SOLVD), which involved 6000 patients, it was observed that, although treatment with enalapril had a protective effect in terms of overall mortality, it was associated with a decrease in hematocrit and an increase in the risk of new-onset anemia. Strikingly, in this study, the overall mortality rate was 108% higher in the patients who developed new-onset anemia. In contrast, in a Spanish series involving the prospective follow-up of 337 patients who had been admitted to the hospital for HF over a 20-month period, the relationship between ACE inhibitors and anemia did not reach statistical significance.[37] Other authors have reported a decrease in the circulating EPO in patients treated with ACE inhibitors.[38-40]

The pharmacological inhibition of the RAAS can produce a decrease in the hematocrit, which is negligible in patients with normal renal function,[41] but more marked in individuals with CRD.[42] While angiotensin I (AT1) antagonists[17] produce effects similar to those of the ACE inhibitors,[43] there are no formal studies comparing the latter with ARB in the anemia of HF.

The decrease in hematocrit as a consequence of the use of ACE inhibitors reaches its nadir in the first 3 months of treatment, but the level tends to remain stable over the long term. The discontinuation of these drugs leads to the normalization of the hematocrit within 3 to 4 months. Table 2 shows the possible mechanisms by which blocking the RAAS can produce anemia.

Prostaglandins have a stimulatory effect on erythropoiesis; thus, the administration of nonsteroidal antiinflammatory drugs can pave the way for anemia through events other than the chronic blood loss also caused by these drugs. Once again, there are no studies specifically focusing on this aspect, and one relevant issue is the possible role of aspirin in promoting anemia, a question that could be addressed without undertaking new specific studies, by processing the data of the existing large series or by metaanalysis.

HOW DOES ANEMIA INFLUENCE THE PROGNOSIS OF HEART FAILURE?

In patients with HF, anemia is a risk factor for mortality,[10] hospital admission, and severity,[7,29] and doubles the risk associated with other factors, such as diabetes mellitus, age, smoking, and a low ejection fraction.[44] In HF, there a linear relationship between the mortality[6] and the hemoglobin/hematocrit levels.[45] A number of authors report specific values for the increase in risk of death or of a major event, including hospital admission, for each decrease of 1% in hematocrit.[13,44] Others[46-49] affirm that an increase in hemoglobin of 1 g/dL reduces the risk of death at 1 year by 40%, with a decrease in the risk of hospital admission for HF of 21%. These data constitute a solid argument for the treatment of anemia in HF. However, they are not sufficient to enable us to establish the optimal hemoglobin level to achieve the maximum benefit with the least possible

TABLE 2. **Possible Anemia-Inducing Mechanisms of Angiotensin-Converting Enzyme Antagonists and Angiotensin I Antagonists***

Renal
Decrease in the synthesis of endogenous EPO
Bone marrow
Decrease in the response to EPO
Inhibition of the growth of erythroid precursors
Change in the response to treatment with rHuEPO
Decrease in IGF-1 levels
Inhibition of the catabolism of N-acetyl-seryl-aspartyl-proline, a peptide that reduces the proliferation of precursors of the red cell series

*EPO indicates erythropoietin; IGF-1, insulin-like growth factor 1; rHuEPO, recombinant human erythropoietin.

complications. An interesting question is the fact that the decrease in hematocrit could be a marker for other factors that increase the mortality in patients with severe HF, for example, CRD.[44]

MECHANISMS BY WHICH ANEMIA CAN INCREASE THE MORBIDITY AND MORTALITY IN HEART FAILURE

Again, we are faced by the lack of sufficient scientific evidence. Lower hemoglobin concentrations are associated with a poorer hemodynamic function, increases in serum urea nitrogen and creatinine, decreases in albumin, cholesterol and body mass index, a worse functional class, and a lower VO_2 (peak oxygen consumption).[6,10,13] A relationship between anemia and a less favorable course has been reported in patients with CRD,[50] asymptomatic left ventricular dysfunction,[45] and advanced HF.[7,13] There are not sufficient data in cases of severe systolic dysfunction.

In patients with preserved ventricular function, the hyperdynamic state can play a role in ventricular hypertrophy, which, in turn, can promote a disproportion between the oxygen supply to the myocardium and the increased ventricular mass, a circumstance that is critical in the presence of significant coronary artery disease. Cardiac output has been found to be sharply increased, with hemoglobin < 10 mg/dL (hematocrit < 30%-33%).[44] In the Prospective Randomized Amlodipine Survival Evaluation (PRAISE), anemia is associated with death due to pump failure. Studies carried out in animals show that an ischemic or hypertrophic heart is more vulnerable to slight decreases in hemoglobin than a normal heart, with a marked deterioration in the ischemia and the myocardial dysfunction. Under these conditions, the treatment with EPO is even more interesting because of its cytoprotective properties with respect to the myocardium.[9,51,52]

In a recent review, Roig pointed out that anemia could principally be a marker of advanced disease, since it is associated above all with a worse functional class, and that its correction improves the symptoms, but not necessarily the mortality rate.[11] On the other hand, it has been shown that hemoglobin is, in itself, an independent predictive factor of mortality in HF, in both anemic and polycythemic individuals.[53] Even more important, the hematocrit range that we should consider as excessive in patients with HF and, in particular with ischemic heart disease, has yet to be clearly established.

In HF, anemia also influences hospital admissions, and a relationship between the hemoglobin levels and the number of admissions due to HF over the preceding year has been observed; in this respect, low hematocrit may be more of a risk factor for hospital admission than for mortality.[47-49]

Anemia can favor the progression of CRD in patients with HF[38] and be, in itself, a risk factor[50] and a predictor for the development of HF in patients with end-stage CRD.[44] In turn, renal function, together with the ejection fraction and the NYHA class, may be an indirect marker of cardiac function.[25,26] Finally, anemia (hemoglobin < 13 g/dL is an independent predictor of the exercise capacity in HF, whereas in patients with hemoglobin >13 g/dL, there is no correlation between VO_2 and hemoglobin.[54]

Cellular and Molecular Biology of Erythropoietin Production: Hypoxia, Transcription Factors, and Other Regulatory Stimuli

Erythropoiesis

Production characteristics, site, and stimuli. The production and action of EPO are the critical points of erythropoiesis. Erythropoietin is a glycoprotein that, in extrauterine life, is produced nearly exclusively in the peritubular fibroblasts of the renal cortex, without direct contact with the capillaries and tubular cells.[55,56] The EPO gene belongs to a set of hypoxia-sensitive genes that are overexpressed when there is a decrease in the cellular partial pressure of oxygen (pO_2).[57] The production of EPO exhibits circadian fluctuations and the kidney intervenes in its catabolism. Hypoxia can lead to a several hundred-fold increase in EPO. In the experimental setting, the increase in the intensity of hypoxia is accompanied by an exponential increase in the number of fibroblasts expressing the EPO gene.[58] There are no data concerning this finding in models of HF.

Why in the kidneys? The possible physiological reasons and consequences of the localization of EPO production in the kidney is a question that has not been fully resolved. The simple fact that blood is made up of red cells and plasma provides a clue: what the organism regulates is the proportion of plasma that is constituted by circulating red cells (hematocrit). The specific mechanisms of this coordination are relatively unknown, although we assume that there is an intrarenal connection between the pathways that monitor and regulate the fluid volume and those that control the erythroid cell volume.[56] In other words, the capacity of the kidney to detect the status of the extracellular volume (ECV) and produce EPO enables it to "create" a normal hematocrit level. This phenomenon may be of importance in HF, in which the accumulation of extracellular fluid and hemodilution can change the "perception" of the hematocrit on the part of the kidney. On the other hand, the kidney extracts only a small part of the oxygen delivered to it, a circumstance that makes it possible to detect slight changes in oxygenation. Moreover, the constancy of the ratio of the work performed (oxygen consumption) to the renal blood flow (oxygen delivery) makes it possible to separate the renal pO_2 from the metabolic activity, meaning that the regulation of

EPO synthesis can be reasonably, although not totally, independent of the reabsorptive activity.

Baseline hematopoietic stimulation originates in the physiological destruction of red blood cells, but its greatest intensity is observed in hemorrhage. Anemia of HF is usually mild, persistent and adaptive. Thus, its effects would be analogous to minor hemorrhage with limited, but long-term, hemodynamic impact. The kidney has to restore volume depending on the amount of fluid lost and produce red cells to replace those lost, but nothing more. Moreover, it has to be able to discern the proportion in which fluid is conserved and new red cells are produced. The joint regulation of these 2 mechanisms is more complex than that of each separately, and is 1 of the keys to research in a disease like HF.

What makes up the chain of signals and mechanisms that increase endogenous erythropoietin? The physiological sequence involved in the stimulation of EPO production is based on 2 transcription factors that can be activated by hypoxia, hypoxia-inducible factors 1, and 2 (HIF-1 and HIF-2).[59,60] Hypoxia-inducible factor 1 is a molecule that regulates an overall multigenic response, rather than isolated effects. The great advance in recent years has been the discovery that transcriptionally active HIF-1 depends on a group of non-heme-iron-dependent prolyl hydroxylases that constitute the true oxygen-sensing mechanism.[59,61]. In contrast, HIF-2 appears to play a selective role in EPO expression, which is practically abolished in the knockouts for this gene.[61,62]

The activation state of HIF in HF is unknown, and there are no data published concerning its measurement. Thus, references to its role are limited to supposition, firm, but still conjectural. Although the patient with HF can go through periods of systemic hypoxia, they are usually transient; brief hypoxia does not constitute a strong enough stimulus for sustained EPO induction.[57] On the other hand, the measurement of tissue oxygenation is a challenge that has yet to be resolved by clinical research and the tissue oxygen levels reached during periods of decompensation and compensation in HF are basically unknown.

Hypoxia-inducible factor 1 is activated by means of phosphorylation under hypoxic conditions. It acts by transactivating more than 70 genes, at least 4 of which are highly relevant to the final effect of EPO: the transferrin gene, supplying iron to the erythroid cells; the vascular endothelial growth factor (VEGF) gene, a cofactor in the stimulation of these same cells and a determining element in angiogenesis and tissue perfusion; the tyrosine hydroxylase gene, involved in dopamine synthesis and respiratory regulation; and the nitric oxide synthase gene, which maintains normal arterial blood pressure under the potentially pressor effect of EPO.[63]

Hypoxia-inducible factor 1 is activated at oxygen concentrations of around 4% to 5% (saturation of 30 to 35 mm Hg).[64] The presence of elevated EPO levels in patients with HF can be taken as an indirect sign of HIF stimulation, but, as yet, there is no direct scientific evidence in this respect.

Hemodynamics and Erythropoietin

The blood flow rate in the kidneys is elevated (approximately 20% of the cardiac output). In the renal medulla, the pO_2 is permanently under 10 mm Hg, whereas in the cortex, it is more variable, with a mean close to the threshold for HIF stimulation (30 mm Hg). Changes in these concentrations are determinant in induction of the EPO gene.[65] Thus, it is assumed that, in patients with more severe HF (NYHA functional class III-IV), since the renal flow rate is reduced, the decreased peritubular oxygen tension would be the main stimulus to trigger EPO production.[17] Other studies point out the association between EPO synthesis in HF and renal hemodynamic dysfunction, although no correlation between plasma EPO levels and arterial pO_2, oxygen saturation, or glomerular filtration has been found.[66]

Role of Angiotensin II

In HF, the low renal output stimulates renin production, and this, in turn, that of angiotensin II (Ang II). A higher Ang II level results in greater sodium reabsorption and, thus, an increased adenosine triphosphate and oxygen consumption, secondary to the increased tubular reabsorptive function. In healthy subjects, the EPO concentrations are correlated with the proximal tubular sodium reabsorption rate.[67] Diuretics that act on Henle's loop, the distal tubule and collecting duct (furosemide, hydrochlorothiazide, and amiloride, respectively) do not affect EPO formation. In contrast, acetazolamide, which acts on the proximal tubule, significantly decreases EPO production in response to normobaric hypoxia and functional anemia.[67]

These data are consistent with the simultaneity of the increase in EPO levels and the poorer prognosis of HF.[68] Thus, if proximal hyperreabsorption is proportional to the degree of decompensation in HF, it can be assumed that those patients who are decompensated will reabsorb more sodium and will produce more EPO. To date, studies addressing this assumption have not been performed in humans.

One relevant question is whether the changes observed in Ang II are due to a direct stimulation of the peritubular fibroblasts that synthesize EPO or to hemodynamic effects induced by Ang II. In vitro, in HepG2 tumor cells, which express AT1 receptors, Ang II, and the AT1 antagonist, losartan, have no effects on EPO production, a circumstance that supports the idea that the in vivo effects of Ang II are mainly due to hemodynamic changes.[69] However, there are data that indicate that Ang II is capable of activating HIF-1α to levels even higher than those

generated by hypoxia.[70] The administration of exogenous Ang II, which increases HIF-1 and its target gene, VEGF, also increases the EPO concentrations in a dose-dependent manner.[71] Likewise, transgenic mice carrying both human renin and human angiotensinogen have persistent erythrocytosis, via the AT1 receptor.[72] These results can be explained by findings in in vitro studies that establish that Ang II not only plays a role in EPO production, but also stimulates erythroid precursors by means of AT1 activation in the erythroid burst-forming units.[41] Despite the fact that we have all this information, it can be said that, as a whole, the effect of Ang II on anemia has yet to be fully defined.

Signaling in Erythropoietin Production

The regulation of EPO production is controlled by an enhancer that binds HIF-1 and, thus, is referred to as HRE (hypoxia responsive element). In addition, there are elements that coregulate gene expression, the importance of which is still not fully known, but that might explain the differences in EPO mRNA synthesis observed under conditions equivalent to hypoxia. Among them, we should mention two transcription factors, GATA and nuclear factor kappa beta (NFκβ), which negatively regulate the expression of EPO mRNA. These 2 factors can be increased in the presence of inflammation, and thus, constitute plausible explanations for the decrease in EPO synthesis in inflammatory conditions. With respect to the stimulation of EPO gene expression, it should be mentioned that there is an inverse relationship between GATA and nitric oxide, a circumstance that indicates a role of the decrease in the latter in the reduced EPO production.[73]

Erythropoietin Signaling in its Target Cells

Erythropoietin signals through a receptor with protein kinase activity, which mediates proliferative and antiapoptotic effects in the erythroid precursors. For the former, EPO stimulates the Ras/mitogen-activated protein kinase (MAPK) pathway and, above all, the Janus kinase 2 (JAK2) pathway, which acts without intermediary metabolites on transcription factors of the STAT (signal transducer and activator of transcription) family, especially STAT5. The antiapoptotic effects can also employ the JAK2/STAT pathway, but the main enzymatic step occurs in phosphatidylinositol-3 kinase. There are EPO receptors (EPO-R) in the erythroblasts, but they are also found in the placenta, heart, retina, brain, and endothelial cells.[74] The EPO-R is a transmembrane receptor that shares properties with the receptors of other hematopoietic factors. While the intracellular transduction of EPO is known to take place, there are considerable gaps in our knowledge with respect to how this hormone regulates the survival, proliferation and differentiation of erythroid progenitor cells.[75]

The inhibitory effect of apoptosis does not only concern the erythroblasts. In this respect, it has recently been demonstrated that EPO is a potent trophic and apoptotic factor, with protective effects in brain and retinal cells, in the renal epithelium and, as mentioned above, in myocardial cells, as well.[51]

Resistance to Erythropoietin

In studies that relate an increase in EPO to a poorer prognosis in HF (severity and mortality), the patients with elevated erythropoietin levels are usually more anemic. In the absence of bleeding, this suggests peripheral resistance to the action of endogenous EPO.[68] The circulating EPO concentrations, as well as the severity of anemia, increase in parallel with the NYHA functional class[38] and, thus, we should look for additional pathogenic elements to explain the anemia.

Other factors that have been implicated in the pathogenesis of resistance to EPO in HF are the mediation of cytokines and generalized bone marrow dysfunction.[11,44] The latter has been postulated on the basis of the decreased leukocyte/lymphocyte counts in patients with HF and anemia, but its importance is marginal.

The causes for resistance to EPO in HF should be studied in accordance with findings associated with other diseases (Table 3). There are no studies that systematically analyze resistance to exogenous EPO in HF, a subject that requires a specific examination.

PHARMACOLOGICAL TREATMENT OF ANEMIA IN HEART FAILURE

To date, there are no studies that systematically compare different treatment regimens in the anemia of HF. The results of the survey that we mentioned above indicate that only a minority of cardiologists and internists employ recombinant erythropoiesis-stimulating agents (ESA) and intravenous iron. For this reason, we have based our study on the experience accumulated in the nephrology setting, with over 15 years of continuous use of ESA, intravenous iron, and other supplements. The nephrology societies have at least 2 large practice guidelines, the Kidney Disease Outcome Quality Initiative (KDOQI)[76-78] in the United States, and the European guidelines, which provide a considerable body of knowledge, with aspects that are reasonably adaptable to the cardiology setting.

Existing Erythropoiesis-Stimulating Agents and Those Being Developed

The first generation of ESA included recombinant EPO-alpha (Epogen, Amgen; and Procrit/Eprex, Johnson & Johnson/Janssen-Cilag) and EPO-beta (NeoRecormon, Roche). The demand for longer-acting ESA led to the development of darbepoetin (Aranesp, Amgen), a

hyperglycosylated derivative with a 3-fold longer half-life, thus making it possible to administer weekly, bimonthly, or even monthly injections; the efficacy of the latter periodicity has been tested in HF patients.[80] A pegylated derivative, a continuous erythropoiesis receptor activator (CERA, Roche), with an even longer half-life,[52,81] will soon be made commercially available. Other highly interesting products capable of inducing hematopoiesis are in an advanced state of development. These include the synthetic EPO receptor agonist developed by Affymax, Hematide, a peptide that is not related to the compounds utilized up to now and that has been shown to produce long-term erythroid induction (1 month), with good tolerance and stability at room temperature. Finally, FibroGen is developing a new approach to the problem of anemia, which involves the stabilization of HIF-1 by means of prolyl hydroxylase inhibitors of small molecular size. In HF, the important potential advantages of agents of this type do not lie solely in their oral administration, but in the induction not only of EPO, but of the multiple genes involved in the antianemic and hypoxic response.[52,81] Recently, for the purpose of acting exclusively on the trophic effects (see below), EPO derivatives that have a weak action in erythroid precursors, but are potent in other cell types, are being developed.

TREATMENT WITH ERYTHROPOIETIN IN HEART FAILURE PATIENTS AND ITS CONSEQUENCES

The critical points of the treatment of anemia in HF are the pharmacological tools and the therapeutic target, that is, the point of optimal yield. While this target has to be individualized, to date, there is not sufficient consensus as to the optimal hemoglobin and hematocrit values to be reached and maintained.[6,7] There are hemoglobin levels that are not considered to be harmful in normal subjects, but are deleterious in HF.[6] It is also necessary to take into account the fact that a rapid increase in hematocrit or its increase beyond normal levels worsens the prognosis. Available studies indicate that a hematocrit value of around 35%-36% and a hemoglobin level of approximately 12 g/dL are safe.[7,9]

Among the beneficial effects of treatment with EPO,[7,11] different authors point out that the correction of anemia increases the ejection fraction, decreases left ventricular mass and improves the oxygen-carrying capacity and oxygen utilization (peak consumption) during exercise, thus lengthening its duration.[82] It also improves the NYHA class and myocardial ischemia during the stress test, stabilizes the creatinine levels, enables the reduction of the doses of diuretics and iron, and improves the quality of life index. All these effects play a role in the reduction in the number and duration of hospital stays.[13,29,82] In a study in which subcutaneous EPO and intravenous iron were administered to HF patients in NYHA function class III-IV with a hemoglobin level < 12 g/dL who had not responded to conventional therapy, the correction of anemia was associated with a marked improvement in cardiac function and a reduction in the number of hospital admissions and the use of diuretics.[9]

Of special interest is the improvement in the exercise capacity in patients with moderate-to-severe HF being treated with EPO, with an increase in the oxygen delivery and a reduction in oxidative stress.[82] In patients with chronic HF, a functionally important relationship has been reported between a hemoglobin < 13 g/dL and exercise capacity; it is highly interesting to observe that this relationship does not exist when the hemoglobin is > 13 g/dL.[54]

Some of the undesirable effects of EPO reported in the past, such as, for example, hypertension, thromboses, and pure red cell aplasia, have disappeared or have been reduced to a minimum. Despite its angiogenic effects,[74] there are no consistent data that allow us to attribute a worsening of malignant tumors or diabetic retinopathy to EPO.

In the economic aspect, the cost of treatment with EPO and iron is lower than that of hospital readmission,[29] but there are no specific studies on this question, and they are necessary.

TABLE 3. Common Causes of Resistance to Erythropoietin*

Blood loss
Iron deficiency: absolute and relative
Chronic renal disease: also provokes endogenous EPO deficiency
Acute and chronic inflammation
Antagonist agents: ACE inhibitors, nonsteroidal antiinflammatory drugs
Malnutrition and deficiency of factors, such as vitamin B_{12}, folic acid
Bone marrow depression

*ACE indicates angiotensin-converting enzyme; EPO, erythropoietin.

THE PHARMACOLOGY OF ERYTHROPOIETIN

The existing preparations can be administered either subcutaneously or intravenously, although for practical reasons, the former is preferred; discomfort at the injection site is minimal. It is essential to keep in mind the importance of maintaining the cold chain at 4ºC to preserve a high efficacy.[83]

The duration of the effect of EPO (up to 1 week) is shorter than that of darbepoetin, which lasts 15 to 30 days, although the therapeutic yield is equivalent. The indications for EPO, originally limited to patients with CRD, are being extended to all the groups that might benefit, including patients with myelodysplastic syndrome or human immunodeficiency virus, premature infants and cancer patients undergoing chemotherapy, as well as in cases of sickle cell anemia, prior to autologous

donation, as perioperative adjuvant therapy and in HF.[52] In CRD, a condition in which the experience is more extensive, the standard doses for initial EPO therapy are 400 U/kg bw/week and 15-200 U/kg bw/week, administered intravenously and subcutaneously, respectively, divided into 1 to 3 doses per week.[84] However, there is less information on the doses to be administered in HF. Thus, regimens similar to those tested in CRD are being used, and will have to be verified over the course of time.

IRON THERAPY

Some authors sustain that it is practically obligatory to add intravenous iron therapy to treatment with EPO[9,29] to prevent possible iron deficiency secondary to the increase in hematopoiesis. The additive effect of the combination of iron and EPO may not be achieved with oral iron.[85,86] However, there is a lack of data to enable us to predict which patients will fail to absorb oral iron, and it is to be hoped that hepcidin measurements will help to resolve this question. The existing intravenous iron preparations, iron gluconate (Ferrlecit, Rhone-Poulenc, 62.5 mg elemental iron), and iron sucrose (Venofer, Uriach, 100 mg elemental iron), have practically no collateral effects if used in reasonable doses, for example, 1 vial a week, in cycles of 6 to 9 vials, depending on the ferritin levels. These doses that we mention are more conservative than others that have been employed, but they take into account the possibility of induction of oxidative effects due to the administration of large quantities of iron via a nonphysiological route, the intravenous route. As a comparative datum for dosing, a blood transfusion supplies approximately 250 mg of elemental iron, which eventually becomes part of the iron in the organism due to the gradual destruction of the transfused red blood cells.

On the other hand, certain recent data indicate that, at least in some patients, iron deficiency is the major pathogenic factor in anemia. In these cases, adjuvant treatment with EPO would not be necessary, as is pointed out in recent communications, which demonstrate improvements in anemia with iron therapy alone,[87] and in preliminary results from clinical studies pending publication or still underway, such as FERRIC-HF and IRON-HF.[88,89] The possible iron deficiency, absolute or relative, can become more marked due to other additional factors, such as losses secondary to antiplatelet/anticoagulant therapy and aspirin-induced gastritis.[7,10,20] Moreover, right HF can favor deficient absorption or nutritional deficiencies involving elements necessary for erythroid maturation, such as vitamin B_{12} and folic acid, and iron itself.[11] There are no recent studies on the prevalence of vitamin deficiencies in individuals with HF, but the current appraisal is that these pathogenic elements of anemia are of limited importance in this context.

ERYTHROPOIETIN CAN IMPROVE HEART FAILURE NOT ONLY BY CORRECTING ANEMIA

Erythropoietin has cytoprotective effects on cardiac endothelium[74] and muscle. The EPO-R is present above all during the fetal period, although it is also found in smaller amounts in the adult heart.[83] On this basis, treatment with EPO could prevent cardiomyocyte apoptosis and stimulate the production of myocardial blood vessels. In vitro and animal studies indicate that EPO is capable of reducing the apoptotic cell death associated with coronary ischemia/reperfusion.[90] One highly interesting possibility is that EPO stimulates neovascularization, in part, because it increases the mobilization of endothelial progenitor cells from the bone marrow to the blood.[90]

CURRENT PERSPECTIVES

At this point, the issue can be summarized by saying that multiple partial data indicate that the correction of anemia provides tangible benefits in the natural history and symptoms of heart failure, but that it is necessary to establish the limits of these benefits and develop sufficiently complex models for the application of diagnostic methods and treatment.

The extensive material available justifies undertaking therapeutic efforts to correct anemia in HF. However, the degree of uncertainty in fundamental aspects, both pathogenic and those concerning the therapeutic targets, is still significant. It is clear, as Roig stressed in this same journal,[11] that one relevant aspect of anemia in HF is its condition as a marker of severity. While preliminary studies indicate that the treatment of anemia with ESA has potentially beneficial effects, the available information does not enable us to reach more definitive conclusions.

In recent months, 2 large studies on anemia of CRD have been published[91,92]; the resulting data not only do not encourage the restoration of normal hemoglobin levels (13 g/dL), but indicate that, despite the improvement in the functional class, there may be a greater number of cardiovascular complications in patients in whom the correction of anemia is more complete. Other reports had demonstrated that the tendency toward the development of left ventricular hypertrophy is not reversed with the achievement of hemoglobin over 12 g/dL. To illustrate the degree of controversy involved, these data do not support the elevation of the treatment threshold advocated by the NKF in its 2006 guidelines[15] and have led to the proposal for the new version that we mentioned above.

With regard to issues specifically related to HF, at the European Society of Cardiology meeting held in 2006, data from another 2 double-blind, randomized, placebo-controlled studies focusing on the treatment of anemia of HF with darbepoetin were provided.[93] All the

participants had an ejection fraction of 40% or less and a hemoglobin level of 9-12.5 g/dL, and HF had to have been diagnosed at least 3 months earlier. When the 475 patients were analyzed as a whole, the individuals who had received darbepoetin alfa exhibited a significant increase in their hemoglobin levels. However, while they did not experience more adverse events than the placebo group, they did not show a significant improvement in the 3 parameters for symptom evaluation (NYHA functional class, patient global assessment and the Minnesota Living with Heart Failure Questionnaire). These results provide data to establish the limit to improvement that can be expected with the treatment of anemia.

Together, these studies constitute a call to order to prevent us from overrating the positive role of the treatments for anemia or, more exactly, support the need to improve the diagnostic accuracy and the correct choice of the therapeutic tools. In summary, we can conclude that the correction of values indicative of severe anemia (hemoglobin less than 10 g/dL) is beneficial, but that the achievement of values that surpass that level may not offer additional advantages. The results of the large multicenter studies that are currently being performed or are getting underway will probably help us to clarify important practical aspects that have yet to be resolved. The largest of these studies (RED-HF) is now being carried out. Its objective is to evaluate the effects of darbepoetin alfa on the morbidity and mortality rates in 3400 patients with HF using a randomized, placebo-controlled design, which we hope will make it possible to consolidate evidence-based treatment strategies.

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