

# Mechanisms of Disease: erythropoietin resistance in patients with both heart and kidney failure

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## SUMMARY

Anemia is common in patients who have both heart failure and chronic kidney disease, and there is an association between anemia and progression of both diseases. The main causes of anemia are deficient production of erythropoietin (EPO), iron deficiency, and chronic disease with endogenous EPO resistance. EPO has been successfully used for over a decade to treat anemia in patients with chronic kidney disease. Less obvious are the safety and efficacy of EPO treatment in patients with both heart failure and renal disease. Up to 10% of patients receiving EPO are hyporesponsive to therapy and require large doses of the agent. Several mechanisms could explain resistance to endogenous and exogenous EPO. Proinflammatory cytokines antagonize the action of EPO by exerting an inhibitory effect on erythroid progenitor cells and by disrupting iron metabolism (a process in which hepcidin has a central role). EPO resistance might also be caused by inflammation, which has a negative effect on EPO receptors. Furthermore, neocytolysis could have a role. As resistance to exogenous EPO is associated with an increased risk of death, it is important to understand how cardiorenal failure affects EPO production and function.

**KEYWORDS** anemia, chronic kidney disease, erythropoietin resistance, heart failure, inflammation

## REVIEW CRITERIA

The PubMed database was searched using the following terms: "chronic kidney disease", "heart failure", "anemia", "erythropoietin", "erythropoietin resistance", "inflammation", "hepcidin" and "erythropoietin receptor". The bibliographies of retrieved articles were searched for relevant references. We focused on English-language articles published in the past 20 years.

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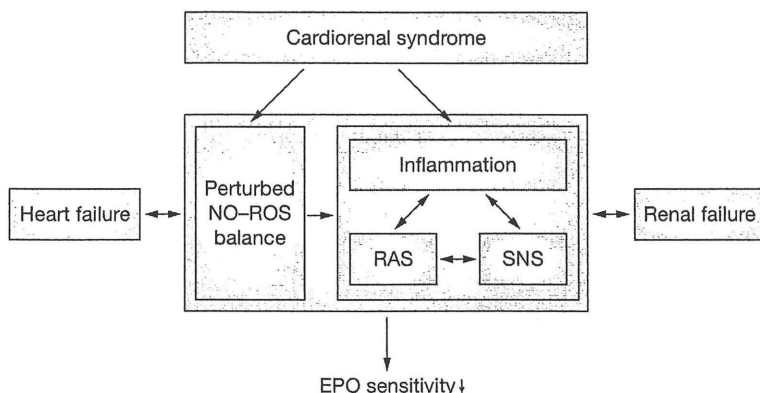
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## INTRODUCTION

Anemia is common in patients with both heart failure and chronic kidney disease (CKD) and is associated with a negative outcome. Whether treatment with erythropoietin (EPO) is of benefit is the subject of several ongoing studies. Therapy with EPO does not increase hemoglobin levels in a considerable proportion of patients. This fact is clinically important because resistance to EPO is associated with an increased risk of death in people with CKD. In this Review, we address these issues with a focus on the mechanisms that modulate the response to EPO. First, we briefly discuss the pathogenesis of the cardiorenal syndrome, and explore the causes and consequences of anemia in CKD, heart failure and the combination of the two. Thereafter, we elucidate the physiological role of EPO and discuss the mechanisms that might underlie the variability in sensitivity to endogenous, as well as exogenous, EPO.

## ERYTHROPOIETIN AND THE CARDIORENAL SYNDROME

We recently proposed a model for the pathogenesis of the cardiorenal syndrome, in which cardiac and renal dysfunction mutually amplify progressive failure of both systems.<sup>1</sup> Inflammation, the balance between nitric oxide and reactive oxygen species, the sympathetic nervous system, and the renin-angiotensin system (RAS), are the 'cardiorenal connectors', cornerstones in the pathophysiology of the cardiorenal syndrome (Figure 1).<sup>1</sup> More recently, we have expanded the model and explored the hypothesis that EPO could dampen the cardiorenal connectors.<sup>2</sup> In this Review, we address the means by which the cardiorenal connectors influence the function of EPO. In acknowledgment of its pivotal role, we will specifically focus on the mechanism by which inflammation affects the physiological function of EPO. The other cardiorenal connectors are only discussed when relevant to this mechanism.



**Figure 1** The pathophysiology of the cardiorenal syndrome and its effect on erythropoietin. The cardiorenal syndrome is characterized by an imbalance between nitric oxide and reactive oxygen species, by increased inflammation, by increased activity of the renin-angiotensin system, and by increased activity of the sympathetic nervous system. Together, these 'cardiorenal connectors' decrease sensitivity to EPO. Abbreviations: EPO, erythropoietin; NO, nitric oxide; RAS, renin-angiotensin system; ROS, reactive oxygen species; SNS, sympathetic nervous system.

#### ANEMIA IN CKD, HEART FAILURE AND THE CARDIORENAL SYNDROME Causes

Anemia associated with CKD has several causes. The main factor is inappropriate synthesis of EPO, with serum levels of EPO being disproportionately low for the degree of anemia (i.e. 'renal anemia').<sup>3</sup> The 'anemia of chronic disease' phenomenon also has an important role in CKD.<sup>4</sup> Several pathophysiological mechanisms underlie this condition, including limited availability of iron for erythropoiesis, impaired proliferation of erythroid precursor cells, reduced expression of EPO and EPO receptors, and, possibly, perturbed EPO signal transduction.<sup>5</sup> These mechanisms will be discussed in detail later in this article. Other causes of anemia in patients with CKD are infection and absolute iron deficiency.<sup>6</sup> Blood loss is a common cause of anemia in patients with renal failure. This blood loss includes occult gastrointestinal blood loss, blood being retained in extracorporeal circuits during dialysis, and withdrawal of blood for laboratory tests. Hemolysis, vitamin B<sub>12</sub> or folate deficiency, hyperparathyroidism, hemoglobinopathies and malignancies can also cause anemia in patients with CKD.<sup>6–8</sup> The consequences of concomitant angiotensin-converting-enzyme (ACE) inhibitor therapy are complex and might contribute to suppression of erythropoiesis.<sup>9,10</sup>

Anemia is a common problem in patients with chronic heart failure (CHF). Ezekowitz *et al.* reported that 17% of 12,065 patients with CHF were anemic.<sup>11</sup> Anemia in CHF develops in response to several factors. The most important form is anemia of chronic disease.<sup>12</sup> In addition, the creatinine clearance rates are less than 60 ml/min in approximately 25–50% of patients with CHF,<sup>13</sup> which could contribute to the development of anemia in these individuals. Other causes of anemia in patients with CHF are iron deficiency, and vitamin B<sub>12</sub> or folate deficiency.<sup>8,12</sup>

ACE inhibitors are part of the standard treatment regimens for CHF and, as mentioned above, whether their use contributes to the development of anemia is uncertain. Angiotensin II enhances EPO secretion and might also directly stimulate erythroid precursor cells.<sup>14</sup> ACE catalyzes the breakdown of *N*-acetyl-seryl-aspartyl-lysyl-proline, an inhibitor of erythropoiesis.<sup>15,16</sup> Accordingly, inhibition of the RAS would reduce hemoglobin levels. This effect has been confirmed in clinical trials in which pharmacological inhibition of the RAS was associated with a small but statistically significant reduction in hemoglobin levels.<sup>17–19</sup> By contrast, Androne *et al.* assert that activation of the RAS reduces hemoglobin concentration in patients with CHF, by hemodilution.<sup>20</sup> It seems, therefore, that the effects of ACE inhibition on hematocrit are complex, and available data are conflicting.

It is not surprising that anemia is a common feature of patients with both CKD and CHF. de Silva *et al.* found that, of 955 patients with systolic heart failure, 32% had anemia and 54% had renal insufficiency.<sup>8</sup> Furthermore, 41% of patients with kidney disease and 22% of patients without kidney disease had anemia. Silverberg *et al.* called the triad of anemia, CKD and CHF the 'cardiorenal-anemia syndrome'.<sup>21</sup> In this syndrome, the three components form a vicious circle, with each constituent capable of causing or worsening the other two.

#### Consequences

Renal anemia has serious clinical consequences. In addition to reducing patient physical capacity and quality of life, anemia induces adaptive cardiovascular mechanisms that increase the risk of cardiovascular disease and death.<sup>22–25</sup> Observational studies have shown that a decreased level of hemoglobin in patients with

CKD is associated with an increased risk of hospitalization and of death.<sup>26–29</sup> Small interventional trials have shown that treatment of anemia with EPO in CKD patients improves quality of life.<sup>30</sup> Some studies,<sup>31,32</sup> but not all,<sup>25</sup> have detected an association between treatment with EPO and regression of left ventricular hypertrophy. Thus, treating anemia is important in patients with CKD, but much controversy exists about the optimum hemoglobin target concentration. Several studies have been performed to address this issue. In 1998, Besarab *et al.* suggested that treating patients with end-stage renal disease to achieve a target hematocrit of 42% could be harmful.<sup>33</sup> The Cardiovascular Risk Reduction by Early Anemia Treatment with Epoetin Beta (CREATE) study detected no significant effect on cardiovascular events of achieving a target hemoglobin level of 130–150 g/l (13–15 g/dl) in patients with CKD.<sup>34</sup> The Correction of Hemoglobin and Outcomes in Renal Insufficiency (CHOIR) study was terminated early when an increased risk of death and cardiovascular hospitalization was detected in patients with CKD treated to achieve a target hemoglobin level of 135 g/l (13.5 g/dl).<sup>35</sup> In conclusion, no data are currently available to support the normalization of hemoglobin concentrations in patients with CKD, and large prospective trials are needed to determine an optimum target level. Until such evidence becomes available, the National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF K/DOQI) guidelines recommend a target hemoglobin level of 110–120 g/l (11–12 g/dl).<sup>36</sup> The European best practice guidelines recommend a target hemoglobin level of above 110 g/l (11 g/dl), with a maximum of 120 g/l (12 g/dl) for patients with concomitant cardiovascular disease or diabetes.<sup>37</sup>

A low hemoglobin concentration in patients with CHF is associated with more-severe disease, greater left ventricular mass index, and higher hospitalization and mortality rates.<sup>11,38,39</sup> The Anemia in Chronic Heart Failure: Outcomes and Resource Utilization (ANCHOR) study, however, showed that a very high hemoglobin level (>170 g/l [17 g/dl]) is also predictive of an increased risk of death and hospitalization in patients with CHF.<sup>39</sup> Several small controlled trials have been performed to assess the effect of treatment with EPO on anemic patients with CHF. EPO treatment improved exercise capacity and left ventricular

function, and reduced hospitalization rate.<sup>40–43</sup>

In the Studies of Anemia in Heart Failure—Heart Failure Trial (STAMINA-HeFT), 319 patients with CHF were randomly assigned to receive either darbepoetin alfa or placebo.<sup>44</sup> No significant differences were observed between the two groups in exercise duration, New York Heart Association class, or hospitalization, after 27 weeks. Available evidence is, therefore, contradictory, and further studies are needed to determine whether, and to what extent, correction of anemia with EPO is beneficial in patients with CHF.

### ERYTHROPOIETIN, ERYTHROPOIESIS AND BEYOND

EPO acts as an erythropoietic protein; it is produced in the kidneys by peritubular cells and can also be produced in the liver.<sup>45</sup> The primary stimulus for production of EPO is hypoxia. A deficiency in tissue oxygen levels increases the activity of hypoxia-inducible factor 2 $\alpha$ , which binds to hypoxia-responsive elements located in the enhancer region of the *EPO* gene in order to activate transcription.<sup>46,47</sup> Other transcription factors, such as GATA2 and nuclear factor kappa B (NF $\kappa$ B), can modulate *EPO* expression; evidence indicates that both factors inhibit transcription of the *EPO* gene.<sup>48,49</sup> The transcription factor Sox6 has been shown in mice to be an important enhancer of erythropoiesis at multiple levels.<sup>50</sup> In the bone marrow, EPO acts synergistically with stem cell factor, granulocyte macrophage colony-stimulating factor, interleukin (IL)-3, IL-4, IL-9 and insulin-like growth factor 1 on erythroid progenitor cells to prevent their programmed cell death, thereby stimulating proliferation and maturation of erythroid progenitors through the normoblast stage into reticulocytes and mature erythrocytes.<sup>51</sup> The EPO receptor is expressed primarily on erythroid cells that are between the colony-forming-unit erythroid stage and the pronormoblast stage of development. The number of EPO receptors per cell gradually decreases during erythroid differentiation.<sup>52</sup>

In addition to acting as an erythropoietic factor, EPO has been shown to have an important role in tissues outside of the erythropoietic system. Expression of EPO receptors and a biological response to EPO have been observed in endothelial, neural, cardiac and other cell types.<sup>53–55</sup> One non-hematopoietic effect of EPO is its stimulation of dose-dependent

proliferation and chemotaxis of endothelial progenitor cells *in vitro*, which promotes vascular reparative processes and neoangiogenesis.<sup>56</sup> This process has also been shown to occur in patients with CKD<sup>57</sup> and in patients with CHF.<sup>58</sup> EPO can also induce nitric oxide production by endothelial cells, which might exert anti-apoptotic effects.<sup>2,59</sup> EPO protects cardiomyocytes against ischemic injury by inhibiting apoptosis.<sup>60</sup> Administration of EPO to ischemic renal cells reduced apoptosis and enhanced regeneration in a study by Vesey and colleagues.<sup>61</sup> Thus, local binding of EPO to endothelial cells, cardiac cells and renal cells exerts cytoprotective and proliferative effects.

Erythropoietic agents were introduced in about 1990 for the treatment of anemia associated with CKD. Nowadays, EPO is also registered for use in patients with nonmyeloid malignancies treated with chemotherapy, in AIDS patients with anemia due to treatment with zidovudine, and in the perioperative stage for surgical patients. Several studies that aim to define the role of EPO treatment in CHF, in the combination of CHF and CKD, and in acute myocardial infarction, are underway.

#### ERYTHROPOIETIN RESISTANCE

In 1979, Caro *et al.* measured endogenous EPO levels in healthy people with or without anemia and compared these with levels in CKD patients with anemia.<sup>62</sup> It seemed that EPO levels were higher in patients with CKD than in those without CKD, but were inappropriately low for the degree of anemia. Serum levels of EPO in normal individuals range between 1 and 27 mU/ml (mean  $6.2 \pm 4.3$  mU/ml), whereas those in patients with CKD are between 4.2 and 102 mU/ml (mean  $29.5 \pm 4.0$  mU/ml);<sup>63</sup> anemia persists in patients with CKD despite average EPO levels being approximately five times higher than those in healthy individuals. This disparity indicates that, in addition to relative EPO deficiency, bone marrow response to endogenous EPO is suppressed in people with CKD. Erythropoietic agents have been used since the 1990s to treat patients with CKD, in an effort to overcome the relative EPO deficiency. Up to 10% of patients, however, have an inadequate response to therapy.<sup>64</sup> This finding is clinically important, because resistance to EPO is associated with an increased risk of death in patients with CKD. This effect persists after adjustment for the generally decreased hematocrit in these patients.<sup>29</sup>

EPO levels have also been measured in people with CHF, and have been found to be high.<sup>65–67</sup> Analogous to the situation in patients with CKD, the high EPO levels in patients with CHF are inappropriately low for the degree of anemia.<sup>68</sup> These data indicate that in CHF, as well as in CKD, there is a relative EPO deficiency as well as resistance of bone marrow to endogenous EPO.

Several mechanisms for resistance to endogenous, as well as exogenous, EPO have been proposed. The processes that cause anemia of chronic disease have a role. The primary hypothesis is that proinflammatory cytokines antagonize the actions of endogenous and exogenous EPO by directly inhibiting erythroid progenitor cells and by disrupting iron metabolism. Interaction between EPO and its receptor, and intracellular EPO signaling, could also have a role. Little is known about the putative contribution of neocytolysis (selective hemolysis of young circulating red blood cells) to EPO resistance. These possible causes of EPO resistance are discussed in the following section.

#### MECHANISMS OF ERYTHROPOIETIN RESISTANCE

##### Cytokines and erythropoiesis

CKD involves a chronic inflammatory state. Patients with CKD have increased levels of markers of inflammation such as C-reactive protein and the cytokines IL-1, IL-6, interferon (IFN)- $\gamma$  and tumor necrosis factor (TNF).<sup>69,70</sup> Increased levels of TNF and IL-6 have also been measured in patients with heart failure.<sup>71–73</sup> These cytokines inhibit the growth of erythroid precursor cells *in vitro*, an effect that is probably brought about through cytokine-mediated induction of apoptosis.<sup>74,75</sup> Taniguchi *et al.* showed that IFN- $\gamma$  downregulates messenger RNA for EPO receptor expression, indicating that the number of EPO receptors can influence apoptosis of erythroid precursor cells.<sup>76</sup> In addition, IL-1 and TNF have been shown to inhibit EPO production, another mechanism by which cytokines induce apoptosis of precursor cells.<sup>77</sup> Moreover, cytokines have a direct toxic effect on progenitor cells, which is generated at least in part by inducing the labile free radical nitric oxide, produced by inducible nitric oxide synthase.<sup>78</sup>

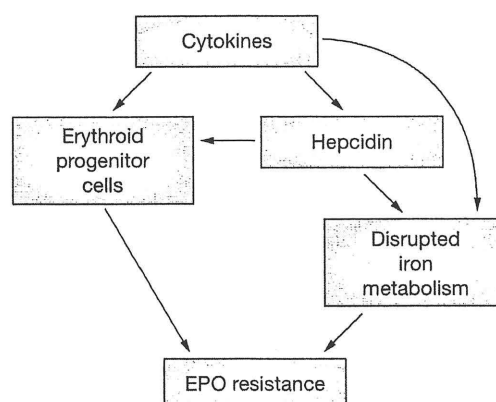
In addition to their direct inhibitory effect on erythroid progenitor cells, cytokines can cause EPO resistance by disrupting iron metabolism (Figure 2). Iron is essential for the production



of hemoglobin. Iron homeostasis involves the following three sources that supply the plasma compartment: iron absorption from the diet; recycled iron from red blood cells; and iron moved from storage sites in the liver (Figure 3). As most of the body's iron is contained in red blood cells, nearly all of the iron for erythropoiesis is supplied by the recycling of iron from senescent red cells. Macrophages phagocytize erythrocytes, and iron is released from hemoglobin in the phagolysosome. From the macrophage, iron is transferred to the circulation by the carrier protein ferroportin.<sup>79</sup> Iron is transported in the plasma by transferrin, which donates iron to cells through its interaction with a specific membrane receptor, the transferrin receptor.<sup>80</sup>

Proinflammatory cytokines, mainly IL-6, affect iron metabolism by stimulating the synthesis of hepcidin. Hepcidin is a type II acute-phase protein produced in the liver that has been proposed to be the central regulator of iron metabolism (Figure 3).<sup>81</sup> Hepcidin is thought to control the efflux of iron into plasma transferrin by downregulating ferroportin, the efflux channel for iron in macrophages and enterocytes. It has also been suggested that hepcidin negatively regulates the expression of the apical divalent metal transporter 1 in the enterocyte.<sup>82</sup> The synthesis of hepcidin thus leads to inhibition of iron absorption in the small intestine and sequestering of iron in macrophages. These actions lead to a decreased iron concentration in the circulation. In addition to its effect on iron metabolism, hepcidin might contribute to EPO resistance by directly inhibiting erythroid-progenitor proliferation and survival (Figure 2).<sup>83</sup> In accordance with these findings, it has been shown that levels of hepcidin, as well as of pro-hepcidin (the precursor of hepcidin), are negatively correlated with hematocrit in hemodialysis patients.<sup>84,85</sup>

Proinflammatory cytokines also have a direct effect on iron homeostasis (Figure 2). TNF, IL-1 and IL-6 upregulate the expression of divalent metal transporter 1 in macrophages, induce ferritin expression, and downregulate ferroportin. In addition, cytokines stimulate an increase in transferrin-receptor-mediated uptake of transferrin-bound iron into macrophages. TNF and IL-1 damage erythrocyte membranes and stimulate erythrophagocytosis. Together, these processes promote intracellular iron



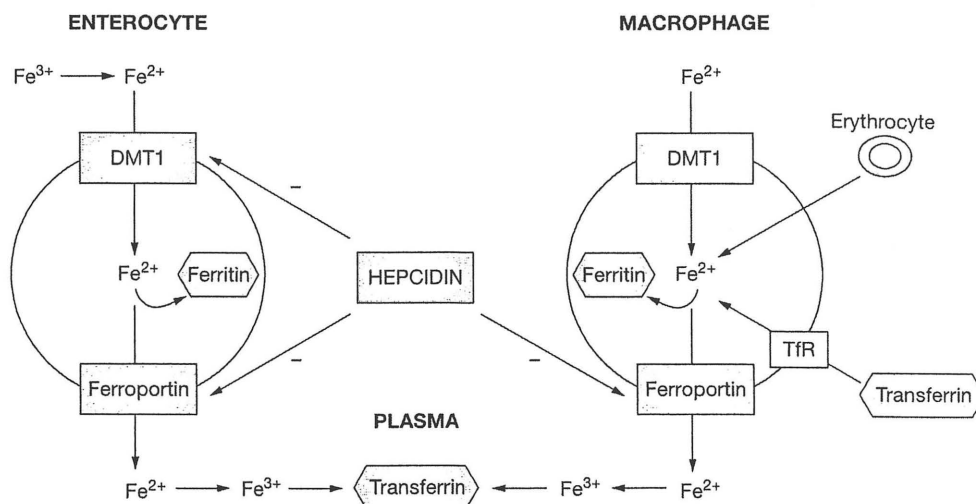
**Figure 2** The role of cytokines in erythropoietin resistance. Proinflammatory cytokines induce EPO resistance by exerting both direct inhibitory effects on erythroid progenitor cells, and indirect inhibitory effects by upregulating hepcidin. Cytokines also induce EPO resistance by disrupting iron metabolism. This effect occurs via upregulation of hepcidin and via a direct effect of proinflammatory cytokines on iron homeostasis. Abbreviation: EPO, erythropoietin.

storage and decrease the plasma concentration of iron.<sup>5</sup>

To sum up, the chronic inflammatory state of CKD plus CHF limits the availability of iron for erythropoiesis, thereby leading to EPO resistance via upregulation of hepcidin and the direct effects of cytokines on iron homeostasis.

### Receptor interactions

Binding of EPO to the EPO receptor is essential for the production of mature red blood cells. The EPO receptor is a type 1 transmembrane protein that belongs to the hematopoietic cytokine receptor superfamily. The EPO-bound receptor is a dimer; however, there has been debate about whether, in the absence of ligand, the EPO receptor is a monomer or oligomer. One model proposes that two monomeric receptors become a dimer after the binding of EPO, resulting in signal transduction.<sup>86</sup> Another model describes a process whereby the EPO receptor appears at the cell surface as a preformed dimer. In this now generally accepted scheme, binding of ligand shifts the receptor from an inactive to an active conformation (Figure 4).<sup>87,88</sup> Activation of the EPO receptor is transient; it is rapidly deactivated by downregulating mechanisms, including receptor internalization and degradation.



**Figure 3** Iron homeostasis and the role of hepcidin.  $\text{Fe}^{3+}$  is reduced to  $\text{Fe}^{2+}$  and moves into the enterocyte via DMT1. In the enterocyte, iron can either be stored as ferritin or can be transported out by ferroportin. In plasma,  $\text{Fe}^{2+}$  is oxidized to  $\text{Fe}^{3+}$ , which can bind to transferrin. Macrophages take up iron by phagocytosis of erythrocytes, as  $\text{Fe}^{2+}$  via DMT1, and from transferrin via the transferrin receptor. In the macrophage, iron is stored as ferritin or is transported to the circulation via ferroportin. Hepcidin negatively regulates ferroportin in the enterocyte and macrophage, and the expression of DMT1 in the enterocyte, leading to decreased plasma iron availability. Abbreviations: DMT1, divalent metal transporter 1; TfR, transferrin receptor.

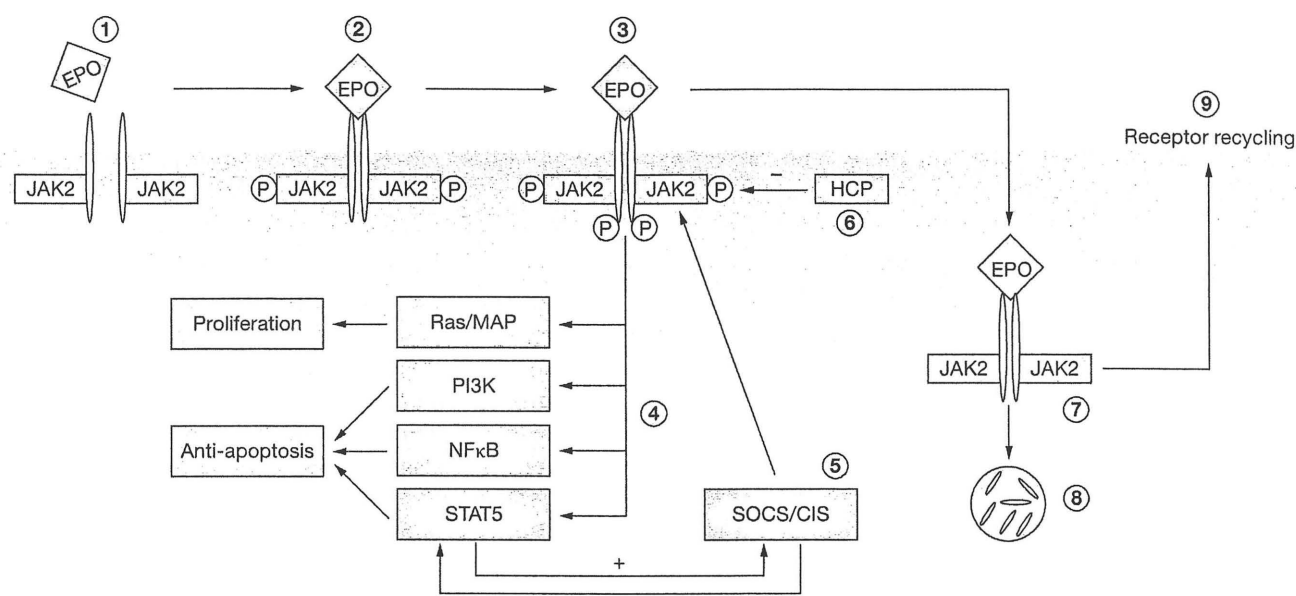
Mechanisms that might underlie EPO resistance are defective dimerization or defective activation of the EPO receptor. It has been shown that the transmembrane domain of the EPO receptor has a powerful ligand-independent dimerizing capacity.<sup>87</sup> Although the role of preformed dimers is still unclear, it could be that the dimerizing activity of the EPO receptor transmembrane domain sensitizes the EPO receptor such that it functions at low EPO concentrations. According to this model, absence of spontaneous dimerization of the EPO receptor could lead to EPO resistance.

Naranda *et al.* described how the EPO receptor can be activated in the absence of its natural ligand by a peptide that binds to a domain that differs from the EPO-binding site.<sup>89</sup> Mimetic peptides that bind to the EPO-binding site activate the same signaling pathways as EPO itself.<sup>90,91</sup> EPO resistance could be induced by the presence of antagonistic peptides that bind to the EPO receptor. For example, it has been shown that cytokine-inducible SH2-containing protein (CIS) binds directly to the cytoplasmic domain of the EPO receptor and inhibits EPO-dependent proliferation.<sup>92</sup> CIS production is induced by

cytokines such as IFN- $\gamma$ , IL-2 and IL-6. As such, cytokines might cause EPO resistance in cardiorenal failure.

Another possible cause of blunted EPO response is a decreased number of EPO receptors. IFN- $\gamma$  inhibits messenger RNA for EPO-receptor expression. The number of EPO receptors can also drop in response to receptor internalization and degradation, which are poorly understood mechanisms of downregulation. Beckman *et al.* studied the contribution of EPO-induced receptor internalization to modulation of EPO intracellular signals.<sup>93</sup> They showed that neither EPO activation of Janus kinase 2 (JAK2), nor tyrosine phosphorylation of the EPO receptor, was required for EPO-induced receptor downregulation. So, in contrast to other classes of growth factor receptors,<sup>94</sup> internalization of the EPO receptor seems to be regulated independently to EPO signaling. An increase in the rate of internalization could decrease the number of EPO receptors and lead to EPO resistance. The means by which ligand-occupied EPO-receptor internalization is regulated, and whether cytokines have a role in this process, is unknown.

After internalization, proteosomes and lysosomes degrade both EPO and the EPO



**Figure 4** Overview of erythropoietin receptor activation and intracellular pathways. The EPO receptor is a preformed dimer ([1]). Binding of EPO leads to activation of JAK2 by transphosphorylation ([2]) and subsequent tyrosine phosphorylation of the cytoplasmic domain of the EPO receptor ([3]). This phosphorylation leads to the initiation of intracellular signaling with activation of STAT5, Ras/MAP, PI3K and NFκB ([4]). STAT5 induces SOCS/CIS, which attenuates EPO signaling by binding to JAK2 and by inhibiting phosphorylation of STAT5 ([5]). EPO signaling is terminated by HCP, which dephosphorylates JAK2 ([6]). The EPO-EPO-receptor complex is internalized following dephosphorylation of the receptor ([7]). The receptor is then degraded in the proteasome ([8]) or recycled to the cell surface ([9]). Abbreviations: CIS, cytokine-inducible SH2-containing protein; EPO, erythropoietin; HCP, hematopoietic cell phosphatase; JAK2, Janus kinase 2; MAP, mitogen-activated protein; NFκB, nuclear factor kappa B; PI3K, phosphatidylinositol 3 kinase; SOCS, suppressor of cytokine signaling; STAT5, signal transducer and activator of transcription 5.

receptor, thereby regulating the degree of EPO signaling. In contrast to internalization, EPO and EPO-receptor degradation has been shown to be dependent on JAK2 activity; blocking JAK2-induced recycling of internalized EPO-EPO-receptor complexes to the cell, indicating perturbation of degradative processes.<sup>95</sup> Enhanced degradation of the EPO receptor could be important to the development of EPO resistance as it would diminish the rate of EPO-EPO-receptor-complex recycling; however, no data are available to support this hypothesis.

### Intracellular signaling

After binding to its receptor, EPO promotes activation of receptor-associated JAK2 tyrosine kinase and subsequent tyrosine phosphorylation of the EPO receptor.<sup>96</sup> This phosphorylation initiates intracellular signaling. Hematopoietic cell phosphatase (HCP; also known as protein-tyrosine phosphatase [SHP-1]) is a cytoplasmic protein that negatively regulates intracellular

signal transduction.<sup>97</sup> HCP dephosphorylates JAK2, preventing tyrosine phosphorylation of the EPO receptor (Figure 4). Upregulation of HCP could, therefore, attenuate the EPO signaling cascade and contribute to EPO hyporesponsiveness. Indeed, levels of HCP are increased in hemodialysis patients who are resistant to EPO therapy.<sup>98</sup> Mechanisms that upregulate the expression of HCP have not been elucidated.

Several signaling pathways are activated in response to tyrosine phosphorylation of the EPO receptor, including those that involve signal transducer and activator of transcription 5 (STAT5), Ras/mitogen-activated protein (MAP), phosphatidylinositol 3 kinase (PI3K) and NFκB (Figure 4). Binding of EPO to its receptor induces phosphorylation of STAT5, which has a crucial role in cytokine-induced survival of hematopoietic cells. Phosphorylation of STAT5 leads to expression of anti-apoptotic proteins including Bcl-x<sub>1</sub> and Bcl-2.<sup>99</sup> Furthermore, STAT5 induces suppressor of

cytokine signaling (SOCS), also known as CIS. SOCS/CIS is a component of a negative feedback mechanism that attenuates EPO signaling by binding to JAK2 or by inhibiting tyrosine phosphorylation of STAT5.<sup>92,100,101</sup> SOCS/CIS is induced by proinflammatory cytokines. Thus, the presence of inflammatory cytokines could cause EPO resistance by affecting intracellular signaling.

The Ras/MAP kinase pathway is activated by EPO and is involved in cell proliferation.<sup>102,103</sup> EPO also activates the PI3K pathway via phosphorylation, which leads to phosphorylation and activation of protein kinase B (Akt).<sup>104,105</sup> Akt can then phosphorylate and inactivate proapoptotic molecules. Prevention of Akt phosphorylation would block the cellular protection afforded by EPO and could lead to EPO resistance; however, no studies have been performed to determine whether Akt is inhibited in cardiorenal failure.

EPO can induce the phosphorylation of inhibitor of NFκB (IκB), thereby activating NFκB, which in turn enhances the transcriptional activity of target genes that encode anti-apoptotic molecules.<sup>105</sup> NFκB also inhibits transcription of the EPO gene. NFκB activity is enhanced by IL-1 and TNF, another mechanism by which inflammatory cytokines affect intracellular signaling and lead to EPO resistance in cardiorenal failure.<sup>49</sup>

### Neocytolysis

Red blood cell mass is maintained within a narrow range in order to optimize tissue oxygenation. Among the processes that control this parameter is neocytolysis. This physiological process is initiated by a drop in EPO levels, which leads to selective hemolysis of young circulating red blood cells (neocytes) and subsequent downregulation of red cell mass when it is excessive.<sup>106</sup> Two mechanisms could explain neocytolysis. EPO might regulate the secretion from endothelial cells of transforming growth factor beta, which influences macrophage-mediated phagocytosis. Alternatively, EPO withdrawal could induce secretion of inflammatory mediators from endothelial cells and macrophages, which would expose phosphatidylserine on young red cells. Phosphatidylserine is an aminophospholipid that is normally localized to the inner leaflet of the red cell membrane; once it is exposed on the red cell surface, it marks

these non-nucleated cells for destruction.<sup>107</sup> Neocytolysis could have a role in resistance to exogenous EPO. A small trial found that neocytolysis occurred in patients with CKD and anemia from whom EPO therapy was withheld.<sup>108</sup> Although the current information about the relevance of neocytolysis is limited, available data indicate that further exploration of this issue is warranted.

### CONCLUSIONS

The pathophysiological basis of the frequently occurring phenomenon of cardiorenal anemia is complex, and includes inadequate EPO production as well as EPO resistance. We propose that inflammation has a key role in EPO resistance. Cardiorenal failure is a low-grade inflammatory condition in which proinflammatory cytokines antagonize the action of EPO by directly inhibiting erythroid progenitor cells and by disrupting iron metabolism, in which hepcidin has a central role. EPO resistance could also be caused by inflammation-induced changes in EPO-receptor properties, assembly and recycling, and by interference with post-receptor signaling routes. This latter subject is largely unexplored. Neocytolysis might also have a role in EPO resistance.

Recombinant human EPO has been registered for more than a decade for the treatment of renal anemia in patients with CKD, and has proven to be a safe and beneficial therapy. Less clear is whether treatment with EPO in patients with heart failure and kidney disease is effective and safe. Resistance to exogenous EPO is associated with an increased risk of death in hemodialysis patients. It is, therefore, important to understand in more detail how cardiorenal failure affects EPO production and function.

### KEY POINTS

- Anemia is common in patients with chronic kidney disease and chronic heart failure, and is associated with a negative outcome
- Resistance to erythropoietin is a major cause of the anemia that affects patients with both chronic heart failure and chronic kidney disease
- Inflammation has a key role in resistance to erythropoietin
- Resistance to exogenous erythropoietin is associated with an increased risk of death in patients with chronic kidney disease



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**Competing interests**

The authors declared no competing interests.

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