Int Urol Nephrol (2015) 47:1365–1372
DOI 10.1007/s11255-015-1012-2

NEPHROLOGY - REVIEW

Less known pathophysiological mechanisms of anemia in patients with diabetic nephropathy

M. Pappa[1] · E. Dounousi[2] · A. Duni[2] · K. Katopodis[1]

Received: 9 January 2015 / Accepted: 12 May 2015 / Published online: 28 May 2015

Abstract Diabetes mellitus (DM) is currently considered a modern global epidemic, and diabetic nephropathy (DN) is the most common cause of chronic kidney disease (CKD). Anemia is one of the most significant complications of CKD, and it is mainly attributed to insufficient erythropoietin (EPO) production. However, anemia develops earlier in the course of CKD among patients with DM, and the severity of anemia tends to be more marked in these patients compared to nondiabetic subjects, regardless of the stage of CKD. In this review, we focus on the "less known" complex interacting mechanisms which are involved in the pathophysiology of anemia associated with DN. Although the major cause of anemia in DN is considered to be an inappropriate response of the plasma EPO concentration to anemia, several other possible mechanisms have been suggested. Glomerular hyperfiltration, proteinuria, renal tubular dysfunction and interstitial fibrosis are among the main culprits. On the other hand, systemic effects such as chronic inflammation, autonomic neuropathy and the renin–angiotensin system are also involved. Finally, several medications are considered to aggravate anemia associated with DN. Since anemia is an important predictor of quality of life and is implicated in the increased burden of cardiovascular morbidity and mortality, further research is required to elucidate its pathogenesis in diabetic patients.

✉ E. Dounousi
evangeldou@gmail.com

[1] Department of Nephrology, General Hospital of Arta, Arta, Greece

[2] Department of Nephrology, University Hospital of Ioannina, Stavrou Niarchou Avenue, PC 45 500 Ioannina, Greece

Keywords Diabetic nephropathy · Anemia · Erythropoietin · Glomerular hyperfiltration · Proteinuria · Tubular dysfunction · Autonomic neuropathy

Introduction

Diabetes mellitus (DM) is currently considered a modern global epidemic with a continuously increasing burden. Registry data show that the incidence of type 2 diabetes mellitus (T2DM) has more than doubled in the last 30 years, and time projections of diabetes burden over the next decades depict further rising trends [1, 2].

Diabetic nephropathy (DN) comprises one of the major microvascular complications of DM [3] and is characterized by albuminuria (>300 mg/24 h), a progressive decline of the glomerular filtration rate (GFR) by 2–20 mL/min per year, arterial hypertension and increased cardiovascular morbidity and mortality [4–6]. The overall prevalence of microalbuminuria and macroalbuminuria is nearly 30–35 % in both types of DM. Diabetic nephropathy in patients with type 1 DM (T1DM) rarely develops earlier than 10 years after diagnosis, whereas approximately 3 % of patients with newly diagnosed type 2 DM (T2DM) already have overt nephropathy [7–9]. Diabetic nephropathy is the most common cause of CKD [10–12], and diabetic patients in some countries account for 40–50 % of patients receiving dialysis [13].

Anemia is one of the most significant consequences of CKD, and it is mainly attributed to insufficient erythropoietin (EPO) production. The control of red blood cell (RBC) mass is based on a classic negative feedback loop mediated by changes in the production of the hormone EPO, which is mainly produced in the kidney and regulates the production of erythrocytes by interaction with

specific EPO receptors (EPOR) on bone marrow erythroid progenitors. Anemia can manifest itself early in the course of CKD, and its prevalence and severity go in parallel with the progression of kidney disease. The National Kidney Foundation's clinical practice guidelines define anemia as a hemoglobin (Hb) concentration lower than 13.5 g/dL for adult men and less than 12.0 g/dL for adult women [14]. However, anemia develops earlier in the course of CKD among patients with DM [15–17] and the severity of anemia tends to be more marked in these patients compared to nondiabetic subjects, regardless of the stage of CKD, while this difference is even most prominent among CKD stage 3 patients [18–21]. Several studies have demonstrated the importance of early detection of anemia in patients with T1DM and T2DM [22–24].

In this review, we focus on the "less known" complex interacting mechanisms which are involved in the pathophysiology of anemia associated with DN.

The role of the kidneys in hematopoiesis

The kidneys play a key role in hematopoiesis since the physiologic regulator of RBC production, the glycoprotein hormone EPO, is produced and released within the kidney, in response to impaired oxygen delivery from the circulating erythrocytes. EPO is a member of the family of class 1 cytokines and is produced by peritubular interstitial cells, which were subsequently identified as peritubular fibroblasts located within the renal cortex [25, 26]. Subsequently, EPO is excreted in the peritubular capillaries and enters the systemic circulation through the renal vein [26]. Although a small quantity of EPO is produced in the liver, the spleen, the brain, the lungs and the testes, its production by these organs cannot substitute for the renal production and cannot meet the increased needs in patients with CKD [27]. The normal plasma EPO concentration ranges from 6 to 32 IU/L [27].

Erythropoietin exerts its action by binding to the EPOR which is present on erythroid progenitors in the bone marrow, from the colony-forming units erythroid stage (CFU-E) to late basophilic erythroblasts. Without EPO present, these cells are rapidly lost via programmed cell death [28, 29]. Thus, EPO is an essential factor determining survival for erythroid progenitors, beginning at the CFU-E stage all the way to basophilic erythroblasts. The production and release of EPO are regulated by a feedback mechanism, which is related to hypoxia, as the fundamental stimulus for EPO production is the availability of O_2 for tissue metabolic needs [30]. Renal cells responsible for the production of EPO possess specific hypoxia-sensing regulatory mechanisms, which are mostly based on transcription factors induced by hypoxia [31, 32] called hypoxia-inducible factors (HIFs).

Pathogenesis of anemia in diabetic nephropathy

The ability of the kidneys to produce EPO is not impaired in renal disease, and the absolute value of EPO can be normal or even high; however, EPO levels will be inappropriately low relative to the degree of anemia, resulting in a functional EPO deficiency. An interplay of pathophysiological mechanisms associated with EPO production and action together with factors unrelated to EPO determines the pathogenesis of anemia in diabetic nephropathy (Fig. 1) [33–35].

Erythropoietin-dependent mechanisms

Glomerular hyperfiltration

The preclinical normoalbuminuric phase of DN is characterized by kidney hypertrophy together with increased renal blood flow and a simultaneous increase in the GFR which augments by 20–40 % above normal levels [36]. The hemodynamic abnormalities in DN have been attributed to hyperglycemia as well as other contributory factors including insulin-like growth factor I, atrial natriuretic peptide, sex hormones, intracellular sorbitol, early glycation products, which cause dilation of the afferent arteriole, constriction of the efferent arteriole and suppression of the tubuloglomerular feedback, thus resulting in increased glomerular pressure and hyperfiltration [3, 37, 38]. Hyperfiltration is observed in 25–75 % of patients with T1DM and 5–40 % of patients with T2DM [36, 39]. Enhanced renal blood flow results in increased oxygen supply in the renal tissue and subsequently removal of the hypoxia stimulus for the production of EPO. Thus, augmented oxygenation of the renal parenchyma, especially at the specific areas of EPO synthesis, results in premature reduction in the renal EPO production [30].

Proteinuria

Severe proteinuria causes loss of the endogenously produced EPO in patients with impaired function of the glomerular barrier, as occurs in DN, since its molecular weight is smaller than albumin. Indeed, patients with DN who present with severe proteinuria appear to have a significant reduction in EPO concentration in the plasma due to increased EPO urinary losses [30, 39–42]. It should be noted that anemia in the setting of proteinuria is not related to the loss of renal function [24]. Finally, both the fractional and the total urinary excretion of EPO are not elevated in patients without significant proteinuria (nonnephrotic type)

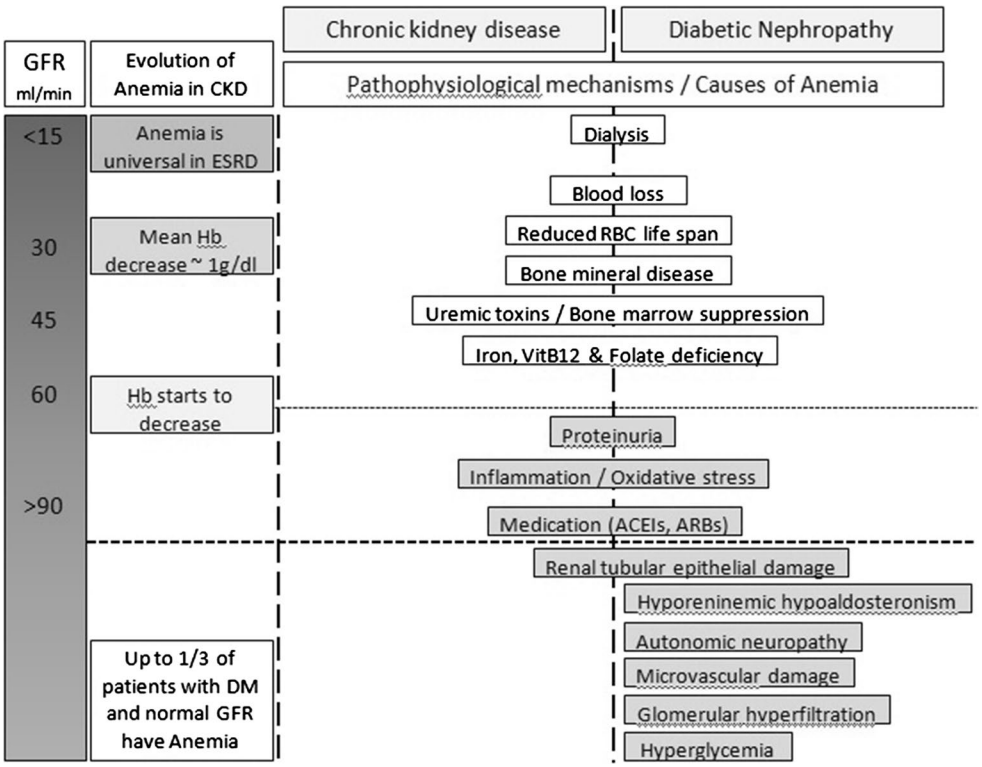

Fig. 1 Suggested pathophysiological mechanisms and causes of anemia in CKD and DN. A number of mechanisms (*white-colored boxes*) are common among all CKD patients independently of the primary renal disease, and they usually appear with the progression of CKD, while other "less known" mechanisms (*gray-colored boxes*) are mainly EPO dependent and initiated at the early, preclinical stages of DN

[43], while hypoalbuminemia has been shown to be significantly associated with severe anemia in patients with DN [44].

Chronic inflammation

In DM, hyperglycemia, arterial hypertension and dyslipidemia stimulate the proinflammatory activity of the endothelium via various mechanisms which involve increased oxidative stress, dysregulation of the nitric oxide synthase (NOS), production of advanced glycosylated end products (AGEs) and the activation of converting factor NF-kappaB [3, 45]. The activated endothelial cells express proinflammatory cytokines such as interleukin-1 (IL-1), tumor necrosis factor α (TNFα) and adhesion molecules such as ICAM-1 and VCAM-1 [30, 46, 47]. It has been suggested that the inflammatory mediators suppress the hypoxia-induced production of EPO [48]; however, there is no solid evidence available to support this hypothesis [49]. A cross-sectional survey in T2DM patients did not reveal any correlation between C-reactive protein (CRP) and EPO levels [50], whereas another study managed to show an inverse significant correlation between CRP and hemoglobin concentration (Hb) in T2DM patients [51]. However, chronic systemic inflammation together with microvascular disease causes a reduction in the activity of EPO on its target cells [52, 53]. The resistance to EPO action involves inhibition of the maturation of erythrocyte precursors, reduction in EPOR expression, inhibition of EPO binding to its receptor by competitors and inhibition of activation of signaling pathways [54]. In addition, EPO can be nonenzymatic glycosylated, similar to apolipoproteins thus causing decreased levels of active circulating EPO [55]. Finally, the degree of hyperglycemia severity appears to modify the expression of EPOR through receptor glycosylation, whereas a significant inverse correlation between serum EPO and the fraction of glycosylated Hb has been found in diabetic patients [13].

Microvascular damage

The early microvascular damage, observed in patients with DM, which is independent of the progressive reduction in the GFR, also contributes to the anemia of DM [30]. Thickening of the glomerular basement membrane together with simultaneous thickening of the tubular basement membrane is reported to be among the first pathological alterations of nephron architecture in patients with DM even in absence of established albuminuria/proteinuria [56]. The changes of the tubular basement membrane disturb the equilibrium between the renal tubules, the peritubular fibroblasts and the endothelium, thus further disrupting the renal excretion of EPO [30]. Inomata et al. [35] reported that interstitial fibrosis also contributes to the anemia of DN. In a recent study, Mise et al. [57] investigated the impact of tubulointerstitial lesions on anemia in diabetic patients with

biopsy-proven DN and demonstrated that higher interstitial fibrosis and tubular atrophy scores were more strongly associated with reduced Hb values, whereas glomerular and vascular lesion scores did not seem to correlate significantly with the presence of anemia. However, it remains to be further clarified whether the process of tubular atrophy and interstitial fibrosis is more severe in DN than in other kidney diseases with underlying severe interstitial lesions.

Autonomic neuropathy

Autonomic neuropathy is one of the culprits considered to play a significant role in the pathogenesis of anemia in DN, although the mechanism has not been completely elucidated. Several studies have demonstrated a positive correlation between polyneuropathy and anemia in patients with DM [58]. These findings are further supported by the fact that patients suffering from multiple system atrophy or pure autonomic neuropathy have EPO deficiency as well [33, 59–61]. Additionally, experimental studies have failed to produce EPO in response to hypoxia after visceral denervation, as occurs in patients with DM [62]. Interestingly, diabetic patients who display signs of autonomic neuropathy such as orthostatic hypotension have lower EPO levels compared to diabetic patients without autonomic neuropathy despite equal Hb concentration levels [63]. On the other hand, an increase in the production of EPO is observed after renal transplantation in the setting of graft denervation, which may cause erythrocytosis [64]. Moreover, in a recent study investigating the response of EPO to anemia in T2DM patients without advanced renal failure, anemic patients had a longer duration of T2DM and a higher cardiovascular autonomic neuropathy score [65]. Although serum EPO level was weakly correlated with hemoglobin values, multiple linear regression analysis revealed that autonomic neuropathy score was independently related to Hb or EPO level, thus suggesting that autonomic neuropathy is associated with a blunted EPO response to anemia in this group of patients [65].

The renin–angiotensin–aldosterone system

The renin–angiotensin–aldosterone system (RAAS), apart from its key role in blood pressure control and cardiovascular homeostasis, is considered to contribute to erythropoiesis as well. Specifically, angiotensin II stimulates erythropoiesis via an increase in the production of EPO and by acting like a growth factor in the bone marrow, especially on erythrocyte precursors [66, 67]. Moreover, it has been suggested that a functional unit of the juxtaglomerular apparatus in which angiotensin II acts is modulated by hematocrit values, thus named the critmeter [68]. Angiotensin II causes increased oxygen demands in the area adjacent to the proximal tubule by stimulating several processes in the area such as sodium reabsorption or ammoniagenesis [68].

Hyporeninemic hypoaldosteronism is very common among patients with T1DM, and it is characterized by low EPO and Hb levels in these patients [69, 70]. Reduced plasma renin activity (PRA) is common among patients with DM and arterial hypertension, but not in diabetic patients who display normal blood pressure levels and have no other complications of DM [71]. In this group of patients, reduced PRA goes in parallel with the occurrence of the other complications of DM such as arterial hypertension, retinopathy and nephropathy [72]. The most likely mechanisms responsible for reduced PRA and consequently suppression of the RAAS system are hyalinosis of the efferent arteriole, diminished levels of circulating catecholamines, nonactive prorenin and expansion of the intravascular volume [69, 72]. Suppression of the RAAS system leads to inhibition of the beneficial role of angiotensin II in erythropoiesis and anemia occurring in patients with mild CKD [70].

The two most widely used groups of antihypertensive drugs in patients with DN, which act on the RAAS system, angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs), might also worsen anemia in these patients [73]. RAAS stimulation causes afferent arteriolar dilation and efferent arteriolar constriction in order to preserve steady levels of the GFR, thus causing reduced renal blood flow and oxygen supply in the peritubular capillaries as well as the renal medulla and subsequently increased EPO production [30]. Moreover, there is a direct effect of angiotensin II in the bone marrow [66]. Consequently, inhibition of the RAAS system reverses all the above mechanisms and results in anemia. Additionally, these medications increase the concentration of the tetrapeptide N-acetyl-seryl-aspartyllysyl-proline, which is a natural inhibitor of erythropoiesis [73]. However, Hayashi et al. [74] did not demonstrate any correlation between Hb levels and the use of ACEI, whereas according to a more recent report, use of ARBs has better correlation with the severity of anemia in diabetic patients with CKD compared to ACEI [75].

Role of renal tubular epithelial cells

The main functions of proximal renal tubule cells include reabsorption of filtered substances such as glucose, sodium or proteins, excretion of metabolic products as well as synthesis and release of growth factors in the peritubular capillaries, such as transforming growth factor-β (TGF-β) and connective tissue growth factor (CTGF) [76, 77]. During the early stages of DN, renal tubular epithelial cell function is suppressed because of both morphological changes

like cell hypertrophy and functional changes involving increased metabolic demands and oxygen consumption [76, 78]. As a result, in the setting of glucosuria and increased supply of glucose in the proximal tubule, there is enhanced reabsorption of sodium through the sodium–glucose cotransporter (SGLT1) [79]. The increased sodium reabsorption in the proximal tubule leads to diminished sodium delivery in the macula densa, stimulation of the RAAS system and augmentation of aldosterone-mediated sodium reabsorption, thus causing expansion of the intravascular volume and increased ultrafiltration [80]. The end result of this energy-consuming process is reduced oxygen supply to the renal parenchyma and tissue hypoxia which stimulate EPO production. Indeed, EPO production is positively correlated with sodium reabsorption in patients with DN [68], whereas inhibition of sodium reabsorption with acetazolamide results in decreased EPO concentrations in normal subjects [81]. However, considering the fact that anemia occurs early during the course of disease in patients with DN, reduced Hb levels and increased sodium reabsorption are probably opposite stimuli for the production of EPO in diabetic nephrons [30].

As already mentioned, in CKD, the EPO plasma concentration is within the normal range, despite low Hb levels [82]. In addition, reduced fractional reabsorption of sodium diminishes oxygen consumption in the epithelial renal tubular cells [83], thus resulting in increased tissue oxygenation of the renal parenchyma [84] and suppression of EPO production and excretion [85].

Finally, recent evidence regarding EPO production by the nephron suggests that EPO is produced by cortical nephrons mainly in the intercalated cells and not in the peritubular cells in normal conditions and by mainly peritubular cells in hypoxia, thus highlighting additional roles of the renal tubular cells in the pathogenesis of anemia [86]. Thus, ongoing research is expected to shed light on the role of renal tubular epithelium on the anemia of DN.

Erythropoietin-independent mechanisms

Renal blood cells disorders

Patients with DM demonstrate significant hyperglycemia-induced metabolic and functional disorders of the RBC such as cell membrane lipid abnormalities [87], suppression of RBC filterability [88] and deformability [89] and alteration of RBC adhesion properties [90].

Impaired activity of the Na+/K+-ATPase pump which leads to RBC swelling [88] together with protein oxidation and accumulation of AGEs on the RBC cell membrane are the main mechanisms responsible for erythrocyte dysfunction [91, 92], thus leading to shortened RBC lifespan [93, 94]. In addition, hyperglycemia promotes the expression of aminophospholipids such as phosphatidylserine on the surface of RBC, which results in their recognition and trapping by the reticuloendothelial system [92].

Medications

There is strong evidence that some of the medications frequently prescribed to diabetic patients may worsen anemia, which is referred to as iatrogenic anemia. Apart from the antihypertensives ACEIs and ARBs mentioned earlier, other culprits are thiazolidinediones and fibrates.

The hypoglycemic agents, thiazolidinediones, decrease glucose plasma levels, improve the action of insulin in the peripheral tissues and augment pancreatic insulin secretion [95]. Fluid retention and weight gain are among the most common side effects of these agents, thus resulting in increased plasma volume and peripheral edema [95]. The main mechanisms responsible for the edema formation are increased sodium reabsorption by the distal nephron, vascular dilation due to improved insulin sensitivity, increased activity of the autonomic nervous system and increased endothelial permeability [96]. Increased plasma volume causes hemodilution, thus reducing plasma Hb values [30]. In addition, large doses of thiazolidinediones may directly suppress the bone marrow [97]. Results from a recent study showed that the occurrence of anemia after use of thiazolidinediones is not only dose dependent, but it also worsens with simultaneous administration of ACEI or ARBs [98].

Accumulating experimental evidence investigates the possible therapeutic potential of fibrates in DN [99]. One the other hand, earlier studies have indicated that fibrates are related to anemia in patients with DN [100]. Their mode of action involves certain nuclear receptors (perixosome proliferator-activated receptors); however, their exact mechanism of action has not been completely elucidated [30, 101]. Fibrates reduce the oxygen affinity of Hb and cause oxygen release in the tissues, thus removing the stimulating role of tissue hypoxia for EPO synthesis [102–104].

Conclusions

Diabetic nephropathy is the most common cause of CKD. Anemia in patients with DN occurs earlier and is more severe than anemia in other CKD patients. On the other hand, anemia in CKD is an important predictor of quality of life and contributes to increased cardiovascular morbidity and mortality in this group of patients. Further research is required to elucidate the pathogenesis of anemia in DN as existing evidence indicates that several, diabetes-related complex and interacting mechanisms are implicated.

Conflict of interest The authors have no conflict of interest to declare.

References

1. Harcourt BE, Penfold SA, Forbes JM (2013) Coming full circle in diabetes mellitus: from complications to initiation. Nat Rev Endocrinol 9:113–123
2. Shaw JE, Sicree RA, Zimmer PZ (2010) Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract 87:4–14
3. Forbes JM, Cooper ME (2013) Mechanisms of diabetic complications. Physiol Rev 93:137–188
4. Molitch ME, DeFronzo RA, Franz MJ, Keane WF, Mogensen CE, Parving HH et al (2004) Nephropathy in diabetes. Diabetes Care 27:S79–S83
5. Mou S, Wang Q, Liu J, Che X, Zhang M, Cao L et al (2010) Prevalence of nondiabetic renal disease in patients with type 2 diabetes. Diabetes Res Clin Pract 87:354–359
6. Parving HH, Smidt UM, Friisberg B, Bonnevie-Nielsen V, Andersen AR (1981) A prospective study of glomerular filtration rate and arterial blood pressure in insulin dependent diabetics with diabetic nephropathy. Diabetologia 20:457–461
7. Gall M-A, Rossing P, Skøtt P, Damsbo P, Vaag A, Bech K et al (1991) Prevalence of micro- and macroalbuminuria, arterial hypertension, retinopathy and large vessel disease in European type 2 (non–insulin-dependent) diabetic patients. Diabetologia 34:655–661
8. Parving HH, Lewis JB, Ravid M, Remuzzi G, Hunsicker LG, DEMAND investigators (2006) Prevalence and risk factors for microalbuminuria in a referred cohort of type II diabetic patients: a global perspective. Kidney Int 69(11):2057–2063
9. Rossing P, Hougaard P, Parving HH (2005) Progression of microalbuminuria in 15 type 1 diabetes: ten-year prospective observational study. Kidney Int 68(4):1446–1450
10. Foley RN, Collins AJ (2007) End-stage renal disease in the United States: an update from the United States Renal Data System. J Am Soc Nephrol 18:2644–2648
11. Stengel B, Billon S, Van Dijk PC, Jager KJ, Dekker FW, Simpson K et al (2003) Trends in the incidence of renal replacement therapy for end-stage renal disease in Europe, 1990–1999. Nephrol Dial Transplant 18:1824–1833
12. Villar E, Chang SH, McDonald SP (2007) Incidences, treatments, outcomes, and sex effect on survival in patients with end-stage renal disease by diabetes status in Australia and New Zealand (1991–2005). Diabetes Care 30:3070–3076
13. Dikow R, Schwenger V, Schömig M, Ritz E (2002) How should we manage anaemia in patients with diabetes? Nephrol Dial Transplant 17(Suppl 1):62–72
14. (2007) KDOQI Clinical Practice Guideline and Clinical Practice Recommendations for anemia in chronic kidney disease: 2007 update of hemoglobin target. Am J Kidney Dis 50(3):471 530
15. Goldhaber A, Ness-Abramof R, Ellis MH (2009) Prevalence of anemia among unselected adults with diabetes mellitus and normal serum creatinine levels. Endocr Pract 15(7):714–719
16. Grossman C, Dovrish Z, Koren-Morag N, Bornstein G, Leibowitz A (2014) Diabetes mellitus with normal renal function is associated with anaemia. Diabetes Metab Res Rev 30(4):291–296
17. Bulum T, Prkacin I, Blaslov K, Zibar K, Duvnjak L (2013) Association between red blood cell count and renal function exist in type 1 diabetic patients in the absence of nephropathy. Coll Antropol 37(3):777–782
18. Ritz E, Haxsen V (2005) Diabetic nephropathy and anaemia. Eur J Clin Invest 35(Suppl 3):66–74
19. Astor BC, Muntner P, Levin A, Eustace JA, Coresh J (2002) Association of kidney function with anemia: the third National Health and Nutritional Examination Survey (1988–1994). Arch Intern Med 162:1401–1408
20. El-Achkar TM, Ohmit SE, McCullough PA, Crook ED, Brown WW, Grimm R et al (2005) Higher prevalence of anemia with diabetes mellitus in moderate kidney insufficiency: the Kidney Early Evaluation Program. Kidney Int 67:1483–1488
21. Bosman DR, Winkler AS, Marsden JT, McDougall IC, Watkins PJ (2001) Anemia with erythropoietin deficiency occurs early in diabetic nephropathy. Diabetes Care 24:495–499
22. Thomas MC, MacIsaac RJ, Tsalamandris C, Molyneaux L, Goubina I, Fulcher G et al (2004) The burden of anemia in the type 2 diabetes and the role of nephropathy: a cross-sectional audit. Nephrol Dial Transplant 19:1792–1797
23. Thomas MC, MacIsaac RJ, Tsalamandris C, Molyneaux L, Goubina I, Fulcher G et al (2004) Anemia in type 2 diabetes. J Clin Endocrinol Metab 89:4359–4363
24. Thomas MC, MacIsaac RJ, Tsalamandris C, Power D, Jerums G (2003) Unrecognized anemia in patients with diabetes: a cross-sectional survey. Diabetes Care 26:1164–1169
25. Schuster SJ, Koury ST, Bohrer M, Salceda S, Caro J (1992) Cellular sites of extrarenal and renal erythropoietin production in anaemic rats. Br J Haematol 81:153–159
26. Maxwell PH, Osmond MK, Pugh CW, Heryet A, Nicholls LG, Tan CC et al (1993) Identification of the renal erythropoietin-producing cells using transgenic mice. Kidney Int 44:1149–1162
27. Jelkmann W (2011) Regulation of erythropoietin production. J Physiol 589:1251–1258
28. Middleton SA, Barbone FP, Johnson DL, Thurmond RL, You Y, McMahon FJ et al (1999) Shared and unique determinants of the erythropoietin (EPO) receptor are important for binding EPO and EPO mimetic peptide. J Biol Chem 274:14163–14169
29. Chasis JA, Mohandas N (2008) Erythroblastic islands: niches for erythropoiesis. Blood 112(3):470–478
30. Thomas M, Tsalamandris C, MacIsaac R, Jerums G (2005) Anaemia in diabetes: an emerging complication of microvascular disease. Curr Diabetes Rev 1:107–126
31. Haase VH (2010) Hypoxic regulation of erythropoiesis and iron metabolism. Am J Physiol Renal Physiol 299(1):F1–F13
32. Wang GL, Jiang BH, Rue EA, Semenza GL (1995) Hypoxia-inducible factor 1 is a basichelix-loop-helix-PAS heterodimer regulated by cellular O2 tension. Proc Natl Acad Sci USA 92(12):5510–5514
33. Winkler AS, Marsden J, Chaudhuri KR, Hambley H, Watkins PJ (1999) Erythropoietin depletion and anaemia in diabetes mellitus. Diabet Med 16:813–819
34. Ishimura E, Nishizawa Y, Okuno S, Matsumoto N, Emoto M, Inaba M et al (1998) Diabetes mellitus increases the severity of anemia in non-dialyzed patients with renal failure. J Nephrol 11:83–86
35. Inomata H, Itoh M, Imai H, Sato T (1997) Serum levels of erythropoietin as a novel marker reflecting the severity of diabetic nephropathy. Nephron 75:426–430
36. Jerums G, Premaratne E, Panagiotopoulos S, MacIsaac RJ (2010) The clinical significance of hyperfiltration in diabetes. Diabetologia 53:2093–2104
37. Sasson AN, Cherney DZ (2012) Renal hyperfiltration related to diabetes mellitus and obesity in human disease. World J Diabetes 3:1–6
38. Blantz RC, Singh P (2014) Glomerular and tubular function in the diabetic kidney. Adv Chronic Kidney Dis 21(3):297–303
39. Tomaszewski M, Charchar FJ, Maric C, McClure J, Crawford L, Grzeszczak W et al (2007) Glomerular hyperfiltration: a new marker of metabolic risk. Kidney Int 71:816–821

40. Vaziri ND, Kaupke CJ, Barton CH, Gonzales E (1992) Plasma concentration and urinary excretion of erythropoietin in adult nephrotic syndrome. Am J Med 92:35–40
41. Bays B, Serra A, Junc J, Lauzurica R (1988) Successful treatment of anaemia of nephritic syndrome with recombinant human erythropoietin. Nephrol Dial Transplant 13:1894–1895
42. Ishimitsu T, Ono H, Sugiyama M, Asakawa H, Oka K, Numabe A et al (1996) Successful erythropoietin treatment for severe anemia in nephrotic syndrome without renal dysfunction. Nephron 74:607–610
43. Nowicki M, Kokot F, Kokot M, Bar A, Dulawa J (1994) Renal clearance of endogenous erythropoietin in patients with proteinuria. Int Urol Nephrol 26:691–699
44. Sasatomi Y, Ito K, Abe Y, Miyake K, Ogahara S, Nakashima H, Saito T (2012) Association of hypoalbuminemia with severe anemia in patients with diabetic nephrosclerosis. Ren Fail 34(2):189–193
45. Thomas MC, Tsalamandris C, MacIsaac R, Medley T, Kingwell B, Cooper ME et al (2004) Low-molecular-weight AGEs are associated with GFR and anemia in patients with type 2 diabetes. Kidney Int 66:1167–1172
46. Okon EB, Chung AW, Rauniyar P, Padilla E, Tejerina T, McManus BM et al (2005) Compromised arterial function in human type 2 diabetic patients. Diabetes 54:2415–2423
47. Téllez Gil L, Roselló AM, Collado Torres A, Moreno RL, Antonio Ferrón Orihuela J (2001) Modulation of soluble phases of endothelial/leukocyte adhesion molecule 1, intercellular adhesion molecule 1, and vascular cell adhesion molecule 1 with interleukin-1 beta after experimental endotoxic challenge. Crit Care Med 29:776–781
48. Faquin WC, Schneider TJ, Goldberg MA (1992) Effect of inflammatory cytokines on hypoxia-induced erythropoietin production. Blood 79:1987–1994
49. Leng HM, Folb PI (1996) Erythropoiesis and erythropoietin synthesis during aseptic acute inflammation. Inflamm Res 45:541–545
50. Thomas MC, Tsalamandris C, Macisaac RJ, Jerums G (2006) Functional erythropoietin deficiency in patients with type 2 diabetes and anaemia. Diabet Med 23:502–509
51. Bhatia V, Chaudhuri A, Tomar R, Dhindsa S, Ghanim H, Dandona P (2006) Low testosterone and high C-reactive protein concentrations predict low hematocrit in type 2 diabetes. Diabetes Care 29:2289–2294
52. Johnson CS, Keckler DJ, Topper MI, Braunscweiger PG, Furmanski P (1989) In vivo hematopoietic effects of recombinant interleukin-1 alpha in mice: stimulation of granulocytic, monocytic, megakaryocytic, and early erythroid progenitors, suppression of late-stage erythropoiesis, and reversal of erythroid suppression with erythropoietin. Blood 73:678–683
53. Maciejewski JP, Selleri C, Sato T, Anderson S, Young NS (1995) Fas antigen expression on CD34+ human marrow cells is induced by interferon gamma and tumor necrosis factor alpha and potentiates cytokine-mediated hematopoietic suppression in vitro. Blood 85:3183–3190
54. Van Der Putten K, Braam B, Jie KE, Gaillard CA (2008) Mechanisms of disease: erythropoietin resistance in patients with both heart and kidney failure. Nat Clin Pract Nephrol 4:47–57
55. Shuvaev VV, Fujii J, Kawasaki Y, Iton H, Hamaoka R, Barbier A et al (1999) Glycation of apolipoprotein E impairs its binding to heparin: identification of the major glycation site. Biochem Biophys Acta 1454:296–308
56. Brito PL, Fioretto P, Drummond K, Kim Y, Steffes MW, Basgen JM et al (1998) Proximal tubular basement membrane width in insulin-dependent diabetes mellitus. Kidney Int 53:754–761
57. Mise K, Hoshino J, Ueno T, Imafuku A, Kawada M, Sumida K, et al (2014) Impact of tubulointerstitial lesions on anaemia in patients with biopsy proven diabetic nephropathy. Diabetes Med. doi:10.1111/dme.12633 (Epub ahead of print)
58. Bosman DR, Osborne CA, Marsden JT, McDougall IC, Gardner WN, Watkins PJ (2002) Erythropoietin response to hypoxia in patients with diabetic autonomic neuropathy and non-diabetic chronic renal failure. Diabet Med 19:65–69
59. Matthews MR (1992) Autonomic ganglia in multiple system atrophy and pure autonomic failure. In: Bannister R, Mathias CJ (eds) Autonomic failure: a textbook of disorders of the autonomic nervous system, 3rd edn. Oxford University Press, Oxford, pp 592–621
60. Van Ingelghem E, Van Zandijcke M, Lammens M (1994) Pure autonomic failure: a new case with clinical, biochemical, and necropsy data. J Neurol Neurosurg Psychiatry 57:745–747
61. Biaggioni I, Robertson D, Krantz S, Jones M, Haile V (1994) The anemia of primary autonomic failure and its reversal with recombinant erythropoietin. Ann Intern Med 121:181–186
62. Finne PH, Skoglund RW (1970) Erythropoietin production in the rat following splanchnic neurectomy. J Lab Clin Med 76:103–106
63. Mayaudon H, Dupuy O, Dolz M, Bordier L, Belmejdoub G, Garcin JM et al (2002) Influence of blood pressure profile on erythropoietin levels in diabetics. Arch Mal Coeur Vaiss 95:743–746
64. Jeffrey RF, Kendall RG, Prabhu P, Norfolk DR, Will EJ, Davison AM (1995) Reestablishment of erythropoietin responsiveness in end-stage renal failure following renal transplantation. Clin Nephrol 44:241–247
65. Kim MK, Baek KH, Lim DJ, Kim YK, Kang MI, Lee KW, Song KH (2010) Erythropoetin response to anemia and its association with autonomic neuropathy in type 2 diabetic patients without advanced renal failure. J Diabetes Complic 24(2):90–95
66. Kim YC, Mungunsukh O, McCart EA, Roehrich PJ, Yee DK, Day RM (2014) Mechanism of erythropoietin regulation by angiotensin II. Mol Pharmacol 85(6):898–908
67. Vlachakos DV, Marathias KP, Madias NE (2010) The role of the rennin angiotensin system in the regulation of erythropoiesis. Am J Kidney Dis 56:558–565
68. Donnelly S (2001) Why is erythropoietin made in the kidney? The kidney functions as a critmeter. Am J Kidney Dis 38:415–425
69. Christlieb AR (1976) Renin–angiotensin–aldosterone system in diabetes mellitus. Diabetes 25(Suppl 2):820–825
70. Donnelly S, Shah BR (1999) Erythropoietin deficiency in hyporeninemia. Am J Kidney Dis 33:947–953
71. Christlieb AR, Munichoodappa C, Braaten JT (1974) Decreased response of plasma renin activity to orthostasis in diabetic patients with orthostatic hypotension. Diabetes 23:835–840
72. Christlieb AR, Kaldany A, D'elia JA (1976) Plasma renin activity and hypertension in diabetes mellitus. Diabetes 25:969–974
73. Azizi M, Rousseau A, Ezan E, Guyene TT, Michelet S, Grognet JM et al (1996) Acute angiotensin-converting enzyme inhibition increases the plasma level of the natural steam cell regulator N-acetyl-seryl-aspartyl-lysyl-proline. J Clin Investig 97:839–844
74. Hayashi T, Suzuki A, Shoji T, Togawa M, Okada N, Tsubakihara Y et al (2000) Cardiovascular effects of normalizing the hematocrit level during erythropoietin therapy in predialysis patients with chronic renal failure. Am J Kidney Dis 35:250–256
75. Inoue A, Babazono T, Iwamoto Y (2008) Effects of the renin–angiotensin system blockade on hemoglobin levels in type 2 diabetic patients with chronic kidney disease. Am J Hypertens 21:317–322
76. Saito A, Kaseda R, Hosojima M, Sato H (2010) Proximal tubule cell hypothesis for cardiorenal syndrome in diabetes. Int J Nephrol 2011:957164. doi:10.4061/2011/957164

77. Wang S, Denichilo M, Brubaker C, Hirschberg R (2001) Connective tissue growth factor in tubulointerstitial injury of diabetic nephropathy. Kidney Int 60:96–105
78. Thomas MC, Burns WC, Cooper ME (2005) Tubular changes in early diabetic nephropathy. Adv Chronic Kidney Dis 12:177–186
79. Vallon V, Thomson SC (2012) Renal function in diabetic disease models: the tubular system in the pathophysiology of the diabetic kidney. Annu Rev Physiol 74:351–375
80. Thomson SC, Vallon V, Blantz RC (2004) Kidney function in early diabetes: the tubular hypothesis of glomerular filtration. Am J Physiol Renal Physiol 286:F8–F15
81. Eckardt KU, Kurtz A, Bauer C (1989) Regulation of erythropoietin production is related to proximal tubular function. Am J Physiol 256:F942–F947
82. Kurtz A, Eckardt KU (1990) Erythropoietin production in chronic renal disease before and after transplantation. Contrib Nephrol 87:15–25
83. Schultze RG, Weisser F, Bricker NS (1972) The influence of uremia on fractional sodium reabsorption by the proximal tubule of rats. Kidney Int 2:59–65
84. Brezis M, Rosen S (1995) Hypoxia of the renal medulla—its implications for disease. N Engl J Med 332:647–655
85. Donnelly S, Shah BR (1996) Erythropoietin deficiency in type I diabetic patients is associated with decrease in fractional sodium reabsorption. J Am Soc Nephrol 7:1356
86. Nagai T, Yasuoka Y, Izumi Y, Horikawa K, Kimura M, Nakayama Y et al (2014) Revealuation of erythropoietin production by the nephron. Biochem Biophys Res Commun 449(2):222–228
87. Baldini P, Incerpi S, Lambert-Gardini S, Spinedi A, Luly P (1989) Membrane lipid alterations and Na+-pumping activity in erythrocytes from IDDM and NIDDM subjects. Diabetes 38:825–831
88. Kowluru R, Bitensky MW, Kowluru A, Dembo M, Keaton PA, Buican T (1989) Reversible sodium pump defect and swelling in the diabetic rat erythrocyte: effect on filterability and implications for microangiopathy. Proc Natl Acad Sci USA 86:3327–3331
89. Brown CD, Ghali HS, Zhao Z, Thomas LL, Friedman EA (2005) Association of reduced red blood cell deformability and diabetic nephropathy. Kidney Int 67:295–300
90. Elishkevitz K, Fusman R, Koffler M, Shapira I, Avitzour D, Arber N et al (2002) Rheological determinants of red blood cell aggregation in diabetic patients in relation to their metabolic control. Diabet Med 19:152–156
91. Resmi H, Akhunlar H, Temiz Artmann A, Güner G (2005) In vitro effects of high glucose concentrations on membrane protein oxidation, G-actin and deformability of 23 human erythrocytes. Cell Biochem Funct 23:163–168
92. Vlassara H, Valinsky J, Brownlee M, Cerami C, Nishimoto S, Cerami A (1987) Advanced glycation endproducts on erythrocyte cell surface induce receptor-mediated phagocytosis by macrophages. A model for turnover of aging cells. J Exp Med 166:539–549
93. Manodori AB, Kuypers FA (2002) Altered red cell turnover in diabetic mice. J Lab Clin Med 140:161–165
94. Rigal D, Monestier M, Baboin-Jaubert M, Chabaud-Sassulas D, Marseglia GL, Ville D et al (1985) Evaluation of erythrocyte survival by the determination of glycosylated haemoglobin. Clinical value. Presse Med 14:521–523
95. Wagstaff AJ, Goa KL (2002) Rosiglitazone: a review of its use in the management of type 2 diabetes mellitus. Drugs 62:1805–1837
96. Nesto RW, Bell D, Bonow RO, Fonseca V, Grundy SM, Horton ES et al (2003) Thiazolidinedion use, fluid retention, and congestive heart failure: a consensus statement from the American Heart Association and American Diabetes Association. October 7, 2003. Circulation 108:2941–2948
97. Maarani Y, Stessman J (2005) Mild reversible pancytopenia induced by rosiglitazone. Diabetes Care 28:1536
98. Raptis AE, Bacharaki D, Mazioti M, Marathias KP, Markakis KP, Raptis SA et al (2012) Anemia due to coadministration of renin–angiotensin-system inhibitors and PPARγ agonists in uncomplicated diabetic patients. Exp Clin Endocrinol Diabetes 120:416–419
99. Kouroumichakis I, Papanas N, Zarogoulidis P, Liakopoulos V, Maltezos E, Mikhailidis DP (2012) Fibrates: therapeutic potential for diabetic nephropathy? Eur J Intern Med 23:309–316
100. Poyart C, Marden MC, Kister J (1994) Bezafibrate derivatives as potent effectors of hemoglobin. Methods Enzymol 232:496–513
101. Fazio S, Linton MF (2010) High-density lipoprotein therapeutics and cardiovascular prevention. J Clin Lipidiol 4:411–419
102. Perutz MF, Poyart C (1983) Bezafibrate lowers oxygen affinity of hemoglobin. Lancet 2(8355):881–882
103. Balakumar P, Kadian S, Mahadevan N (2012) Are PPAR alpha agonists a rational therapeutic strategy for preventing abnormalities of the diabetic kidney? Pharmacol Res 65:430–436
104. Florentin M, Liberopoulos EN, Mikhailidis DP, Elisaf MS (2008) Fibrate associated adverse effects beyond muscle and livertoxicity. Curr Pharm Des 14:574–587