30 Anemia and Diabetic Nephropathy

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CONTENTS

INTRODUCTION

Although anemia is a common problem in patients with chronic kidney disease (CKD), it is only in the last decade that an appreciation of its potential impact in patients with diabetes has emerged *(1,2)*. The prevalence of anemia in patients with diabetes is twice that seen in patients with nondiabetic renal disease and similar renal function. Approximately one in five patients with type 1 or 2 diabetes have hemoglobin (Hb) levels below the gender-specific normal range. Importantly, it is patients with vascular complications that are both at increased risk for anemia, and are most likely to suffer its adverse consequences.

This chapter will consider the relationship between anemia and diabetes in three parts. The first part will deal with the epidemiology and possible mechanisms associated with anemia, in the setting of diabetic nephropathy (DN). It will describe the prevalence of anemia in three Australian clinics and compare this with large community-based studies

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of anemia, including National Health and Nutrition Education Survey-III. The role of "functional erythropoietin deficiency" in which erythropoietin levels are inappropriately low for the ambient Hb, will also be discussed.

The second part of this chapter will deal with the anemia associated with advanced DN, starting with epidemiological data from the US Renal Data System. This will include the emerging concept that anemia is not only a product of the process of renal impairment but also a contributor to the development of congestive heart failure (CHF) and increased cardiovascular mortality associated with end-stage renal disease (ESRD) in patients with diabetes. The frequent coexistence of CHF, renal impairment, and anemia has been called the cardio–renal–anemia syndrome, with the component of anemia offering a potential means of therapy *(3)*. The coexistence of two or more components of this syndrome appears to increase mortality and, interestingly, this may occur in the absence of overt renal disease.

The final section of this chapter will examine the potential role for correction of anemia in patients with diabetes, using iron and erythropoietin, and the difficulties experienced so far in interpreting cardiovascular and renal outcomes of intervention studies.

ERYTHROPOIETIN AND THE ANEMIA OF DIABETIC NEPHROPATHY

Although a number of mechanisms potentially contribute to the development of anemia in diabetes, damage to the renal mechanisms that normal control Hb levels appears to be an essential component. Consistent with this paradigm, at moderate levels of renal impairment, with glomerular filtration rate (GFR) in the range 30–60 mL/min/1.73 m^2, anemia is present in more than one-third of patients with diabetes. Once GFR is below 30 mL/min/1.73 m^2, anemia is more common, affecting up to half of all patients. For the same level of GFR, patients with increased levels of urinary albumin excretion (UAE) have an increased prevalence of anemia, such that those patients with macroalbuminuria are nearly 10-fold more likely to have anemia that those with normoalbuminuria and normal renal function.

The kidney has a central role in the control of hemopoiesis and hence the oxygen-carrying capacity of blood. Erythropoietin is produced predominantly in the kidneys in adults *(4)*, with smaller amounts made in the liver and other sites. Within the kidneys, erythropoietin is expressed in the cortical and outer medullary fibroblasts, located between capillaries and oxygen-consuming tubules *(5,6)*. There is a close correlation between hematocrit, renal erythropoietin gene expression, and serum erythropoietin levels. Increases in erythropoietin secretion result when the oxygen supply is reduced as a result of a decrease in Hb levels, a decrease in oxygen-carrying capacity of Hb, or a decrease in oxygen saturation of Hb. At a local level, erythropoietin synthesis is controlled via the induction of oxygen-sensing mechanisms including hypoxia-inducible factor (HIF)-1 *(7,8)*. HIF-1 increases exponentially as cellular oxygen tension decreases and activates the gene expression of erythropoietin *(9)*. HIF-1 also activates transcription of several hypoxia-inducible genes including vascular endothelial growth factor (VEGF) and several glycolytic enzymes involved in anerobic ATP generation *(10,11)*.

DN is associated with a reduced renal capacity to synthesize and secrete erythropoietin in response to anemia. Erythropoietin levels in diabetes are in the normal range and are not low, as would be consistent in a true deficiency state. However, in the

setting of anemia, in which erythropoietin levels would normally rise exponentially, levels in patients with DN remain "inappropriately" in the normal range. DN therefore represents a state of functional erythropoietin deficiency and may consequently be responsive to supplementation. In our recent studies, erythropoietin levels were measured in 722 patients with diabetes *(12)*. In the 23% of the study population with anemia, 77% had erythropoietin levels inappropriately within the normal range. Although 55% of anemic patients had moderate renal impairment, erythropoietin levels were also inappropriately normal in 18 of 26 of anemic patients with normal renal function (GFR > 90 mL/min/1.73 m^2). However, 17 of these 26 patients had DN as reflected by an increase in albumin excretion rate (AER). It follows that failure to produce erythropoietin in response to a declining Hb level may be one of the earliest manifestations of DN *(2,12)*. In addition, the severity of erythropoietin deficiency, as a cause of anemia in patients with diabetes, is not always proportional to the degree of renal impairment.

The precise mechanisms by which diabetes impairs the renal erythropoietin response to reduced Hb remain to be established. Although "functional erythropoietin deficiency" is clearly linked to renal dysfunction in diabetes, the reduction in synthesis of erythropoietin in response to anemia appears to be greater than that seen in other renal (and particularly glomerular) diseases. Like anemia, tubulo-interstitial damage may be seen in diabetes, independent of and in advance of late changes of declining glomerular filtration. For example, thickening and reduplication of the tubular and epithelial basement membrane can be readily observed in the early diabetic kidney, even among normoalbuminuric patients *(13)*. It is conceivable that damage to the erythropoietin-producing cells in the cortical interstitium or disruption of the delicate interaction between tubule, peritubular fibroblast, and endothelium required for normal hemopoietic function in the kidney, may contribute to impaired erythropoietin release. In fact, endogenous erythropoietin production has been suggested as a possible marker of the severity of tubulo-interstitial damage in diabetes *(1)*.

Renal injury leads to a decrease in erythropoietin production and anemia. Although this may be partly mediated through the loss of erythropoietin-producing cells from the kidney, some patients with CKD are able to mount an appropriate increase in erythropoietin levels in response to hypoxia, suggesting that sensing rather than synthetic pathways are chiefly disrupted *(14)*. Although erythropoietin gene expression has been well documented in peritubular fibroblasts, it has so far not been demonstrated in cultured renal cells. This suggests that an interaction is necessary between proximal tubular epithelial cells and peritubular fibroblasts as a prerequisite for erythropoietin gene activation. Damage to this delicate interaction, as a result of interstitial fibrosis and cytoskeletal disorganization associated with renal injury, may also contribute to functional erythropoietin deficiency.

Renal injury also causes a transformation of peritubular fibroblasts into myofibroblasts. One hypothesis is that myofibroblasts can still generate erythropoietin but less well than fibroblasts *(11)*. This is of interest in relation to diabetic nephropathy, as advanced glycation endproducts (AGEs) have been shown to modulate myofibroblast transformation *(15)*. This may be one explanation for the occurrence of anemia in DN at an earlier stage than in nondiabetic renal disease with a similar degree of renal impairment.

Erythropoietin deficiency in diabetes may also be caused by autonomic dysfunction. Watkins et al. *(16)* originally demonstrated that patients with primary autonomic failure suffer from erythropoietin deficiency and anemia. A further study by his group showed

a strong correlation between polyneuropathy and the development of anemia in patients with type 1 diabetes *(17)*. However, these patients also had coexisting nephropathy making it difficult to distinguish cause from association. In addition, denervated kidneys are still able to produce erythropoietin in response to anemia. Nonetheless, a key role for the sympathetic nervous system in impaired erythropoietin responsiveness should not be discounted.

Functional as well as structural changes may also contribute to impaired erythropoietin production. Increased metabolic demand in diabetic tubular cells and chronic hypoxia in the tubulo-interstitium may also be an important mediator of anemia in diabetes *(18)*. Oxidized nucleic acids, endogenous polyamines, and cobalt all inhibit the cellular release of erythropoietin in vitro, and are increased in diabetes. Each of these factors may contribute to a recalibration of the "set-point" for erythropoietin secretion, which can still be overcome with sufficient stimulation. This is phenomenologically similar to impaired glucose sensing in diabetic islets, which may respond normally to acute stimulation with arginine or tolbutamide but inappropriately to a physiological glucose stimulus *(19)*.

Urinary erythropoietin loss, associated with proteinuria in patients with diabetes has also been proposed as a mechanism for reduced circulating erythropoietin levels in patients with DN *(20)*. Patients with heavy proteinuria have substantial loss of erythropoietin in their urine. Our studies have demonstrated that patients with proteinuria have an increased prevalence of anemia, independent to renal function. However, neither the urinary excretion of erythropoietin nor its fractional excretion appears to be significantly increased in diabetes in the absence of heavy protein losses. In addition, erythropoietin deficiency may be observed in diabetes in the absence of proteinuria, although it is most commonly seen in this subgroup.

In our study of 722 patients with diabetes, anemia in association with increased erythropoietin levels (>30 mU/mL) was observed in 37 patients, representing approx 20% of the 165 patients with anemia *(12)*. A recent report has described the association of raised erythropoietin levels and anemia in the absence of nephropathy *(21)*. Anemia was present in 10 of 62 patients with type 2 diabetes. Serum erythropoietin levels increased appropriately with decreasing Hb, but in patients with anemia raised erythropoietin levels were not associated with the expected increase in reticulocyte count. This study raises the possibility that resistance to erythropoietin rather than impaired erythropoietin secretion may contribute to anemia in a subgroup of patients without overt renal disease.

ANEMIA AS A PROGRESSION PROMOTER IN DIABETIC NEPHROPATHY

Several studies have shown that anemia is an independent risk factor for progression in both diabetic and nondiabetic renal disease *(22)*. For example, in the Reduction of Endpoints in NIDDM with Angiotensin II Antagonist Losartan Study (RENAAL) study of patients with type 2 diabetes and nephropathy, anemia at the start of the study was a strong predictor of the rate of doubling of serum creatinine or development of ESRD *(23)*. Similarly, the Canadian multicenter cohort study of 313 predialysis patients with CKD showed that a decrease in baseline Hb of 5 g/L over a median follow-up of 2 yr was associated with a 13% shorter time to initiation of renal replacement *(24)*. The time to renal replacement shortened more than eightfold as Hb fell from 130 to less than 100 g/L.

However, it remains to be established whether patients with anemia simply have more severe renal disease. Anemia *per se* does not result in renal damage. As anemia may be considered a manifestation of renal injury, it is easy to imagine that damaged kidneys may be subject to more aggressive renal disease. Nonetheless, there are several potential mechanisms linking anemia with the progression of DN.

For instance, tissue hypoxia is a common mechanism for progression of various forms of renal disease in addition to high blood pressure and proteinuria *(14)*. Anemia may induce mitogenic and fibrogenic stimuli by lowering the partial oxygen tension in the renal cortex. This could be mediated by HIF-1 which regulates genes involved in angiogenesis (such as the prosclerotic mitogen, VEGF), vasomotor response (inducible nitric oxide synthase), heme oxygenase-1 and endothelins, glycolysis (the glucose transporter GLUT-1 and glycolytic enzymes), matrix metabolism (transforming growth factor-β_1, collagens, and matrix metalloproteinases), and cell survival, all pathways implicated in the pathogenesis of progressive renal disease.

A major consequence of anemia is an increase in oxidative stress *(25)*, a key mediator in the development and progression of diabetic renal disease. This may be mediated by the loss of antioxidant properties of erythrocytes including the enzymes superoxide dismutase, catalase, and other antioxidant proteins. In addition, renal anemia is associated with increased production of free radicals. It is conceivable that the combination of increased oxidative stress and tissue hypoxia associated with anemia may act to stimulate the production of extracellular matrix, increasing interstitial fibrosis and tubular apoptosis, and lead to tubular atrophy associated with progressive renal disease.

ANEMIA AND EXTRA-RENAL MICROVASCULAR DISEASE

Several lines of evidence suggest that anemia may influence the course of extrarenal microvascular disease in diabetes. Anemia is associated with an increased risk of background and proliferative retinopathy in patients with diabetes. Given that renal disease and retinopathy are closely associated, it is perhaps not surprising to see an increased prevalence of anemia in patients with more aggressive microvascular disease. However, anemia may also have a direct effect on the development and progression of diabetic retinal disease. Anemia may act to accelerate diabetic retinopathy by promoting retinal hypoxia and macularedema *(26)*. This may be mediated by VEGF, which is a potent stimulus to new vessel formation and increased capillary permeability *(27)*. Consistent with this hypothesis, a few small studies have demonstrated that correction of anemia in patients with diabetes may be associated with a reduction in macular hard exudates *(28,29)* and edema *(30)*. However, further interventional studies are required to determine if anemia is causally related to diabetic retinopathy.

Similar considerations apply to the association of anemia with DN. Endoneural hypoxia, owing to decreased microvascular blood flow and altered vascular permeability, is observed early in the course of diabetes and the resultant ischemia plays a role in the progressive DN. Factors that exacerbate hypoxia are known to accelerate nerve injury in diabetes and there is evidence that erythropoietin itself has neuroprotective and neurotrophic effects in experimental diabetes *(31)*. In diabetic patients with anemia and polyneuropathy, erythropoietin therapy has been associated with improvements in motor nerve conduction velocity but no correlation was found between the increase in Hb and improvement in nerve function *(32)*. This raises the possibility that

erythropoietin may have a direct impact on microvascular complications, independent of red cell formation. For instance, erythropoietin has been shown to have proangiogenic properties *(33)* and also to protect vascular endothelial cells and smooth muscle cells against apoptosis *(34,35)*. It follows that the pleiotropic effects of erythropoietin may promote new vessel formation and limit vascular injury in peripheral nerves, kidneys, and the heart in addition to correcting anemia.

EPIDEMIOLOGY OF ANEMIA IN DIABETES: COMMUNITY AND CLINIC-BASED STUDIES

Diabetes is responsible for 45% of new patients entering ESRD programs in the United States, 36% in Germany, and 22% in Australia. The majority of these patients will have anemia, although this represents the tip of a much larger problem. Most patients with DN will not survive to reach ESRD, succumbing instead to comorbid vascular complications and infection. It is not yet clear how anemia contributes to their morbidity or mortality, as there have been comparatively few studies in early diabetes. Table 1 summarizes the studies of anemia in patients with diabetes and nephropathy before ESRD.

An early study documenting the presence of normochromic, normocytic anemia in overt DN was reported by Watkins' group in London *(2)*. Anemia was defined as Hb ≤115g/L for women and ≤120g/L for men. Of 27 patients with type 1 diabetes and nephropathy, 13 were anemic compared with none of 27 patients with glomerulonephritis with similar levels of proteinuria and serum creatinine. Serum creatinine levels were similar in the 13 patients with DN and anemia (mean 110, range 63–160 μ*M*) compared with the 14 patients without anemia (mean 88, range 64–133 μ*M*). The majority of patients with type 1 diabetes and anemia were shown to have inappropriately low erythropoietin levels *(1,2,36)*.

Two recent community-based studies from the United States have assessed the prevalence of anemia according to GFR. The Third National Health and Nutrition Examination Survey (NHANES-III), performed between 1988 and 1994, was a large, community-based study of the prevalence of anemia according to kidney function. In the study group as a whole (15,971 adults), a significant decrease in Hb was apparent in men with GFR (estimated as creatinine clearance) ≤70 mL/min and in women with GFR ≤50 mL/min *(37)*. It was estimated from NHANES-III data that 9.7 million adult women and 3.8 million adult men in the United States have a GFR ≤50 mL/min. When anemia was defined as Hb <110 g/L, it was estimated that 610,000 women and 230,000 men had anemia associated with impaired kidney function. When anemia was defined as Hb <120 g/L, the estimated burden of anemia associated with renal insufficiency in the United States was 1.2 million women and 390,000 men.

A further analysis of the NHANES-III data compared the prevalence of anemia, (Hb <120g/L), according to GFR in people with and without diabetes *(38)*. This analysis found that people with diabetes and moderate renal impairment had a higher prevalence of anemia when compared with people without diabetes and similar degrees of renal impairment (Fig. 1). In the GFR range of 60–30 mL/min/1.73 m^2, anemia was present in 6, 12, and 22% of nondiabetic subjects as GFR decreased from 60–50, to 50–40 and to 40–30 mL/min/1.73 m^2, respectively. By contrast, in the same categories of GFR in diabetic subjects, anemia was present in 16% ($p < 0.001$), 24% ($p < 0.01$) and 41% ($p < 0.04$), respectively *(38)*. This study confirmed that anemia was more prevalent in

Table 1
Anemia in Early Diabetic Nephropathy

Study	*Participants*	*Stage of diabetic nephropathy*	*Results*
Inomata (1997) *(1)*	Type 2 DM	N, Micro overt	– Hb and EPO both ↓ with advancing DN – ↓ initial serum EPO and ↓ [Hb × EPO] in six patients with decreasing GFR compared with seven patients with stable GFR over 26 mo
Bosman (2001) *(2)*	Type 1 DM overt DN ($n = 27$) vs glomerular nephritis ($n = 26$)	Overt DN TP 1086 mg/24 h serum creatinine ≤180 μ*M* Gl N TP 1874 mg/24 h	– 13 of 27 DN anemic, Hb 106 ± 9 g/L – 0 of 26 GLN anemic, Hb 137 ± 14 g/L – S EPO in DN failed to ↑ c/w nondiabetic microcytic anemia patients. Therefore anemia with ↓ EPO occurs in DN before advanced renal failure
Winkler (1999) *(69)*	Type 1 DM ($n = 15$) + AN vs Type 1 DM without AN ($n = 15$)	3 Micro, 12 overt serum creatinine <122 μ*M*	– Group with DN + AN had lower Hb (111 ± 12 vs 137 ± 7 g/l) – Results suggest AN as a cause of anemia but role of DN cannot be excluded
Bosman (2002) *(17)*	Type 1 DM + DN + AN ($n = 5$) vs nondiabetic CKD ($n = 4$)	Overt DN	– Diabetic group: inappropriately low EPO for severity of anemia, but normal EPO response to acute hypoxia
Astor (2002) *(38)* NHANES-III	Community survey (Total $N = 15{,}971$) (diabetic $n = 1195$)	EGFR Estimated by MDRD-4 formula	– Twofold ↑ prevalence of anemia (16–41%) in people with diabetes and moderate renal impairment (eGFR 30–60 mL/min/1.73 m^2) than in people without diabetes and similar renal function (6–22%)
Thomas (2003) *(41)*	Type 2 DM in one clinic ($n = 820$)	N, Micro or overt	– Anemia present in 23% – Prevalence two to three times greater than for patients with comparable renal function and iron stress on general population – Predictors of Hb: transferrin saturation, GFR, sex, AER, and HbA_{1c}

(Continued)

Table 1 *(Continued)*

Study	*Participants*	*Stage of diabetic nephropathy*	*Results*
Thomas (2004) *(44)*	Clinic survey Type 2 DM Three Australian Centers (n = 2125)	N, Micro or overt	– Anemia present in 20% – Anemia associated with ↓CC or ↑ AER in >75% of patients – Patients with CC 60–90 twice as likely to have anemia compared with CC > 90 mL/min/1.73 m^2
Thomas (2004) *(45)*	Cross-sectional survey Type 1 DM (n = 312)	N, Micro or overt	– Anemia present in 14% overall <8% of N, 24% of Micro, 52% of overt – Renal impairment associated with sixfold ↑ in anemia
El-Achkar (2005) *(39)* Kidney Early Evaluation Program	Community survey (n = 5380) Mean age 53 yr 27% with diabetes	eGFR using MDRD-4 formula 5% with serum creatinine > 1.4 (F) >1.5 (M) mg/dL 16% with GFR < 60 mL/min/1.73 m^2	– Anemia present in 12% of diabetic and 6% of nondiabetic participants – ↑ Prevalence of anemia with diabetes and eGFR 30–89 mL/min//1.73 m^2 – Prevalence of anemia similar in diabetic + nondiabetic for eGFR >90 or <30 mL/min/1.73 m^2

N, normoalbuminuria; DN, diabetic nephropathy; Micro, microalbuminuria; AN, autonomic neuropathy; CC, creatinine clearance; Overt, overt nephropathy.

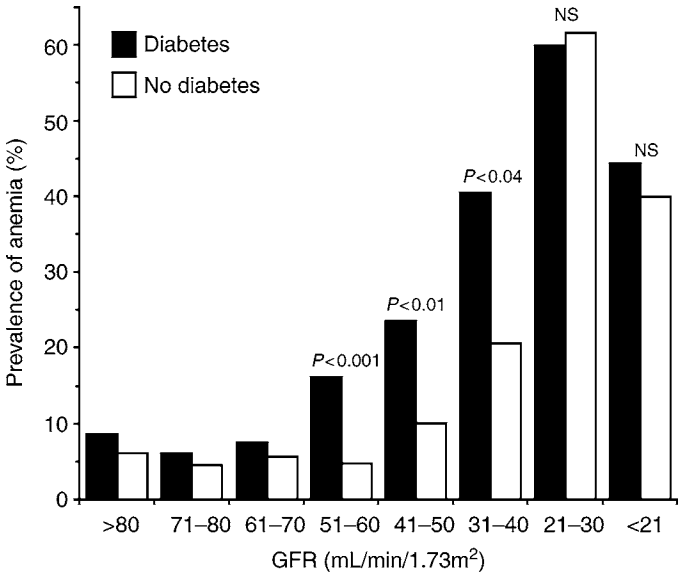

Fig. 1. Doubling of prevalence of anemia in diabetic compared with nondiabetic participants between GFR 30 and 60 mL/min/1.73 m^2 in NHANES-III *(38)*.

people with diabetes even when serum creatinine levels were still in the normal range. By the time that severe renal impairment developed at a GFR of <30 mL/min/1.73 m^2, the prevalence of anemia was nearly 60%. This was roughly that also seen in the nondiabetic population.

The Kidney Early Prevention Program (KEEP 2.0), organized by the National Kidney Foundation, studied 5380 individuals over 18 yr of age including 1769 men and 3611 women *(39)*. Anemia was defined as Hb < 120 g/L in men and women aged older than 51 yr and <110 g/L in women younger than 51 yr. GFR was calculated using the simplified Modification of Diet in Renal Disease (MDRD) formula. Diabetes and anemia were present in 25.7% and 7% of the cohort, respectively. In persons with diabetes, anemia prevalence increased from 6.4, 6.7, 16.5 to 57.1% as GFR decreased from >90, 89–60, 59–30 to <30 mL/min/1.73 m^2. In the GFR category 59–30 mL/min/1.73 m^2, anemia prevalence was significantly higher in persons with diabetes compared with persons without diabetes (16.5 vs 6.8%, $p < 0.001$). In addition, there was a greater risk of anemia among men with diabetes compared with women with diabetes when a gender-specific definition was used. Importantly, because most patients with DN have little overt renal impairment, the majority of patients with anemia are supervised by their primary care physician. In this context, anemia is usually unrecognized and almost always untreated.

In the KEEP 2.0 study *(39)*, there was no difference in the prevalence of anemia in patients with and without diabetes at GFR levels <30 mL/min/1.73 m^2. This is consistent with findings from the Predialysis Survey of Anemia Management (PRESAM) study of advanced kidney disease, which showed the prevalence of anemia appears to be similar in predialysis patients both with and without diabetes *(40)*. However, both these results may underestimate the impact of anemia, because of the premature death of diabetic patients with early anemia.

In our studies, we examined the prevalence and predictors of anemia in 820 patients from a single diabetes clinic in a tertiary referral center *(41)*. Eligibility for inclusion in the study was defined as patients having at least three estimations of AER, with at least one AER having been performed within the previous 2 yr *(41)*. The study included 458 men (56%) and 362 women who had been followed for a median of 4.8 yr (range 1–28). The mean age was 62.2 yr. Of the women in the study population 71% were aged older than 55 yr and were therefore likely to be postmenopausal. More than 95% were of Caucasian descent. The majority of participants had type 2 diabetes (80%), of whom 46% were receiving insulin. The mean HbA_{1c} was 7.9%. In a randomly selected subgroup of 330 patients (40%), a clinical history detailing the presence or absence of specific diabetes complications and treatment modalities was also obtained.

Many participants had experienced complications of diabetes, consistent with the mean known duration of diabetes of 16 yr *(41)*; 37% had evidence of retinopathy, 27% microalbuminuria, and 13% macroalbuminuria; 69% were receiving antihypertensive medication and 44% had evidence of macrovascular disease; 30% had moderate renal impairment defined by a GFR less than 60 mL/min/1.73 m^2. Interestingly, approx 20% of those with moderate renal impairment had normoalbuminuria. We found that in our population of 820 patients nearly one-fourth (23%) had anemia according to World Health Organization guidelines (men Hb < 130 g/L, women Hb < 120g/L). This was very similar to that found in the NHANES-III population with mild-to-moderate renal impairment *(38)*. That is, at every level of renal function in the GFR range of 30–60 mL/min/1.73 m^2 people with diabetes were twice as likely to have anemia (Fig. 2).

Univariate analysis revealed several predictors of anemia in people with diabetes including female gender, low GFR, increased age, low-iron stores, increased AER and, unexpectedly, low HbA_{1c}. Multiple regression revealed that five variables explained approx 42% of the Hb variance in the entire clinic population. Independent predictors for Hb were transferrin saturation, GFR, gender, AER, and HbA_{1c} level. Although gender was an important determinant of raw Hb levels, the most powerful predictors were transferrin saturation and GFR, accounting for 22 and 10% of the variance of Hb, respectively *(41)*. After adjusting for GFR age and all of the potential clinical determinants of anemia lost statistical significance. Although inflammation may cause anemia, no independent association was found between C-reactive protein and Hb.

Decreased transferrin saturation predicted low Hb levels at every level of renal impairment. However, decreased iron stores do not explain the increased rates of anemia in patients with diabetes. The rates of iron deficiency in our study population were the same or slightly lower than that seen in the general Australian population, and the overall iron stores are slightly higher than that seen in the general population *(42)*. There was an association between higher iron stores and worse glycemic control, consistent with studies by Fernandez-Real *(43)* showing that iron modulates insulin action so that to increase insulin resistance and oxidative stress. However, although reduced iron availability may predict the Hb level in people with diabetes, it is not the reason for the increased prevalence of anemia in people with diabetes and nephropathy.

The prevalence and predictors of anemia in long-term outpatients with type 2 diabetes from three large Australian clinical centers confirmed the findings reported earlier *(44)*. In a combined study population of 2125 patients, roughly one in five patients had anemia. Patients with mild renal impairment were twice as likely to have anemia as those with normal renal function, and patients with moderate renal impairment were twice as likely to have anemia as those with mild renal impairment.

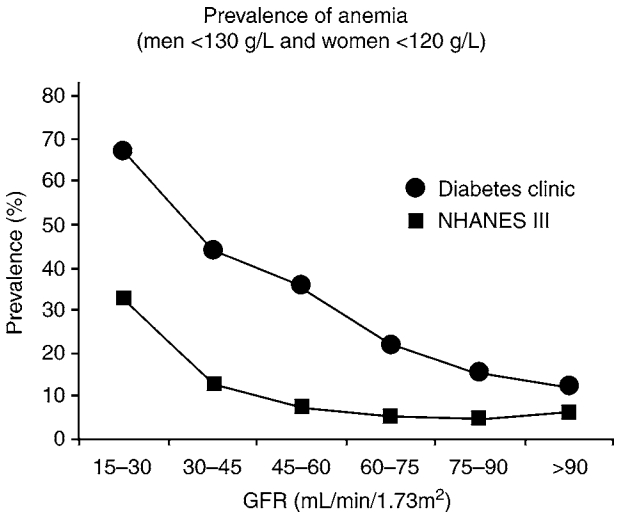

Fig. 2. Prevalence of anemia in an Australian Clinic setting *(41)* compared with NHANES-III *(37)*.

In a study population of 312 patients with type 1 diabetes from the same three centers, one in seven patients had anemia *(45)*. Patients at greatest risk were identified either by renal impairment or albuminuria. Patients with renal impairment were six times more likely to have anemia than those with normal renal function. Fifty-two percent of patients with macroalbuminuria had anemia compared with 24% of patients with microalbuminuria but less than 8% of normoalbuminuric patients.

Recent studies in the same cohort have examined the potential role of AGEs mediating the interaction between anemia and nephropathy. In this study, serum levels of low-molecular-weight fluorescent AGEs in 604 patients with type 2 diabetes were 34% higher than in nondiabetic subjects *(46)*. Notably, independent predictors of low-molecular-weight AGEs were low GFR and Hb. Whether AGEs contribute to anemia or are a marker of renal damage in diabetes will need to be determined by prospective studies.

ANEMIA, CARDIAC FAILURE, AND CARDIOVASCULAR OUTCOMES IN RENAL DISEASE

Patients with diabetes have an increased risk of cardiovascular events (Table 2) and heart failure (Table 3). Early in the course of diabetes, cardiac fibrosis and hypertrophy occur, leading to diastolic dysfunction, characterized by impaired relaxation and a stiff ventricle *(47,48)*. Anemia has the potential to exacerbate cardiac dysfunction in diabetes. In normal subjects, a fall in Hb from 140 to 40 g/L evokes an adaptive response in the heart. In the short term, oxygen extraction increases from 24 to 31% and cardiac output increases from 3 to 5.5 L/min/m^2 *(49)*. In the long term, these adaptive changes may lead to left ventricular hypertrophy (LVH), a known risk factor for adverse clinical outcomes. Several observational studies have demonstrated that anemia in combination with severe renal insufficiency *(50,51)* or cardiac failure *(52)* is an independent risk factor for worse cardiovascular outcomes (Table 2). For example, in a Canadian study of patients with ESRD, a decrease in Hb was associated with an increased risk of left ventricular dilatation, new and recurrent heart failure, and death but not with ischemic heart disease *(50)*. This was consistent with the concept that the long-term response to

Table 2
Observational Studies: Anemia and Cardiovascular and Renal Outcomes in Patients With Advanced Renal Disease

Study	*Participants*	*Duration*	*Results*
Foley (1996) *(50)*	ESRD ($n = 432$)	Prospective echo study 41 mo	– Each 10 g/L ↓ Hb independently associated with 46% ↑ risk of LV dilatation on repeat echo, 28% ↑ risk of new CHF and 20% ↑ risk of recurrent CHF – Each 10 g/L ↓ Hb associated with 14% increase in mortality
Madore (1997) *(56)*	H-Dialysis + EPO if indicated ($n = 21,899$)	Multiple linear regression	– Hb < 100–110 g/L associated with ↓ survival – Hb > 110 g/L not associated with further improvements in survival – Fe stores albumin, dialysis dose predicts Hb level
Levin (1999) *(54)*	Early CKD ($n = 246$)	Prospective multicenter echo study Baseline vs 12 mo comparison of LV growth	– LV mass index ↑ in 25% over 12 mo – After adjusting for baseline LVMI, ↓ Hb, and ↑ SBP were independent predictors of LV growth
Levin (2001) *(24)*	Early CKD, mean CC 36 mL/min mean age 56 yr 30% diabetic ($n = 313$)	Canadian multicenter cohort study 23 mo	– Pre-existing CVD 4in 6%, independent of severity of kidney dysfunction – 20% incidence of new or worsening CV events – Best predictors of new CVD: diabetes (OR 5.35), age (OR 1.26) – Progression of existing CVD mediated by low DBP (OR 0.72) and ↑ triglycerides (OR 1.48) – ↓ Hb by 5 g/L predicts shorter time to dialysis (OR 0.87)
Ofsthun (2003) *(59)*	H-Dialysis for at least 6 mo + EPO if indicated ($n = 44,500$)	First 6 mo (baseline) vs second 6 mo	– Risk of death + hospitalization inversely associated with Hb, no additional risk with Hb > 120 g/L 84% survival if baseline Hb < 90 g/L 94% survival if baseline Hb ≥ 130 g/L

Table 3
Observational Studies of Anemia and Cardiovascular Outcomes in Patients With Cardiac Failure and Early CKD

Study	*Participants*	*Design/duration*	*Results*
Al-Ahmad (2001) *(70)*	CHF (ACE_I vs placebo) (n = 6000)	5 yr Retrospective analysis of SOLVD database	– 2% lower Hct and 10 mL/min/1.73 m^2 lower eGFR each independently associated with 6% ↑ in mortality
Horwich (2002) *(52)*	CHF (NYHA class III or IV) and LVEF < 40% (n = 1061)	Retrospective cohort study 1 yr	– Anemia associated with ↓ GFR – Hb associated with more severe CHF – Survival 55.6% with Hb < 123 g/L 74.4% with Hb > 148 g/L
McClellan (2002) *(71)*	CHF (+ CKD in 38%) Admitted to community hospitals N = 665 (mean age 76 yr)	Retrospective cohort study 1 yr	– Mortality 44.9% with CKD, 31.4% without CKD – Mortality 31.2% with Hct ≥ 40%, 33.8% with Hct 36–39%, 36.7% with Hct 30–35% and 50% with Hct 30–35%
Ezekowitz (2003) *(53)*	New onset CHF Discharged from community hospitals, Canada N = 12,065 (median age 78 yr)	Retrospective cohort study 5 yr	– Anemia present in approx 30% women, 17% men (defined as Hb < 120 g/L in women, Hb < 130 g/L in Men) – ↓ survival if anemia present
Collins (2003) *(72)*	CHF Diabetic + nondiabetic (N = 665) Medicare beneficiaries	Observational 2 yr	– CKD (RR 2.0), anemia (RR 2.0), diabetes (RR 1.5) and CHF (RR 2.9) are independent risk factors for mortality – Combination of risk factors increase mortality: diabetes + CHF (RR 3.7), diabetes + CKD (RR 2.4), CKD + anemia (RR 3.7)

CHF frequently leads to plasma volume expansion, which could itself falsely lower Hb concentration. In a study of plasma volume in 37 patients with CHF and low haematocrit (Hct), haemodilution, and true anemia were present in equal proportions *(76)*.

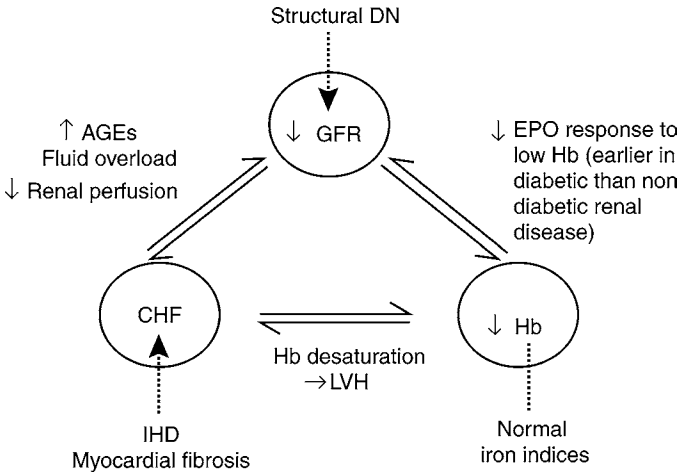

Fig. 3. Contributing factors and interrelationships in cardio–renal anemia *(60)*.

anemia involves a cardiomyopathic rather than an ischaemic mechanism *(49)*. Similarly, in a study of survival of patients with heart failure, the Hb at the time of initial diagnosis of heart failure was a significant predictor of survival *(53)*. Another cross-sectional study in patients with early renal disease showed that left ventricular mass index (LVMI) increased by 33% for every 5 g/L decrease in Hb and for every 15 mmHg increase in systolic blood pressure *(54)*. The extent of risk associated with anemia was, therefore, similar to traditional risk factors such as hypertension and dyslipidemia.

The prognosis of heart failure is significantly worse in patients with diabetes than in nondiabetic patients. It is conceivable that anemia may contribute to this risk, as part of the "cardio–renal–anemia syndrome," a triad of complications that often occur in the one patient *(3)* (Fig. 3). Two recent epidemiological studies have suggested that cardiovascular outcomes appear to worsen in patients with two or more components of this triad when compared with patients with only one component. For example, the atherosclerosis risk in communities study showed a twofold higher risk of coronary heart disease in patients with elevated serum creatinine levels, but this was evident only in the presence of anemia *(19)*. Another study of 2000 patients with heart failure showed that anemia has a high prognostic impact. Each 1% drop in hematocrit below normal was associated with a 2% increase in mortality over 12 mo *(55)*.

The association of anemia with cardiovascular and other outcomes has also been studied in patients with ESRD as part of the US Renal Data System, which included a database of 66,671 patients of which more than 40% had diabetes. A Hb < 110 g/L predicted increased risk of death after adjustment for clinical and laboratory parameters *(56)*. Another study of incident hemodialysis patients showed that a hematocrit of 36–39%, approximately equivalent to a Hb of 130 g/L, was associated with a 22% lower risk of admission to hospital when compared with patients with a hematocrit in the range of 33–36% *(57)*.

In patients with renal disease, anemia is also a risk factor for cerebrovascular disease. The Atherosclerosis Risk in Community study assessed the risk of incident stroke in a middle-aged population *(58)*. Patients with renal insufficiency alone had an increased hazard ratio for cerebrovascular accident of 1.81 (1.26–2.02, 95% confidence interval [CI]), but the hazard ratio increased to 5.48 (2.04–14.41, 95% CI) in patients

with renal insufficiency and anemia. A large observational study of 44,000 patients receiving hemodialysis has been performed recently in the United States *(59)*. All participants had survived 6 mo of dialysis and erythropoietin therapy, with 6 mo of follow-up. Patients with Hb >120 g/L had a reduced mortality compared with patients with Hb <110 g/L. For every decile of Hb from 90 to more than 130 g/L, there was a graded increase in the proportion of surviving patients *(59)*.

Caution should be taken in extrapolating data in patients with ESRD, to patients with diabetes and earlier stages of renal disease. Although the mechanism of anemia may be similar, a number of risk factors work in different ways in these two settings. For example, elevated lipid levels are associated with an increased risk of adverse outcomes in patients with diabetes and early nephropathy, whereas in ESRD elevated levels of cholesterol and obesity are associated with improved outcomes. Further prospective studies are required to address the role of anemia in pre-ESRD patients with diabetes.

In summary, renal impairment worsens heart function and compromises its therapy, heart failure worsens renal function and anemia worsens prognosis in people with combined cardiac and renal disease *(60)*. It remains to be seen if diabetes-specific pathogenetic links such as AGEs modulate the prevalence and expression of this syndrome in diabetic nephropathy.

THE POTENTIAL UTILITY OF ANEMIA CORRECTION IN PATIENTS WITH DIABETES

Although there is a clear rationale for the use of supplemental erythropoietin in diabetic patients with "functional erythropoietin deficiency," there is no conclusive evidence that correcting anemia significantly improves clinical outcomes in patients with CKD, apart from quality-of-life indices (Table 4). Because of difficulties in separating cause-and-effect in observational studies, several studies have attempted to determine if correction of anemia ameliorates cardiovascular and/or renal outcomes in patients with ESRD. Two early studies were performed in cardiac patients with the aim of normalizing Hb. The US Normalized Hematocrit trial in patients with overt cardiac failure *(61)* showed no overall benefit, a trend to reduced survival in the intervention group, and a high incidence of vascular access thrombosis. However, a posthoc subgroup analysis in each treatment group showed that higher Hb levels conferred a survival advantage. The Canadian Normal Hemoglobin study in patients with asymptomatic LVH found that erythropoietin treatment conferred no benefit in regressing LVH or in changing vascular access thrombosis *(50)*.

A controlled but not randomized study was performed in 153 hemodialysis patients who received anemia therapy with erythropoietin as well as antihypertensive medication *(62)*. After a follow-up of 54 ± 37 mo, the mean Hb rose from 86 to 105 g/L and mean blood pressure fell from 169/90 to 146/78. In patients who showed a decrease in LVMI, survival was improved so that a 10% decrease in LVMI was associated with a 22% decrease in all-cause mortality *(62)*.

A randomized controlled trial of normalization of Hb was performed in 416 Scandinavian patients with renal disease. Participants were recruited in three categories: predialysis, peritoneal dialysis, and hemodialysis *(63)*. Participants from each category were randomly allocated to normalization of Hb or to a subnormal Hb group. Approximately 20% of participants had diabetes, which was a much lower prevalence

Table 4
Interventional Studies of Anemia in Advanced Renal Disease

Study	*Participants*	*Design/duration*	*Study groups*	*Results*
Kuriyama (1997) *(73)*	Predialysis, serum creatinine 2–4 mg/dL Hct < 30% (n = 108)	Prospective randomized end point: Doubling serum creatinine over 36 mo	Gp1—untreated anemia (n = 31) vs Gp 2—anemia treated with EPO (n = 42) vs Gp 3—untreated, nonanemic control (n = 35)	– Rate of doubling of serum creatinine significantly greater in untreated group (Gp 1–84%, Gp 2–52%, Gp 3–60%) – Anemia *per se* is a factor in progression of ESRD – Better survival rate in Gp 2 attributed to nondiabetic patients
Besarab (1998) *(61)* "Normal Hct Cardiac Trial"	H-Dialysis (+ EPO) With CHF or IHD Mainly diabetic (n = 1233)	Randomized prospective 14 mo (median)	"Normal" Hct, 42 ± 3% vs 'Low' Hct, 30 ± 3%	– Study terminated prematurely – Trend for ↑ deaths + ↑ non fatal MI in normal Hct gp (RR 1.3, CI 0.9 - 1.9) – *Post hoc* within-group analysis – ↑ survival with ↑ Hb levels
London (2001) *(62)*	H-Dialysis (+ EPO) (n = 153)	Pre vs post comparison 54 ± 37 mo LV mass estimated by echocardiogram	EPO, Fe and BP therapy for all SBP 169 → 146 mmHg Hb 86 → 105 g/L	– Overall mortality 58/153 – Every 10% ↓ LVMI reduced mortality by 22%, CV mortality by 28% – ↓ in LVMI correlated with ↑ Hb – Uncertainty regarding relative roles of anemia and BP control
Collins (2001) *(57)* USRDS	Incident H-dialysis (n = 66,671)	Before/after EPO/Fe 12 mo		– CV mortality rate in ESRD 20-40 times higher than in general population – Risk of hospitalisation 16-22% lower if Hct 36–39% compared with Hct 33–36%, but mortality not different

Furuland (2003) *(63)*	Three groups of CKD patients – Predialysis three groups of CKD patients – PD – Haemodialysis ($n = 416$) approx 20% diabetic	Randomized, controlled 48–76 wk	EPO, Fe therapy for all "Normal" Hb 135–160 g/L vs "Subnormal" Hb 90–120 g/L	– Overall mortality 13.4% N-Hb, 13.5 S-Hb – Primary analysis: improved quality of life in normal-Hb group – No difference in survival or hospitalization among groups – Sub-analysis: within each randomized group, ↑ mortality with lower Hb
Silverberg (2003) *(64)*	Severe resistant CHF + early CKD Diabetic ($n = 84$) + nondiabetic ($n = 95$)	Open, uncontrolled 12 mo	EPO, Fe therapy Diabetic Hb 104→ 131 g/L Nondiabetic Hb 105→ 129 g/L	Exercise capacity ↑ (NYHA class ↑ by 34.8% in diabetic ↑ by 32.4% in nondiabetic) ↓ diuretic use ↓ hospitalization Serum creatinine stable during intervention
Roger (2004) *(74)*	CKD (cc 15–20 mL/min) predialysis ($n = 155$)	Prospective, randomized end point change in LVMI 2 yr	Targets A = Hb 120–130 g/L vs B = Hb 90–100 g/L	– Achieved Hb A = 121 ± 14 g/L B = 108 ± 13 g/L – No difference in changes in LVMI or GFR between A and B
Gouva (2004) *(75)*	Nondiabetic predialysis serum creatinine 2–6 mg/dL Hb 90–116 g/L ($n = 88$)	Prospective, randomized primary end point: Doubling of serum creatinine, renal replacement or death (22.5 mo)	Early EPO ($n = 45$) vs late EPO ($n = 43$)	– Primary end point: Early EPO 13/45 ($p = 0.0078$) Late EPO 23/43 – Adjusted for baseline serum creatinine, relative hazard for primary end point early vs late = 0.37 ($p = 0.004$)
Strippoli (2004) *(65)*	19 trials Meta-analysis by random effects model	Randomized trial evidence from Cochrane registry, Medline embase	12 trials EPO vs placebo ($n = 638$) seven trials Higher vs lower Hb targets ($n = 2058$)	– Compared with Hb > 130 g/L, Hb < 120 g/L associated with lower all cause mortality (RR 0.84, CI 0.71–1.00) – Hb ≤ 100 g/L reduced hypertension by half but increased seizures fivefold

In CKD with CV disease, benefits associated with higher Hb (↓ seizures) are outweighed by ↑ risk of hypertension and death.

than in studies performed in the United States. In each of the three study groups, normalization of Hb was associated with improved quality of life after a follow-up of 48–76 wk but there were no differences in thrombosis, blood pressure, hospitalization, or survival. A *post hoc* analysis within the normalized and subnormal groups showed that the lower the Hb the lower the survival rate *(63)*.

Another study assessed the effects of correction of anemia with erythropoietin and intravenous iron in a mixed group of diabetic and nondiabetic patients with CHF and CKD *(64)*. As there was no concurrent control group, the study was based on before and after comparisons. The baseline serum creatinine was approx 200 μ*M*, corresponding to a GFR of between 10 and 20 mL/min/1.73 m^2. After 12 mo of therapy, Hb increased from 102 to 120 g/L. In both diabetic and nondiabetic patients left ventricular ejection fraction, hospital admission rates, and heart functional class all improved. In addition, self reported symptoms of heart failure and fatigue score improved in both groups. GFR was estimated by the Cockcroft-Gault formula (eGFR) in the 12 mo before intervention and during 12 mo of intervention. Serum creatinine levels remained unchanged from 0 to 12 mo, suggesting an apparent arrest of decline in eGFR *(64)*. However, the authors did not exclude the possibility that erythropoietin induced weight gain rather than a change in GFR was responsible for the apparent stabilization of eGFR.

In treating patients with anemia, any potential benefits need to be carefully balanced against the significant financial cost involved. Although exogenous erythropoietin or erythropoietin analogues are generally well tolerated, there is a potential for significant adverse events. The impact of hypertension, increased blood viscosity and peripheral resistance associated with increase Hb levels may offset any benefit arising from correction of anemia. Increasing the Hb level to the high normal range may also be associated with increased mortality. Correcting anemia via other means such as transfusion also carries associated risks including HLA sensitisation, which may render a patient ineligible for transplantation. Repletion of iron may also act to promote intracellular oxidative stress and impair insulin sensitivity.

In summary, the balance of risk and potential benefits form correcting anemia in patients with diabetes remains to be established. A recent meta-analysis of randomized controlled trials has addressed cardiovascular outcomes in intervention studies for the anemia of CKD *(65)*. This has shown that in CKD patients with existing cardiovascular disease, the benefits associated with higher Hb targets (reduced seizures) are outweighed by the harms (increased risk of hypertension and death). However, there are insufficient data to guide decisions in patients without existing cardiovascular disease or in patients with early diabetic renal disease.

At present, at least four ongoing trials (CREATE *[66]*, TREAT, CHOIR *[67]* and ACORD *[68]*) are addressing the potential vascular and renal impact of different Hb targets for erythropoietin therapy. Results of these studies should provide a definitive guide to the role of intervention for anemia in patients with overt diabetic nephropathy. However, additional diabetes-specific trials will be necessary to address the effects of correction of anemia on the progression of early renal and extrarenal diabetic microvascular disease.

CONCLUSION

Anemia is a common complication of diabetic renal disease, seen with a two to three times greater prevalence and earlier onset than in patients with renal impairment from other causes. At least one in five patients with type 1 or 2 diabetes in tertiary referral clinics have anemia, in which it constitutes a significant additional burden. In these

patients, erythropoietin levels are normal but inappropriately low in the context of the subnormal Hb level (functional erythropoietin deficiency). This may be owing to the predominance of damage to cells and vascular architecture of the renal tubulo-interstitium associated with DN. These changes may be apparent, like albuminuria, before demonstrable changes in GFR. In addition, systemic inflammation, autonomic neuropathy, and reduced red cell survival may compound the effects of anemia in diabetes. In contrast to the inappropriately low erythropoietin levels in the majority of diabetic patients with anemia, a small subgroup of patients with anemia and normal renal function may have resistance to erythropoietin.

Although anemia may be considered a marker of diabetic kidney disease, reduced Hb levels, even within the normal range, identify diabetic patients with an increased risk of hospitalization and mortality. As with macrovascular disease, several observational studies indicate a worse outcome for diabetic retinopathy and neuropathy. Anemia may also be significant in determining the outcome of heart failure and hypoxia-induced organ damage in patients with diabetes as part of the cardiorenal anemia syndrome. Upcoming studies will determine whether correction of anemia will lead to improved outcomes in patients with diabetes.

REFERENCES

1. Inomata S, Itoh M, Imai H, Sato T. Serum levels of erythropoietin as a novel marker reflecting the severity of diabetic nephropathy. Nephron 1997;75:426–430.
2. Bosman DR, Winkler AS, Marsden JT, Macdougall IC, Watkins PJ. Anemia with erythropoietin deficiency occurs early in diabetic nephropathy. Diabetes Care 2001;24:495–499.
3. Silverberg D. Outcomes of anemia management in renal insufficiency and cardiac disease. Nephrol Dial Transplant 2003;18(2):ii7–ii12.
4. Koury ST, Koury MJ, Bondurant MC, Caro J, Graber SE. Quantitation of erythropoietin-producing cells in kidneys of mice by in situ hybridization: correlation with hematocrit, renal erythropoietin mRNA, and serum erythropoietin concentration. Blood 1989;74:645–651.
5. Lacombe C, Da Silva JL, Bruneval P, et al. Peritubular cells are the site of erythropoietin synthesis in the murine hypoxic kidney. J Clin Invest 1988;81:620–623.
6. Maxwell PH, Osmond MK, Pugh CW, et al. Identification of the renal erythropoietin-producing cells using transgenic mice. Kidney Int 1993;44:1149–1162.
7. Maxwell PH, Pugh CW, Ratcliffe PJ. Inducible operation of the erythropoietin 3′ enhancer in multiple cell lines: evidence for a widespread oxygen-sensing mechanism. Proc Natl Acad Sci USA 1993;90:2423–2427.
8. Wang GL, Semenza GL. General involvement of hypoxia-inducible factor 1 in transcriptional response to hypoxia. Proc Natl Acad Sci USA 1993;90:4304–4308.
9. Jiang BH, Rue E, Wang GL, Roe R, Semenza GL. Dimerization, DNA binding, and transactivation properties of hypoxia-inducible factor 1. J Biol Chem 1996;271:17,771–17,778.
10. Semenza GL, Agani F, Booth G, et al. Structural and functional analysis of hypoxia-inducible factor 1. Kidney Int 1997;51:553–555.
11. Eckardt KU. Biology of erythropoietin production. Nephrol Dial Transplant 1995;10:1572–1574.
12. Thomas MC, Cooper ME, Tsalamandris C, MacIsaac R, Jerums G. Anemia with impaired erythropoietin response in diabetic patients. Arch Intern Med 2005;165:466–469.
13. Thomas M, Burns W, Cooper M. Tubular changes in early diabetic nephropathy. Adv Chronic Kidney Dis 2005;12:177–186.
14. Fine LG, Bandyopadhay D, Norman JT. Is there a common mechanism for the progression of different types of renal diseases other than proteinuria? Towards the unifying theme of chronic hypoxia. Kidney Int Suppl 2000;75:S22–S226.
15. Oldfield MD, Bach LA, Forbes JM, et al. Advanced glycation end products cause epithelial-myofibroblast transdifferentiation via the receptor for advanced glycation end products (RAGE). J Clin Invest 2001;108:1853–1863.
16. Yoshida H, Kuriyama S, Atsumi Y, et al. Angiotensin I converting enzyme gene polymorphism in non-insulin dependent diabetes mellitus. Kidney Int 1996;50:657–664.

17. Bosman DR, Osborne CA, Marsden JT, Macdougall IC, Gardner WN, Watkins PJ. Erythropoietin response to hypoxia in patients with diabetic autonomic neuropathy and non-diabetic chronic renal failure. Diabet Med 2002;19:65–69.
18. Baba T, Nakajima S, Yajima Y. Beta3-adrenergic receptor gene polymorphism is not associated with hypertension in NIDDM patients without nephropathy. Horm Metab Res 1998;30:629–632.
19. Jurkovitz CT, Abramson JL, Vaccarino LV, Weintraub WS, McClellan WM. Association of high serum creatinine and anemia increases the risk of coronary events: results from the prospective community-based atherosclerosis risk in communities (ARIC) study. J Am Soc Nephrol 2003;14:2919–2925.
20. Thomas MC, Tsalamandris C, MacIsaac R, Jerums G. Anemia in diabetes: An emerging complication of microvascuar disease. Current Diabetes Reviews 2005;1:107–126.
21. Craig KJ, Williams JD, Riley SG, et al. Anemia and diabetes in the absence of nephropathy. Diabetes Care 2005;28:1118–1123.
22. Deicher R, Horl WH. Anemia as a risk factor for the progression of chronic kidney disease. Curr Opin Nephrol Hypertens 2003;12:139–143.
23. Keane WF, Lyle PA. Recent advances in management of type 2 diabetes and nephropathy: lessons from the RENAAL study. Am J Kidney Dis 2003;41:S22–S25.
24. Levin A, Djurdjev O, Barrett B, et al. Cardiovascular disease in patients with chronic kidney disease: getting to the heart of the matter. Am J Kidney Dis 2001;38:1398–1407.
25. Guest CS, Ratnaike S, Larkins RG. Albuminuria in aborigines and Europids of south-eastern Australia. Med J Aust 1993;159:335–338.
26. Sinclair SH. Diabetic macular edema: New concepts of pathology and treatment. Semin Ophthalmol 1999;14:197–199.
27. Aiello LP, Pierce EA, Foley ED, et al. Suppression of retinal neovascularization in vivo by inhibition of vascular endothelial growth factor (VEGF) using soluble VEGF-receptor chimeric proteins. Proc Natl Acad Sci USA 1995;92:10,457–10,461.
28. Friedman EA, L'Esperance FA, Brown CD, Berman DH. Treating azotemia-induced anemia with erythropoietin improves diabetic eye disease. Kidney Int Suppl 2003:S57–S63.
29. Berman DH, Friedman EA. Partial absorption of hard exudates in patients with diabetic end-stage renal disease and severe anemia after treatment with erythropoietin. Retina 1994;14:1–5.
30. Friedman EA, Brown CD, Berman DH. Erythropoietin in diabetic macular edema and renal insufficiency. Am J Kidney Dis 1995;26:202–208.
31. Bianchi R, Buyukakilli B, Brines M, et al. Erythropoietin both protects from and reverses experimental diabetic neuropathy. Proc Natl Acad Sci USA 2004;101:823–828.
32. Hassan K, Simri W, Rubenchik I, et al. Effect of erythropoietin therapy on polyneuropathy in predialytic patients. J Nephrol 2003;16:121–125.
33. Ribatti D, Presta M, Vacca A, et al. Human erythropoietin induces a pro-angiogenic phenotype in cultured endothelial cells and stimulates neovascularization in vivo. Blood 1999;93:2627–2636.
34. Carlini RG, Alonzo EJ, Dominguez J, et al. Effect of recombinant human erythropoietin on endothelial cell apoptosis. Kidney Int 1999;55:546–553.
35. Akimoto T, Kusano E, Inaba T, et al. Erythropoietin regulates vascular smooth muscle cell apoptosis by a phosphatidylinositol 3 kinase-dependent pathway. Kidney Int 2000;58:269–282.
36. Cotroneo P, Maria Ricerca B, Todaro L, et al. Blunted erythropoietin response to anemia in patients with Type 1 diabetes. Diabetes Metab Res Rev 2000;16:172–176.
37. Hsu CY, McCulloch CE, Curhan GC. Epidemiology of anemia associated with chronic renal insufficiency among adults in the United States: results from the Third National Health and Nutrition Examination Survey. J Am Soc Nephrol 2002;13:504–510.
38. Astor BC, Muntner P, Levin A, Eustace JA, Coresh J. Association of kidney function with anemia: the Third National Health and Nutrition Examination Survey (1988–1994). Arch Intern Med 2002;162:1401–1408.
39. El-Achkar TM, Ohmit SE, McCullough PA, et al. Higher prevalence of anemia with diabetes mellitus in moderate kidney insufficiency: The Kidney Early Evaluation Program. Kidney Int 2005;67:1483–1488.
40. Valderrabano F. Anemia management in chronic kidney disease patients: an overview of current clinical practice. Nephrol Dial Transplant 2002;17(1):13–18.
41. Thomas MC, MacIsaac RJ, Tsalamandris C, Power D, Jerums G. Unrecognized anemia in patients with diabetes: a cross-sectional survey. Diabetes Care 2003;26:1164–1169.
42. Thomas MC, MacIsaac RJ, Tsalamandris C, Jerums G. Elevated iron indices in patients with diabetes. Diabet Med 2004;21:798–802.
43. Fernandez-Real JM, Lopez-Bermejo A, Ricart W. Cross-talk between iron metabolism and diabetes. Diabetes 2002;51:2348–2354.

44. Thomas MC, MacIsaac RJ, Tsalamandris C, et al. The burden of anemia in type 2 diabetes and the role of nephropathy: a cross-sectional audit. Nephrol Dial Transplant 2004;19:1792–1797.
45. Thomas MC, MacIsaac RJ, Tsalamandris C, et al. Anemia in patients with type 1 diabetes. J Clin Endocrinol Metab 2004;89:4359–4363.
46. Thomas MC, Tsalamandris C, MacIsaac R, et al. Low-molecular-weight AGEs are associated with GFR and anemia in patients with type 2 diabetes. Kidney Int 2004;66:1167–1172.
47. Poirier P, Bogaty P, Garneau C, Marois L, Dumesnil JG. Diastolic dysfunction in normotensive men with well-controlled type 2 diabetes: importance of maneuvers in echocardiographic screening for preclinical diabetic cardiomyopathy. Diabetes Care 2001;24:5–10.
48. Sarnak MJ, Levey AS, Schoolwerth AC, et al. Kidney disease as a risk factor for development of cardiovascular disease: a statement from the American Heart Association Councils on Kidney in Cardiovascular Disease, High Blood Pressure Research, Clinical Cardiology, and Epidemiology and Prevention. Circulation 2003;108:2154–2169.
49. Weiskopf RB, Viele MK, Feiner J, et al. Human cardiovascular and metabolic response to acute, severe isovolemic anemia. JAMA 1998;279:217–221.
50. Foley RN, Parfrey PS, Harnett JD, Kent GM, Murray DC, Barre PE. The impact of anemia on cardiomyopathy, morbidity, and and mortality in end-stage renal disease. Am J Kidney Dis 1996;28: 53–61.
51. Mann JF. What are the short-term and long-term consequences of anemia in CRF patients? Nephrol Dial Transplant 1999;14(2):29–36.
52. Horwich TB, Fonarow GC, Hamilton MA, MacLellan WR, Borenstein J. Anemia is associated with worse symptoms, greater impairment in functional capacity and a significant increase in mortality in patients with advanced heart failure. J Am Coll Cardiol 2002;39:1780–1786.
53. Ezekowitz JA, McAlister FA, Armstrong PW. Anemia is common in heart failure and is associated with poor outcomes: insights from a cohort of 12,065 patients with new-onset heart failure. Circulation 2003;107:223–225.
54. Levin A, Thompson CR, Ethier J, et al. Left ventricular mass index increase in early renal disease: impact of decline in hemoglobin. Am J Kidney Dis 1999;34:125–134.
55. Kosiborod M, Smith GL, Radford MJ, Foody JM, Krumholz HM. The prognostic importance of anemia in patients with heart failure. Am J Med 2003;114:112–119.
56. Madore F, Lowrie EG, Brugnara C, et al. Anemia in hemodialysis patients: variables affecting this outcome predictor. J Am Soc Nephrol 1997;8:1921–1929.
57. Collins AJ, Li S, St Peter W, et al. Death, hospitalization, and economic associations among incident hemodialysis patients with hematocrit values of 36 to 39%. J Am Soc Nephrol 2001;12:2465–2473.
58. Abramson JL, Jurkovitz CT, Vaccarino V, Weintraub WS, McClellan W. Chronic kidney disease, anemia, and incident stroke in a middle-aged, community-based population: the ARIC Study. Kidney Int 2003;64:610–615.
59. Ofsthun N, Labrecque J, Lacson E, Keen M, Lazarus JM. The effects of higher hemoglobin levels on mortality and hospitalization in hemodialysis patients. Kidney Int 2003;63:1908–1914.
60. Silverberg DS, Wexler D, Blum M, Iaina A. The cardio renal anemia syndrome: correcting anemia in patients with resistant congestive heart failure can improve both cardiac and renal function and reduce hospitalizations. Clin Nephrol 2003;60(1):S93–S102.
61. Besarab A, Bolton WK, Browne JK, et al. The effects of normal as compared with low hematocrit values in patients with cardiac disease who are receiving hemodialysis and epoetin. N Engl J Med 1998;339:584–590.
62. London GM, Pannier B, Guerin AP, et al. Alterations of left ventricular hypertrophy in and survival of patients receiving hemodialysis: follow-up of an interventional study. J Am Soc Nephrol 2001;12:2759–2767.
63. Furuland H, Linde T, Ahlmen J, Christensson A, Strombom U, Danielson BG. A randomized controlled trial of haemoglobin normalization with epoetin alfa in pre-dialysis and dialysis patients. Nephrol Dial Transplant 2003;18:353–361.
64. Silverberg DS, Wexler D, Blum M, et al. The effect of correction of anemia in diabetics and nondiabetics with severe resistant congestive heart failure and chronic renal failure by subcutaneous erythropoietin and intravenous iron. Nephrol Dial Transplant 2003;18:141–146.
65. Strippoli GF, Craig JC, Manno C, Schena FP. Hemoglobin targets for the anemia of chronic kidney disease: a meta-analysis of randomized, controlled trials. J Am Soc Nephrol 2004;15:3154–3165.
66. Macdougall IC. CREATE: new strategies for early anemia management in renal insufficiency. Nephrol Dial Transplant 2003;18(2):ii13–ii16.

67. Reddan D, Tran L-L, Jollis L, Singh A. Anemia correction and left ventricular hypertrophy: an echocardiographic substudy of the Correction of Hemoglobin and Outcomes in Renal Insufficiency (CHOIR) study. J Am Soc Nephrol 2002;13:676A.
68. Laville M. New strategies in anemia management: ACORD (Anemia Correction in Diabetes) trial. Acta Diabetol 2004;41(1):S18–S22.
69. Winkler AS, Marsden J, Chaudhuri KR, Hambley H, Watkins PJ. Erythropoietin depletion and anemia in diabetes mellitus. Diabet Med 1999;16:813–819.
70. Al-Ahmad A, Rand WM, Manjunath G, et al. Reduced kidney function and anemia as risk factors for mortality in patients with left ventricular dysfunction. J Am Coll Cardiol 2001;38:955–962.
71. McClellan WM, Flanders WD, Langston RD, Jurkovitz C, Presley R. Anemia and renal insufficiency are independent risk factors for death among patients with congestive heart failure admitted to community hospitals: a population-based study. J Am Soc Nephrol 2002;13:1928–1936.
72. Collins AJ. The hemoglobin link to adverse outcomes. Adv Studies in Med 2003;3:S14–S17.
73. Kuriyama S, Tomonari H, Yoshida H, Hashimoto T, Kawaguchi Y, Sakai O. Reversal of anemia by erythropoietin therapy retards the progression of chronic renal failure, especially in nondiabetic patients. Nephron 1997;77:176–185.
74. Roger SD, McMahon LP, Clarkson A, et al. Effects of early and late intervention with epoetin alpha on left ventricular mass among patients with chronic kidney disease (stage 3 or 4): results of a randomized clinical trial. J Am Soc Nephrol 2004;15:148–156.
75. Gouva C, Nikolopoulos P, Ioannidis JP, Siamopoulos KC. Treating anemia early in renal failure patients slows the decline of renal function: a randomized controlled trial. Kidney Int 2004;66:753–760.
76. Androne AS, Katz SD, Lund L, et al. Hemodilution is common in patients with advanced heart failure. Circulation 2003;107:226–229.