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REVIEW

# Erythropoietin and the heart: facts and perspectives

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## ABSTRACT

EPO (erythropoietin) has long been identified as a primary regulator of erythropoiesis. Subsequently, EPO has been recognized as playing a role in a broad variety of processes in cardiovascular pathophysiology. In particular, the tight interactions of EPO with the nitric oxide pathway, apoptosis, ischaemia, cell proliferation and platelet activation appear of great interest. Although enhanced EPO synthesis is viewed as an appropriate compensatory mechanism in the cardio–renal syndrome, which features CHF (congestive heart failure) and CRF (chronic renal failure), maladaptative excessive EPO synthesis in the advanced stages of these diseases appears to be predictive of higher mortality. Clinical trials based on the use of EPO in both heart and renal failure have so far produced contradictory results, whereas treatment targeted to restore low Hb levels appears rational and is supported by regulatory authorities. New areas for therapeutic use of EPO, such as acute coronary syndromes, are under investigation, and they are discussed in the present review together with other clinical applications in cardiovascular diseases. The revisited concept of a potential use of endogenous EPO levels as a predictor of CHF severity, as well as in the monitoring of responses to treatment, deserves appropriate investigation, as this may identify EPO as a useful biomarker in the clinical management of cardiovascular diseases.

## INTRODUCTION

EPO (erythropoietin) is a haemopoietic hormone produced by the kidney in response to hypoxia and is the primary regulator of erythropoiesis: in the bone marrow EPO promotes the proliferation of erythroid progenitor cells and increases the production of red blood cells. rhEPO (recombinant human EPO; epoetin-$\alpha$) has been used since the late 1980s in patients with anaemia due to EPO deficiency as a consequence of CRF (chronic renal failure). Subsequently its use was approved for the treatment of anaemia, induced by

**Key words:** biomarker, congestive heart failure (CHF), chronic renal failure (CRF), endothelium, erythropoietin (EPO), myocardial infarction (MI).

**Abbreviations:** ACE, angiotensin-converting enzyme; Ac-SDKP, *N*-acetyl-Ser-Asp-Lys-Pro; AngII, angiotensin II; BNP, brain natriuretic peptide; CEPO, carbamylated derivative of EPO; CHF, congestive heart failure; CK, creatine kinase; CRF, chronic renal failure; eNOS, endothelial nitric oxide synthase; EPC, endothelial progenitor cell; EPO, erythropoietin; EPO-R, EPO receptor; ESA, erythropoiesis-stimulating agent; GFR, glomerular filtration rate; HIF1, hypoxia-inducible factor 1; I/R, ischaemia/reperfusion; IGF-1, insulin-like growth factor-1; IL, interleukin; $IP_3$, inositol trisphosphate; JAK-2, Janus kinase-2; LV, left ventricular; MAPK, mitogen-activated protein kinase; MI, myocardial infarction; MRI, magnetic resonance imaging; NF-$\kappa$B, nuclear factor $\kappa$B; NT-proBNP, N-terminal proBNP; NYHA, New York Heart Association; O/P, observed/predicted; PCI, percutaneous coronary intervention; PI3K, phosphoinositide 3-kinase; RAS, renin–angiotensin system; rhEPO, recombinant human EPO; STAT, signal transducer and activator of transcription; STEMI, ST segment elevation MI; TNF$\alpha$, tumour necrosis factor $\alpha$; VEGF, vascular endothelial growth factor.

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zidovudine in HIV/AIDS patients, and by chemotherapy and radiotherapy in patients with cancer, and also to reduce the need for transfusions before major surgery [1].

Discovery of EPO-Rs (EPO receptors) in non-haemopoietic tissue raised the hypothesis that, besides its effect on erythropoiesis, this glycoprotein might exert non-haemopoietic functions. Studies in experimental models have indeed shown that systemic administration of rhEPO in a rat model of focal brain ischaemia reduced the volume of cerebral infarction by 50–75 % [2]. Furthermore, in patients with acute ischaemic stroke, EPO treatment was safe, well-tolerated and associated with significant improvement in clinical outcomes after 1 month [3]. EPO also produced beneficial effects in various clinical conditions, including hypoxic retinal disease [4], gastrointestinal ischaemia [5], ischaemic spinal cord injury [6] and renal ischaemic injury [7–9].

As EPO-Rs are also expressed in the heart, several studies have investigated the effects of EPO on the heart and it has been shown that EPO exerts cardioprotective actions. In fact early studies showed that EPO treatment in anaemic patients with chronic kidney disease, whom often develop CHF (congestive heart failure), produces a reduction in cardiac size, a regression of LV (left ventricular) hypertrophy and improves LV ejection fraction and myocardial contractility [10–13]. These findings, together with several other observations, generated a growing interest for the use of EPO in ischaemic heart disease, particularly in the treatment of MI (myocardial infarction).

In the present review we have summarized the current knowledge on the relationships between EPO and cardiovascular disease, with the aim of highlighting the potential therapeutic use of EPO in cardiovascular disease. We provide a concise overview of the molecular mechanism of action of EPO and discuss ongoing and future potential clinical applications of EPO in cardiovascular diseases. Finally, we briefly analyse the use of endogenous EPO as a prognostic biomarker in cardiac diseases.

## EPO BIOSYNTHESIS AND SIGNALLING

EPO is a 34 000 Da 165-amino-acid glycoprotein synthesized primarily by the peritubular cells in the kidney. The main stimulus for the production of EPO is hypoxia. Anaemia, blood loss, congenital heart disease and conditions characterized by reduced renal perfusion, cause renal hypoxia and subsequent increases of HIF1 (hypoxia-inducible factor 1). HIF1 is a transcription factor and regulates the expression of a large number of hypoxia-inducible genes, including the EPO gene on chromosome 7, and genes encoding VEGF (vascular endothelial growth factor) and PDGF (platelet-derived growth factor). All these growth factors represent downstream signals of IGF-1 (insulin-like growth factor-1), via HIF1 [14], but, whereas IGF-1 increases EPO synthesis [14], EPO does not appear to exert significant modulation on IGF-1 concentrations [15].

When oxygen is present, the HIF1$\alpha$ subunit rapidly undergoes ubiquitination by von Hippel–Lindau protein and it is consequently degraded by proteasome proteolysis; in the absence of oxygen HIF1$\alpha$ is not degraded and it translocates into the nucleus where it binds to HIF1$\beta$. The HIF1$\alpha$–HIF1$\beta$ heterodimer activates the HRE (hypoxia-responsive element) of hypoxia-sensitive genes, inducing EPO transcription [16,17].

Serum EPO concentrations vary between 10 and 25 units/l [16], but EPO levels rise in an exponential manner with a decrease in Hb levels.

EPO binds its specific EPO-R on committed erythroid progenitor cells and promotes their survival and proliferation, thus increasing the production of red blood cells and improving tissue oxygen supply. EPO-R is also expressed in cardiomyocytes, fibroblasts, and on retinal, neuronal, renal, vascular smooth muscle and endothelial cells [2,4,18,19]. On erythroid cells, the receptor exists in a preformed dimerized configuration, in which a single ligand molecule engages two identical receptor extracellular domains. Monomeric EPO-R, however, is able to interact with other receptor proteins, forming heteromeric complexes [e.g. with the $\beta$-receptor (CD131), which is also a component of the GM-CSF (granulocyte/macrophage colony-stimulating factor), IL (interleukin)-3 and IL-5 receptors]. In a recent study, a heteromeric complex consisting of EPO-R and $\beta$-receptor has been shown to mediate tissue protection signals [20]. This type of receptor has no effect on erythropoiesis and it is probably only involved in the non-haemopoietic effects of EPO. This could allow the distinguishing of EPO-induced tissue-protective effects from erythropoietic effects when given at pharmacological doses.

### Intracellular signalling pathways downstream from EPO-Rs

The interaction between EPO and its receptors leads to dimerization of the receptors and autophosphorylation of JAK-2 (Janus kinase-2), which is constitutively associated with the receptor. JAK-2 then phosphorylates different signal transducers and activates several downstream signalling pathways [17,21–23].

EPO stimulates cell proliferation by activating the RAS/MAPK (mitogen-activated protein-kinase) and STAT5 (signal transducer and activator of transcription 5) pathways [24]. Activation of STAT5 results in increased cell-survival signals by up-regulation of the anti-apoptotic proteins Bcl-2 and Bcl-$X_L$ [25,26]. EPO

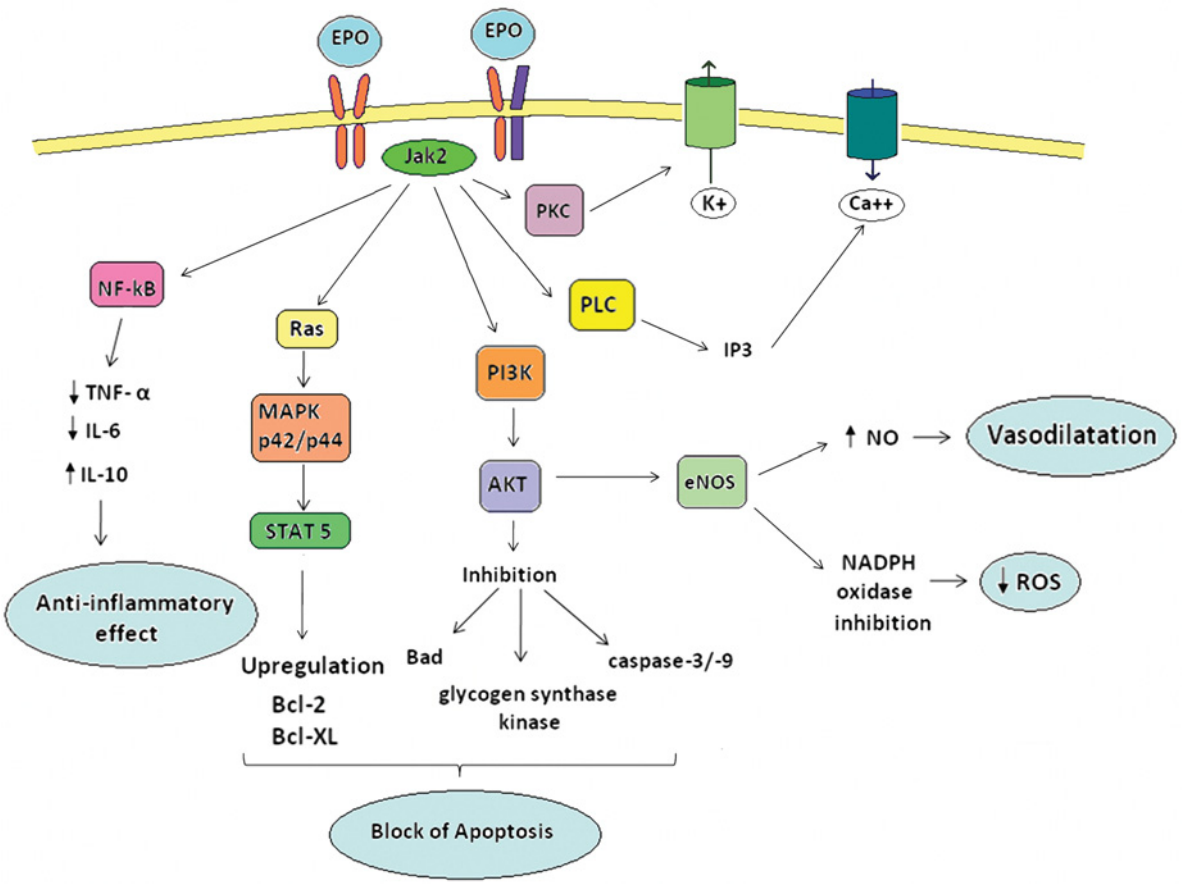


**Figure 1 Activation of intracellular signalling pathways by EPO**

PLC, phospholipase C; PKC, protein kinase C.

also inhibits apoptosis by activation of the PI3K (phosphoinositide 3-kinase)/AKT signalling pathway. AKT [also known as PKB (protein kinase B)] is a serine/threonine kinase that inhibits pro-apoptotic molecules, such as BAD (Bcl-2/Bcl-$X_L$-antagonist, causing cell death), caspase 3/9 and glycogen synthase kinase 3$\beta$, thereby preventing mitochondrial cytochrome *c* release [27,28]. The PI3K/Akt pathway also enhances the expression of eNOS (endothelial nitric oxide synthase) thus increasing the levels of nitric oxide. Nitric oxide promotes vasorelaxation and reduces apoptosis by reducing oxidative stress by NADPH oxidase inhibition, inhibiting the pro-apoptotic caspase 3/8 proteins and modulating expression of protective genes, such as heat-shock protein 70 or Bcl-2 [16,29].

EPO also mediates a rise in intracellular calcium concentrations by activating phospholipase C$\gamma$1 and hence increasing $IP_3$ (inositol trisphosphate) levels [30]. In addition, by activating protein kinase C, EPO promotes the opening of the potassium channels $K^+$/ATP and $K^+/Ca^{2+}$, which are probably involved in cardioprotective responses during ischaemic injury [31,32]. Finally EPO has anti-inflammatory effects, linked to down-regulation of NF-$\kappa$B (nuclear factor $\kappa$B) with suppression of pro-inflammatory cytokines, such as TNF$\alpha$ (tumour necrosis factor $\alpha$) and IL-6. In addition, it increases IL-10, an anti-inflammatory cytokine [22,33].

Tissue distribution of EPO-Rs together with the features of the downstream intracellular pathways described above (Figure 1) and the biological effects of the signals have generated growing interest for the potentially relevant effects of EPO in cardiovascular disease.

## HAEMOPOIETIC EPO EFFECT AND CHF

Anaemia is defined, according to WHO (World Health Organization) criteria, as an Hb concentration <13 g/dl in adult men and <12 g/dl in adult women. Anaemia is very common in patients with CHF; its prevalence ranges from 15 to 70 % depending upon the definition of anaemia, the population considered and the severity of CHF.

There are several possible causes of anaemia in CHF [34–37]. (i) Renal dysfunction: the decrease of cardiac

output causes a reduction of renal perfusion, leading to chronic renal ischaemia. Chronic kidney disease is associated with a reduced production of EPO. (ii) Elevated production of cytokines: TNF$\alpha$ and IL-6 are often elevated in CHF as in many other chronic inflammatory states. These cytokines cause anaemia in different ways; they inhibit EPO production and proliferation of bone marrow erythroid progenitor cells, but also interfere with iron metabolism. By inducing the expression of hepcidin, they also cause macrophage iron retention and decreased duodenal absorption, thus making less iron available for erythropoiesis (a reticulo-endothelial iron block) [35,38]. (iii) Haematinic deficiency (of iron, folate and vitamin B12) due to poor intake, malabsorption or chronic blood loss, is a frequent condition seen in patients treated with aspirin or oral anticoagulation agents. (iv) Haemodilution: the activation of the RAS (renin–angiotensin system), consequent to reduced renal perfusion, leads to an expanded plasma volume, with a subsequent reduction in haematocrit levels while red blood cell volume is increased, resulting in so-called pseudo-anaemia [39,40]. These patients seem to have a worse prognosis than patients with 'true' anaemia [41]. (iv) Use of ACE (angiotensin-converting enzyme) inhibitors: since AngII (angiotensin II) stimulates EPO secretion and erythopoiesis, ACE inhibitors reduce both EPO production in the kidney and EPO activity in the bone marrow. They also increase the level of the haemopoiesis inhibitor Ac-SDKP (*N*-acetyl-Ser-Asp-Lys-Pro), which is degraded by ACE [42].

## Role of anaemia in the 'vicious' cycle of CHF

Anaemia is an independent risk factor for increased mortality in CHF patients [43]. Lower Hb levels are associated with more severe symptoms, a worse haemodynamic profile (low blood pressure, and high heart rate and pulmonary capillary wedge pressure), and severely impaired exercise capacity [44]. Anaemia worsens the natural history of CHF as tissue hypoxia causes systemic vasodilatation and reduction of arterial and perfusion pressures. This activates the sympathetic nervous system, which leads to reflex vasoconstriction and tachycardia; renal vasoconstriction activates the RAS leading to water and sodium retention. The increased plasma volume contributes, in turn, to peripheral oedema and increases cardiac load. The heart progressively undergoes remodelling, LV dilatation and hypertrophy, associated with myocardial cell death and fibrosis, to which the renin–angiotensin–aldosterone axis activation also contributes, together with the production of pro-inflammatory cytokines [36,43]. Moreover, reduced oxygen delivery determines pulmonary vasoconstriction, leading to pulmonary hypertension, which further contributes to progression of cardiac failure.

CHF is often associated with chronic kidney disease, which is mostly a consequence of the decreased renal perfusion, as well as of the chronic use of diuretics. CRF (chronic renal failure) is an independent risk factor for death in CHF, and it can produce anaemia as a consequence of the reduced EPO production.

Therefore CHF causes anaemia, which in turn worsens cardiac function, leading to a further deterioration of renal function. This results in worsening anaemia and leads to a vicious pathophysiological cycle that is defined as the 'cardio–renal anaemia syndrome'. Normalization of Hb concentrations may contribute to the interruption of this cycle (Figure 2).

These considerations help us to understand the key importance of treating anaemia in CHF. For this purpose, the available tools include intravenous iron therapy and treatment with ESAs (erythropoiesis-stimulating agents), both of which have been studied extensively.

Silverberg et al. [45,46] were the first to prove that a combination of subcutaneous EPO and intravenous iron not only increases Hb level, but also improves LV ejection fraction, NYHA (New York Heart Association) functional class and GFR (glomerular filtration rate). This strategy also reduces mortality, morbidity, days of hospitalization and the need for diuretics.

Subsequent studies showed that EPO is also able to improve quality of life and exercise capacity, as assessed by exercise duration, the 6-min walking test and peak $\dot{V}O_2$ assessment [47]. EPO administration also reduces LV systolic diameter, LV systolic volume and LV mass, and is associated with a lower plasma BNP (brain natriuretic peptide) level, which is a reliable marker of reduced severity in CHF [48–50]. Ventricular geometry improvement was confirmed in the setting of mitral regurgitation in a recent human study based on a 2-month EPO administration [51].

These favourable results could be related to an increased myocardial oxygen supply, but probably they also reflect the direct beneficial effects that EPO exerts directly on the myocardium, such as the reduction of oxidative stress and myocardial cell apoptosis, and promotion of neovascularization in the coronary circulation. Although these data appeared very promising, recent larger multi-centre trials have, however, generated contradictory results. For instance, therapy with darbepoetin-$\alpha$ did not improve quality of life, NYHA class and exercise tolerance compared with placebo in patients with CHF [52–54]. In the same period, the FDA (Food and Drug Administration) released advice to restrict EPO administration, according to revised product labelling, at the recommended dose and only for approved indications. In the TREAT (Trial to Reduce Cardiovascular Endpoints with Aransep$^{TM}$ Therapy) study, a large ($n = 4038$) trial based on long-term darbepoetin administration, more than 4000 patients with anaemia, diabetes and chronic kidney disease, not

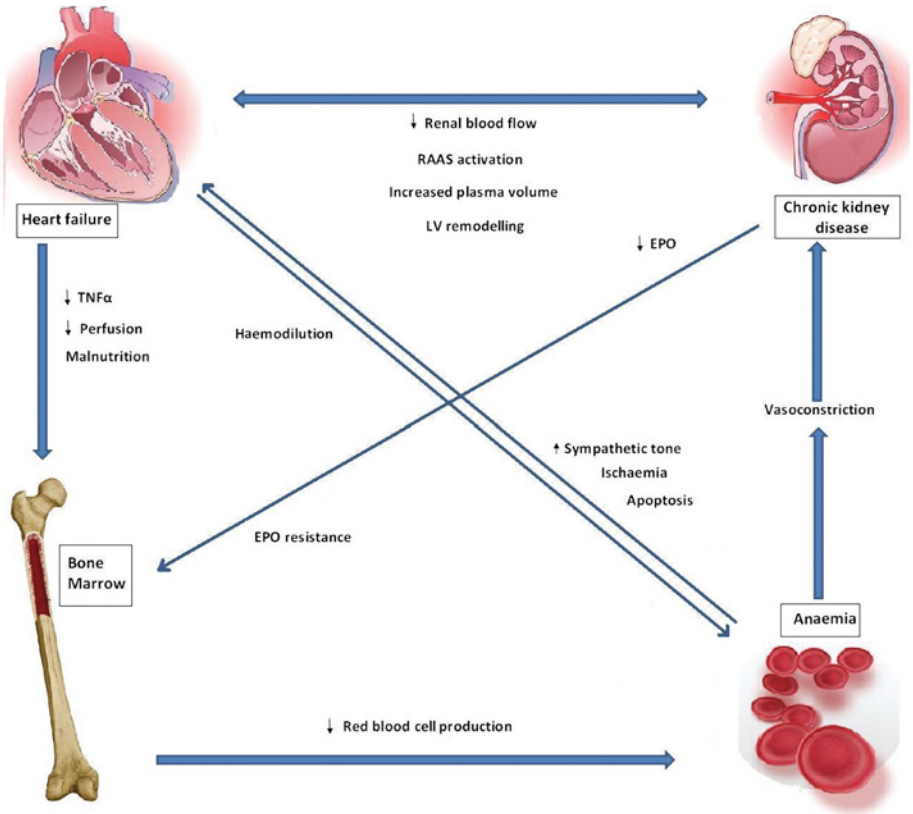


**Figure 2 The cardio-renal anaemia syndrome**

RAAS, renin–angiotensin–aldosterone system.

undergoing dialysis, were treated to a Hb level target of 13 g/dl. After a median follow-up of 29 months, the study showed a doubled risk of fatal and non-fatal stroke with darbepoetin compared with placebo. Only a modest improvement in patient fatigue was reported, whereas no differences were detected in death rate, end-stage renal disease and cardiovascular composite end point or MI [55]. A recent meta-analysis, summarizing all studies with EPO in anaemic CHF patients, and an analysis from the Cochrane database, has revealed a non-significant reduction in mortality, as well as improvements in exercise tolerance, symptoms, hospitalization rates and all-cause mortality [56,57].

## Safety of EPO treatment in patients with cardiac disease

EPO treatment in anaemic patients with CHF has been generally well tolerated. The most common non-specific side effects that have been reported are musculo-skeletal and neurological signs and symptoms, as well as fatigue, worsening of CHF and upper respiratory tract infections. The degree of adverse events, such as hypertension, MI, stroke and seizures, appears to be similar in treated and in control patients [47,52–54]. However recently, concerns have been raised about the safety of chronic EPO treatment in patients. In the CHOIR (Correction of Hemoglobin and Outcomes in Renal Insufficiency) trial, patients with chronic kidney disease were randomized and received a dose of epoetin-$\alpha$, targeted to achieve either a higher (13.5 g/dl) or a lower Hb level (11.3 g/dl). The study was terminated early because there was an increased mortality risk in patients showing higher Hb levels: in 17.5 % of patients in the high-Hb group death, MI and hospitalizations for CHF or stroke were reported. No difference in the quality of life was found between the two groups [58].

In the CREATE (Cardiovascular Risk Reduction by Early Anaemia Treatment with Epoetin-$\beta$) trial the authors investigated whether, in patients with chronic kidney disease, the complete correction of anaemia with epoetin-$\beta$ to target levels of 13–15 g/dl improved the outcomes when compared with a partial correction of anaemia (epoetin-$\beta$ was only given when Hb was below 10.5 g/dl). This study confirmed that the complete correction of anaemia did not improve outcomes [59].

The mechanisms underlying a potential increased mortality are unclear, though they could be partially attributable to an increased thromboembolic risk or to raised blood pressure.

EPO treatment not only increases blood viscosity as a result of increased red cell mass, but also exerts a

direct effect on platelets, by enhancing platelet activation. EPO stimulates the synthesis of endothelin in vascular endothelial cells, raises calcium concentrations in vascular smooth muscle cells and increases the expression of AngII receptors on the endothelium, thereby increasing vasomotor tone [60].

These side effects are more frequent with chronic high-dose EPO treatment, which is associated with an increased haematocrit. A recent study [61] showed that low-dose EPO treatment after MI in rats significantly improves cardiac function, without increasing haematocrit levels. These observations indicate a potential strategy to limit the adverse effects of EPO treatment. Another possibility is the use of CEPO (carbamylated derivative of EPO). CEPO does not have effects on erythropoiesis, but retains the tissue-protective effects of EPO by stimulating a separate portion of the EPO-R (the PI3K). Fiordaliso et al. [62] demonstrated that 50 $\mu$g/kg of body weight of subcutaneous daily CEPO administration for 7 days before reperfusion in rats with MI, reduced cardiomyocyte loss and prevented LV dysfunction, without increasing hematocrit levels. The RED-HF (Reduction of Events with Darbepoetin-$\alpha$ in Heart Failure) trial is a large-scale ($n = 2600$) Phase III morbidity and mortality trial currently under way, with the aim of clarifying the safety and potential benefits of treating anaemia with EPO in patients with CHF [63]. Hence, CEPO could thus represent a safer and more effective alternative for the treatment of cardiovascular diseases such as CHF and MI.

## Pleiotropic EPO effects and acute MI

Growing evidence supports the hypothesis that rhEPO may improve cardiac function in patients with acute MI. In experimental studies, rhEPO acutely reduced infarct size by inhibiting apoptotic cell death, thereby preserving ventricular function; in addition, cardioprotective long-term effects, such as prevention of ventricular remodelling and CHF have been described.

### Acute cardioprotective effects of EPO in ischemic heart disease

Ischaemic myocardial injury is characterized by a central region of cellular necrosis surrounded by an area of variable size, called the 'area at risk' or 'penumbra', which displays myocyte apoptosis. Several studies have investigated whether EPO could limit infarct size by preventing apoptosis within the 'area at risk'.

Calvillo et al. investigated the protective role of rhEPO in a rat model of cardiac I/R (ischaemia/reperfusion) injury. The administration of rhEPO (intraperitoneally, 5000 units/kg of body weight per day for 7 days) reduced cardiomyocyte loss by 50 % and preserved cardiac haemodynamic function [64]. Subsequent studies in similar models confirmed that EPO treatment, even through a single administration at different time points (from pre-ischaemia throughout the onset of reperfusion), decreased infarct size and significantly enhanced post-ischaemic systolic and diastolic ventricular function, independent of hematocrit increments [65–68].

The minimum effective rhEPO dose and the therapeutic window were investigated by Moon et al. [69] in a rat model after permanent coronary artery ligation. These authors established that the lowest effective dose to reduce apoptosis and the resulting MI size was 150 units/kg of body weight, if administrated immediately after ischaemia. This efficacy was lost when the administration was delayed by 8 h, although a much higher dose (3000 units/kg of body weight) was still effective up to 12 h, but not if the treatment was delayed for 24 h.

In view of the observation that EPO-Rs are more densely expressed in cardiac fibroblasts and endothelial cells than in cardiomyocytes [68], and that apoptosis is known to start in endothelial cells and then to gradually spread to surrounding cardiomyocytes [70], van der Meer et al. [18] suggested that EPO also protects the myocardium by preventing apoptosis in endothelial cells thus preserving blood flow.

In addition, EPO exerts an anti-inflammatory action, which may contribute to limit the ischaemic damage, thus preventing subsequent progression of CHF. I/R injury induces severe inflammatory responses [22], by activating the NF-$\kappa$B pathway and AP-1 (activator protein 1), which translates into an up-regulation of TNF$\alpha$. This, in turn, triggers the cytokine cascade and an accumulation of neutrophils, which accentuates tissue damage. The cardioprotective effect of EPO might be partially due to the suppression of the myocardial I/R-induced inflammatory response [33]. In a small study conducted in patients undergoing cardiopulmonary bypass for a coronary artery bypass graft, however, EPO administration (500 units/kg of body weight intravenous epoetin-$\alpha$) did not reduce perioperative release of inflammatory cytokines [71].

### Long-term effects of EPO

In addition to its effects in limiting infarct size in the acute phase, EPO promotes the formation of new blood vessels, through two different mechanisms. EPO stimulates both angiogenesis, by acting directly on *in situ* endothelial cells [72,73] and vasculogenesis, that is the formation of blood vessels from the EPCs (endothelial progenitor cells) derived from the bone marrow. These properties are paralleled by an activation of the PI3K/Akt pathway that has a proliferative, promigratory and anti-apoptotic effect in mature endothelial cells and EPCs [74,75]. In a rat model of post-MI CHF, van der Meer et al. [76] demonstrated that EPO treatment, started 3 weeks after MI, albeit not reducing infarct size, was able to improve cardiac function. This improvement was related to increases in capillary density and capillary-to-myocyte ratio, which suggests neovascularization.

**Table 1 Overview of current clinical studies investigating the role of EPO in MI**

i.v., intravenous.

| Acronym (Reference) | Design | Patients | Dosing and type of EPO | Follow up | Primary end point | Secondary end point |
|---|---|---|---|---|---|---|
| EPAMINONDAS [83] | Randomized placebo-controlled double-blind dose-finding weight-adjusted | 102 first STEMI after successful primary PCI | Epoetin-$\alpha$; 100 or 200 units/kg of body weight per day; triple 30 min i.v. infusion within 12 h after PCI and on next 2 days | 12 months | Infarct size by CK-MB; LV ejection fraction and cardiac MR | Major adverse events; LV remodelling |
| HEBE III [84] | Randomized controlled open-label | 466 first STEMI for primary PCI | Unspecified EPO; 60 000 units; single i.v. bolus within 3 h after PCI | 1 and 12 months | LV ejection fraction by radionuclide ventriculography | Infarct size; major adverse events |
| REVEAL [85] | Randomized placebo-controlled double-blind dose-finding | 210 first STEMI for primary or rescue PCI | Epoetin-$\alpha$, dose escalation of 15, 30 or 60 ($\times 10^3$) units (safety phase) followed by efficacy-phase single i.v. dose | 3 months | Infarct size by cardiac MR | |
| REVIVAL-3 [86] | Randomized placebo-controlled double-blind | 138 first STEMI for primary PCI | Epoetin-$\beta$; 33 000 units; triple i.v. bolus after PCI and after 24 and 48 h | 6 months | LV ejection fraction by cardiac MR | |

The disproportion between cardiac hypertrophy and vascular growth is a major factor in impaired myocardial perfusion, which leads to myocardial remodelling and progressive deterioration of cardiac function. By stimulating the formation of new vessels in the non-infarcted wall of the ventricle, EPO attenuates ventricular remodelling and improves cardiac function [77]. EPO has also been shown to increase the number of circulating EPCs by mobilizing bone-marrow-derived haemopoietic stem cells and by promoting their incorporation in the newly formed endothelium.

VEGF appears of key importance in the translation of cardioprotective EPO signals. Indeed, EPO treatment was shown to increase the expression of VEGF leading to enhanced proliferation of endothelial cells, as well as increasing the levels of EPC chemotaxis, which subsequently return into damaged myocardium by a homing mechanism [78].

Li et al. have also reported that late treatment with EPO is able to dampen inflammation and cytokine production related to the degree of CHF, thereby improving cardiac performance; EPO has been shown to limit fibrosis by limiting the increase in TGF$\beta$ (transforming growth factor $\beta$)-1, a major stimulator for tissue fibrosis, as well as by attenuating the overproduction of reactive oxygen species that are involved in myocardial remodelling [79].

To date, few data are available about the cardioprotective effects of EPO in human cardiac ischaemia. In an early single-centre study, a 300 $\mu$g dose of the long-acting EPO analogue darbepoetin-$\alpha$, given intravenously in patients with acute MI before PCI (percutaneous coronary intervention), increased the number of EPCs, although there was no significant improvement in LV function or clinical outcomes [80]. The administration of EPO was safe and well tolerated. In a later study, Ferrario et al. [81] investigated the effects of a short-term high-dose EPO administration in patients with acute MI. EPO was administered before PCI, and 24 h and 48 h later, reaching a total dose of 100 000 units. EPO increased circulating $CD34^+$ cells (endothelial cells and EPCs), shifted the gene expression profile of PBCs (peripheral blood cells) towards an anti-apoptotic, proangiogenetic and anti-inflammatory phenotype, and reduced the release of myocardial enzymes. There was also a trend towards a more favourable LV remodelling. Conflicting results have been reported by Suh et al. [82]. In that study an intravenous bolus of rhEPO (50 units/kg of body weight), administered before revascularization to patients with acute STEMI (ST segment elevation MI), did not reduce infarct size, in the absence of thrombotic or hypertensive side effects.

Currently, several trials are testing the effects of intravenous EPO administration on infarct size in the attempt to translate the cardioprotective action observed in experimental models to patients with acute MI. The main characteristics of all these studies are shown in Table 1.

EPAMINONDAS (Exogenous erythroPoietin in Acute Myocardial Infarction: New Outlook aNd dose Association Study) [83] is a double-blind placebo-controlled dose-finding Phase II trial, conducted in three centres in Italy, including ours, which aims to test the effects of two intravenous doses of rhEPO (100 or 200 units/kg of body weight of epoetin-$\alpha$) on infarct size. This was administered on the first 3 days of hospitalization to 102 patients with a first STEMI. The first dose of the drug or placebo is given within 12 h of PCI. The duration of follow-up is 12 months. The primary end point is to assess the effects of EPO on infarct size, estimated by the CK (creatine kinase)-MB 24 h time–concentration curve, LV wall motion score index, LV ejection fraction and the extent of 'late enhancement' by contrast-enhanced MRI (magnetic resonance imaging). The secondary end point is the assessment of the effects of EPO on the incidence of major adverse events and on LV remodelling at the 12-month follow-up. LV remodelling is defined as a >20 % change in end-diastolic volume at follow-up compared with the pre-discharge values. The results of these Phase II studies will help to define the safety and potential efficacy of EPO in reducing infarct size in STEMI patients.

The HEBE III trial is a multicentre prospective open-label trial with blinded evaluation of the primary end point. Patients with STEMI ($n$ = 466) are randomly assigned to receive a single 60 000 unit bolus of EPO or placebo within 3 h of a successful PCI. The primary end point is to study the effect of EPO on LV ejection fraction, assessed by planar radionuclide ventriculography after 6 weeks. Secondary study end points include assessment of myocardial infarct size and the incidence of major adverse events [84].

REVEAL (Reduction of infarct Expansion and Ventricular remodelling with Erythropoietin After Large myocardial infarction) is a randomized double-blind placebo-controlled parallel Phase II clinical study that evaluates the effects of a single administration of epoetin-$\alpha$ on infarct size, LV remodelling and circulating EPCs in patients with STEMI. EPO will be given at doses of 15 000, 30 000 or 60 000 units in the first dose-escalation safety phase, then a single dose efficacy phase will follow. The infarct size and the dimensions of the heart are assessed by cardiac MRI within 2–6 days and after 3 months [85].

REVIVAL-3 is a German double-blind placebo-controlled randomized trial investigating additional benefits of epoetin-$\beta$ administration (33 000 units/dose, given immediately, and 24 h and 48 h after PCI) in patients with acute MI after successful primary PCI, by evaluating LV ejection fraction. The study has shown a trend towards a higher rate of adverse events in the EPO-treated group at the 6-month follow-up [86].

Therefore, in both CHF and acute MI, EPO treatment is currently being actively investigated. For this reason, as well as on the basis of the available data, treatment with EPO should not yet be considered for clinical use in cardiovascular disease in patients not included in an investigational setting.

## EPO AS A PROGNOSTIC MARKER

Endogenous EPO levels are a prognostic marker in patients with CHF. Although it appears that EPO could be a potentially important clinical biomarker, as it could be informative in the follow-up of CHF patients, this application has been poorly investigated and not yet applied in clinical practice.

In CHF, cardiac output gradually decreases, leading to chronic hypoperfusion of the kidney. To maintain the GFR, renal arteries constrict. This results in renal ischaemia and reduction of renal blood flow, followed by the activation of the RAS that contributes to the progressive deterioration of cardiac performance and clinical condition. The reduction of $Po_2$ (partial pressure of $O_2$) in peritubular cells also stimulates the release of EPO. Hence, there is an inverse correlation between serum EPO and renal plasma flow, as well as between serum EPO and GFR in CHF patients [87]. With worsening of cardiac function and renal hypoperfusion therefore serum EPO levels progressively rise.

We first demonstrated that increased plasma EPO levels are related to the progression of cardiac failure and correlates with the NYHA class and plasma natriuretic peptide levels, as well as to blood urea and serum creatinine levels [88]. Serum EPO levels are reduced by treatment with ACE inhibitors in patients with CHF, and this reduction is paralleled by clinical and haemodynamic improvements [88,89]. Later studies have demonstrated that high EPO levels are associated with increased mortality and hospitalization in CHF patients, representing an independent prognostic marker. EPO levels are also directly related to NT-proBNP (N-terminal proBNP) levels [90,91].

Although EPO stimulates erythropoiesis and increases the number of circulating red blood cells, in CHF patients haematocrit levels are normal or even lower than in normal subjects, but total blood, plasma and packed red blood cell volume are increased [88]. This can be explained by the haemodilution, which characterizes this pathological condition. The activation of the renin–angiotensin–aldosterone system causes fluid retention and plasma volume expansion, with a consequent reduction of haematocrit, which causes the so-called 'pseudo-anaemia'.

In normal subjects there is an inverse correlation between log[EPO] and Hb levels, explained by well-preserved negative-feedback mechanisms; in CHF patients this correlation is poor and there is a wide range of EPO levels for the same Hb concentration. The extent of EPO-resistance (assessed as the weekly

weight-adjusted dose of EPO divided by the Hb level) was found to be predictive of survival in a large ($n$ = 1710) prospective cohort at a 12-month follow-up (79 compared with 92 %, $P < 0.001$ in the higher compared with the lower EPO-resistance group) [92].

In recent studies, van der Meer et al. [93,94] divided CHF patients into three groups on the basis of the O/P (observed/predicted) ratio, which is a useful index to assess the adequacy of EPO levels on the basis of Hb level. In a population of 605 patients they found that the majority of patients had EPO levels that were lower than expected. This can be explained by the renal dysfunction that commonly accompanies the progressive worsening of heart function, and may be by haemodilution. At total of 12 % of patients showed EPO levels consistent with their Hb levels, whereas 9 % had EPO levels that were higher than expected. Those authors suggested different mechanisms to explain the higher than predicted EPO levels. First of all, EPO is produced by the kidney in response to renal hypoperfusion when cardiac output decreases, and not only in response to anaemia. Metabolic disorders, such as acidosis, hypocapnia and hyperphosphataemia can shift the oxygen–Hb dissociation curve to the right, modify oxygen delivery and influence EPO levels [94,95]. The presence of an EPO resistance in the bone marrow is another possibility; this could be due to the action of inflammatory cytokines, which are often elevated in CHF patients, or maybe due to serum factors, such as Ac-SDKP, a strong haemopoiesis inhibitor. Finally, AngII, which remains elevated despite chronic treatment with ACE inhibitors, stimulates EPO secretion. Patients with a higher O/P EPO ratio showed an increased mortality risk compared with other patients [8,83], and both elevated baseline EPO levels and persistently elevated EPO levels were shown to be predictors of impaired survival [94]. A small study in cardiac resynchronization therapy confirmed that high EPO levels measured prior to the procedure were able to discriminate between responders and non-responders in patients with similar ejection fractions, NYHA classification, 6-min walking test, renal function, serum sodium and MLHFQ (Minnesota Living with Heart Failure questionnaire) outcomes [96].

Studies have also investigated whether endogenous EPO levels could predict long-term outcomes in patients with acute MI after successful PCI. Early results are promising. Namiuchi et al. [97] reported that, in 101 patients with a first MI, who underwent successful PCI within 12 h, high serum EPO levels, as assessed after primary PCI and within 24 h of pain onset, were associated with smaller infarct size as determined by the cumulative CK release. Consistent with that study was a small study conducted by Nakamura et al. [98], who found that, in patients with MI undergoing primary PCI, there was a positive correlation between time-dependent increases in serum EPO levels (assessed until day 7) and changes of ejection fraction, thus suggesting a role for reactive EPO synthesis in improving cardiac function in the chronic phase. Other trials, however, did not show any correlation between serum EPO levels and indexes of cardiac damage and did not confirm the prognostic significance of EPO in patients with acute coronary syndrome [99]. Conversely, a large study ($n$ = 627), in which EPO levels were assessed at admission for a suspected acute coronary syndrome (both unstable angina and non-STEMI), generated different results. In fact, a 47 % increase in the mortality level was observed in patients with the highest EPO levels. This was no longer significant when adjusted for cardiac TnT (troponin) and NT-proBNP levels [100]. Further studies are thus needed to better elucidate the role of endogenous EPO in patients with acute MI.

## CONCLUSIONS

According to current guidelines, the use of ESAs and supplemental iron should only be considered in patients with chronic kidney disease, in order to increase the Hb level to a target of 11.0–12.0 g/dl. Growing evidence indicates that EPO is not only a haemopoietic hormone, but has pleiotropic effects that may make it useful for the treatment of cardiac disease, especially CHF. This clinical application is being actively investigated to explore the possibility that EPO safely improves cardiac function and quality of life. EPO seems to be very promising in the treatment of MI to reduce infarct size and to prevent ventricular remodelling, and several clinical studies based on short-term administration are under way. Further studies will be required to clarify both the efficacy and safety of EPO treatment, as well as the appropriate indications, doses, therapeutic window and target Hb levels. The prognostic significance of endogenous EPO levels in patients with CHF is another potentially important field of clinical application that could potentially better predict the response to treatment; in this perspective also, further studies are required to investigate this area of application related to EPO.

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