Chapter 8

Anemia in Chronic Kidney Disease and After Kidney Allotransplantation (Systematic Review)

Yuriy S. Milovanov, Lidia V. Lysenko (Kozlovskaya), Ludmila Y. Milovanova, Victor Fomin, Nikolay A. Mukhin, Elena I. Kozevnikova, Marina V. Taranova, Marina V. Lebedeva, Svetlana Y. Milovanova, Vasiliy V. Kozlov and Aigul Zh. Usubalieva

Additional information is available at the end of the chapter

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Abstract

Anemia in chronic kidney disease (CKD) has been recognized as a separate independent risk factor of cardiovascular (CV) events. The aim of the review is to provide a literature summary concerning early diagnosis and treatment of anemia in CKD that may be useful for clinicians and contribute to decrease CV mortality. Literature searches were made in such major databases as: PubMed, Medline, Embase, Cochrane Library, CINAHL, Wiley Online Library, Scopus, Web of Science, e-library, and website of WHO. This search encompassed original articles, systematic reviews, and meta-analyses relevant to CKD and anemia over recent 15 years. A total of 54 references from 562 reviewed articles were selected as they met to the search criteria (anemia and CKD, including diabetes mellitus, systemic diseases and post-transplant anemia). The publications included 27 randomized controlled trials, 20 experimental studies representing new data on the links of CKD anemia and cardiovascular risk markers (cytokines, Klotho, fibroblast growth factor (FGF-23), hyperglycemia, hypoalbuminemia and some others), 4 systematic reviews and 3 clinical practice guidelines. The main attention was devoted to the analysis of the studies provided an early diagnosis of anemia, an ability to minimize the factors contributing to its severity that have allowed to improve CV and total outcomes and to reduce costs of hospital treatment of CKD patients with anemia.

Keywords: chronic kidney disease, anemia, post-transplant anemia, HIF, sKlotho, FGF-23

1. Introduction

Anemia associated with chronic kidney disease (CKD) has been recognized over recent years as a powerful independent predictor of cardiovascular (CV) complications which is the main cause of mortality in CKD patients [1–3].

Numerous studies demonstrated the multifactorial causes of anemia in CKD. Some CKD patients have more severe anemia than it would be expected according to their degree of severity of renal insufficiency [4–7]. In these cases, it is necessary to consider other factors that may contribute to the renal anemia, such as inflammation and infection (excessive production of cytokines), effect of drugs (renin-angiotensin-aldosterone system (RAAS) blockers, cytostatics and some others), nutritional disorders (hypoalbuminemia, deficiency of vitamin B_{12}, folic acid, iron) [2, 8, 9]. Chronic inflammation and pro-inflammatory cytokines (IL-1β, TNF-α, INF-γ) are the factors that may aggravate anemia development in patients with diabetes mellitus (DM), as well as in CKD due to systemic diseases (systemic lupus erythematosus (SLE) and systemic vasculitis) [5, 7].

The role of hypoxia inducible factor (HIF) in renal anemia genesis has been better identified. HIF regulates oxygen-sensitive genes' transcription erythropoietins (EPO gene) and activity of the others important mediators, particularly vascular endothelial growth factor (VEGF), glucose transporters, and nitrogen oxide synthetases [10]. It was studied the relation of HIF with the main iron homeostasis regulator—hepcidin [11]. Active oxygen radicals, which content is always increased in CKD, accelerate HIF degradation and inhibit EPO gene expression, depressing tubular cells adaptation to hypoxia [12]. Hyperglycemia may also lead to HIF degradation and incomplete EPO production, especially in autonomous polyneuropathy in DM [5, 6].

It is discussed the role of lowering sKlotho in CKD anemia that significantly contributes to CV risk increase [13]. Hemoglobin (Hb) levels ≥ 110 g/l was associated with more increased sKlotho levels as a cardiorenoprotective factor in anemia management with epoetin and iron in CKD patients with renal anemia [7, 9].

CKD anemia has normocytic normochromic character. During the course of long-term hemodialysis treatment, anemia may transforms into the hypochromic microcytic iron deficiency anemia due to blood losses and EPO treatment that requires a proper treatment [9, 14].

In recent decades, indications for kidney transplantation have been extended by the taking of high-risk patients (diabetic nephropathy, rapidly progressive glomerulonephritis-RPGN, elderly age) that lead to an increase in the prevalence of post-transplant anemia [15] and increase in CV risk.

If long-term anemia is not completely corrected in CKD patients, eccentric left ventricular hypertrophy (LVH) is formed with developing chronic heart failure (CHF) [1, 16, 17] because of decreased Hb level and an insufficient O_2 supply to the tissues leading to the sympathetic nervous system activation, to the increased heartbeat, the renin-angiotensin system and antidiuretic hormone activation, sodium and water retention and edema occurrence, to the

increased venous return to heart and the eccentric LVH. In addition, lowering sKlotho leads to increasing serum fibroblast growth factor (FGF-23), which is considered now as a new uremic toxin, which level elevates earlier than parathyroid hormone (PTH) as the progression of the CKD [18, 19]. Increased FGF-23 and lack oxygen supply to cardiomyocytes enhance their apoptosis with the development of myocardial fibrosis and CHF [18, 19].

Early diagnosis of anemia, minimizing and eliminating as much as possible the factors contributing to its development and timely initiation of treatment are considered today as an important strategy to reduce CV and total mortality of patients, to improve their quality of life, and to reduce costs of a hospital treatment of CKD patients with anemia [1, 9, 20, 21]. However, since the publication of the KDIGO 'Clinical Practice Guideline for Anemia' in 2012, significant advancement has been achieved in our understanding of anemia mechanisms in CKD including kidney transplant patients. At the same time, there is a burning need for randomized clinical trials for better informed decisions and future optimization of CKD patients care. The aim of the review is to provide a literature summary concerning the early diagnosis and treatment of anemia in CKD and after kidney transplantation, which may be useful for clinicians in their clinical practice.

2. Methods

Literature searches were made in 10 major databases: Pubmed, Medline, Embase, Cochrane Library, CINAHL, e-library, Wiley Online Library, Scopus, Web of Science, and website of WHO ICTRP. The search was carried out to find all articles relevant to CKD and anemia, including diabetes mellitus, systemic diseases and transplant patients, as well as original experimental data. This search encompassed original articles, systematic reviews and meta-analyses. There was no language restriction.

2.1. Agreed criteria for article inclusion into the review

- Articles should be full text. Brief publications and abstracts were not included
- Research should include at least 20 patients in each group. The minimum mean duration of study was 6 months
- Analyzed literature over last 15 years
- The article should have the detailed research protocol for assessing of its quality
- Patients examination must meet KDIGO 2012 guidelines
- Randomized controlled trials
- Original experimental data over recent years representing the link of CKD anemia and the markers of cardiovascular risk (cytokines, Klotho, FGF-23, hyperglyceamia, hypoalbuminemia and some others)
- Systematic reviews and meta-analyses in this field

3. Results

A total of 54 references from 562 reviewed articles which met to the search criteria (anemia and CKD, including diabetes mellitus, systemic diseases, and transplanted patients, as well as original experimental data) were selected. The publications included 27 randomized controlled trials, 20 experimental studies representing new data over recent years on the link of the anemia in CKD and the markers of cardiovascular risk (cytokines, Klotho, FGF-23, hyperglycemia, hypoalbuminemia, active oxygen radicals, angiotensin II, interleukin-1,TNF-alpha, NO, and some others), 4 systematic reviews and 3 clinical practice guidelines. There were 10 studies in pre-dialysis patients, 10 in dialysis, and 7 after transplantation among selected studies relevant to the prevention and treatment of anemia in CKD. The main attention was devoted to the analysis of the studies on early diagnosis of anemia, ability to minimize the factors contributing to its severity, timely treatment initiation, that have allowed improving CV and total outcomes, as well as reducing costs of hospital treatment of CKD patients with anemia.

4. Anemia in chronic kidney disease

Renal anemia (defined as serum Hb levels <130 g/l for men and <120 g/l for women) is an obligatory complication of CKD progression [1, 2], which is usually occurring when glomerular filtration rate (GFR) decreases below 60 ml/min/1.73 m^2 (CKD stage 3–5). At GFR below 43 ml/min/1.73 m^2, linear relationship between GFR and Hb level is noted (decreased GFR of 5 ml/min/1.73 m^2 is accompanied by a fall in Hb level of 3 g/l) [1, 2, 20]. Importantly, the degree of Hb decreasing is not just a marker of CKD progression, but now, it is considered as also a direct independent predictor of cardiovascular (CV) complications, which are the main causes of mortality in CKD patients [1–3].

Appearance of anemia in CKD may be due to the lowering of endogenous erythropoietin (EPO) production, shortening of the survival time of red blood cells, decreasing in erythroid progenitors receptors' sensitivity to EPO, and diminution of iron supply to bone marrow because of iron deficiency or its decreased availability, caused mainly by increased levels of hepcidin, due to inflammation accompanying to the chronic uremia [1, 2, 8]. In addition, the pathogenic mechanism of CKD anemia development may involve folate and vitamin B_{12} deficiency due to malnutrition and chronic inflammation resulting in immature erythroblasts apoptosis [22, 23].

Some CKD patients have more severe anemia than it would be expected according to their degree of renal insufficiency severity [4, 5, 23]. In these patients other factors contributing to the anemia should be considered: inflammation and infection (excess cytokines production), effect of drugs (RAAS blockers, cytostatic agents), nutritional disorders (hypoalbuminemia, vitamin B_{12}, folic acid, iron deficiency). In diabetes mellitus (DM) patients, some other factors may also aggravate anemia: chronic inflammation and pro-inflammatory cytokines (IL-1β, TNF-α, INF-γ), their elevated levels are detected even before the renal failure appearance. Anemia has been found in about 10% of DM patients with normal kidney function [24]. In

the cohort of >9000 patients without renal disease, DM was an independent determinant of Hb levels [24]. Many factors have been suggested additionally contributing to the pathogenesis of anemia in DM patients, such as erythropoietin deficiency due to efferent sympathetic denervation of the kidney in diabetic neuropathy, chronic inflammatory reaction leading to functional iron deficiency, non-selective urinary protein excretion leading to transferrin and erythropoietin loss and the use of RAAS blockers [5, 25]. A direct comparison of anemia between matched diabetic and non-diabetic CKD patients in the epidemiologic study which included particularly large number of patients at CKD Stage 3A found out the difference between the groups, and diabetic patients had anemia two times more often than patients without diabetes (60.4 vs 26.4%). Serum ferritin levels, but not iron, were higher in diabetic than in non-diabetic patients at all stages; the first ones had also more severe anemia and the increased ferritin as an acute-phase protein may signify higher subclinical inflammation in diabetic patients [6].

In patients with CKD due to systemic diseases (SLE, systemic vasculitis), anemia in 10–20% of cases also develops at the earlier CKD stages (1–2 stage). It is suggested that exacerbation of glomerulonephritis as well as an underlying systemic disease may lead to cytokine-mediated disorders of erythropoiesis with the development of anemia of chronic disease (ACD). Recognition of the ACD based on the analysis of the features of iron metabolism characterized this form of anemia [7, 9, 23]. The disorders of the iron metabolism are mainly associated with increased iron absorption and retention iron by reticuloendothelial system cells (RES), followed to lowering of iron admission to the bone marrow [7, 23, 26].

In recent years, it has determined the role of hypoxia-inducible factor (HIF) in the genesis of renal anemia [10, 12]. It was found that HIF regulates transcription of oxygen-sensitive genes (EPO gene) and activity of other important mediators, particularly VEGF (vascular endothelial growth factor), glucose transporters, and nitrogen oxide synthetases. HIF is a heterodimer constantly expressed in kidney and consists of alpha and beta subunits. Expression of HIF β-subunit occurs constantly and plays an important role in the body's response to xenobiotics when heterodimeric transcription complex with aryl gidrocarbon receptor formed. Expression of α-subunits of HIF-complex (HIF-1α and HIF-2α) is regulated by partial oxygen pressure in tissues. In the case of hypoxia absence, HIF-1α and HIF-2α rapidly degraded. In case of sufficient oxygen supply to tissues, enzyme FIH (factor inhibiting HIF) affects asparagine hydroxylation, thereby preventing increase in transcriptional HIF-1α activity. In case of Hb level fall, α-subunit degradation is inhibited that leads to the formation of HIF-1β—cytochrome P300 complex [10, 22]. As a result, active HIF complex binds to the complementary site of gene EPO locus thereby increasing its production.

Besides hypoxia, transcriptional HIF-1 activation is induced by nitrogen oxide (NO), tumor necrosis factor (TNFα), interleukin-1, and angiotensin II. Thereby, HIF-1 also takes part in the regulation of angiogenesis, glucose, and iron homeostasis [10].

It has been currently established that HIF takes part in EPO production, not only in kidney but also in liver. Liver is also involved in EPO synthesis, but less than kidneys, and extrarenal EPO synthesis cannot compensate its renal production deficiency [27].

The relation of HIF-2α and the main regulator of iron homeostasis—hepcidin has been studied: hypoxia and iron deficiency suppress hepcidin synthesis and thereby increase in the possibility of iron absorption in intestine [11]. It is found that HIF and iron interact by iron-regulated proteins—IRP's: IRP-1 and IRP-2. When iron stores in the body decrease, IRP-IRE complex prevents sequestration of transferrin receptor and thereby enhances intracellular intake of iron. If iron stores in the body are sufficient, IRP-IRE complex is inactivated and undergone to protosomal degradation, and iron is not absorbed. Furthermore, HIF-2α post-translationally prevents the development of more severe iron deficiency [10, 22].

Elevating active oxygen radicals in CKD also promotes HIF-1α degradation and inhibits EPO gene expression, thereby decreasing molecular adaptation of tubular cells to hypoxia [12]. Hyperglycemia accompanying to diabetic neuropathy, and especially in case of autonomous polyneuropathy, also leads to HIF-1α degradation and insufficient EPO production [5, 9, 24].

HIF-1α serum content is decreased in CKD patients with anemia (reference ranges are 1.5–6.0 pg/ml in adults and in children older than 14 years and). The murine antiserum and monoclonal antibodies against HIF-1α molecules are used for HIF detection [1, 12].

4.1. Diagnosis of anemia

According to the World Health Organization, criteria for the diagnosis of anemia are [2, 9]:

- Hb <130 g/l, Hct < 39%, red blood cell count < 4.0 mln/mcl, in men;
- Hb <120 g/l, Hct < 36%, red blood cell count < 3.8 mln/mcl, in women;
- Hb <110 g/l, Hct < 33%, in pregnant women.

In CKD patients for the diagnosis and future dynamic anemia control, it is necessary to check: Hb level, main red blood indexes: mean corpuscular volume (MCV), mean cell Hb (MCH), mean cell Hb concentration (MCHC), number of reticulocytes, ferritin level and transferrin saturation (TSAT), serum vitamin B_{12}, and folates, as well as for the other anemia forms [1, 9, 20].

Anemia in CKD is the normocytic and normochromic one. The number of reticulocytes in renal anemia is usually normal or slightly increased that depends on the degree of bone marrow erythropoiesis activity. It is noted increasing of immature reticulocytes fraction (IRF) that representatives of active bone marrow erythropoiesis, despite the EPO deficiency [1, 20, 22]. Perhaps, constant blood loss, associated with hemodialysis procedures, may activates compensatory medullar erythropoiesis. During the long-term hemodialysis, anemia may transforms into hypochromic microcytic iron deficiency anemia due to blood losses or EPO treatment that requires a proper treatment. In case of hypochromic microcytic anemia development, hemoglobin in reticulocytes (Ret-Hb) decreases [14, 28].

Functional iron deficiency for erythropoiesis may be diagnosed using TSAT coefficient, as well as the hypochromic red blood cells percentage (HRS), determined by flow cytometry [1, 9, 20]. A high serum ferritin level in combination with low-transferrin saturation are the evidence of increased hepcidin activity that may often be caused by inflammation, confirmable by increased C-reactive protein (CRP) concentration (more than 50 mg/dl) [8, 23, 29]. If

CRP level is high, CKD patient should be examined to detect inflammation (acute infection, active systemic inflammatory disease) and subsequent anti-bacterial and/or anti-inflammatory treatment should be provided before starting EPO therapy [1, 9, 20, 23, 29].

Renal anemia is accompanied by various clinical symptoms (dyspnoa, dizziness, poor appetite, depressed mood, decrease in performance, exercise tolerance, breakdown of cognitive, and sexual functions) and led to poor quality of life, increased hospital admissions, progression of kidney, and CV diseases, and increased mortality. Results of observational studies over last years have been strongly confirmed that Hb decreasing is a direct independent predictor of the eccentric LVH and CHF development (**Figure 1**), because of the falling of Hb level in blood and accompanied insufficient oxygen supply to tissues lead to sympathetic nervous system activation, increased heart rate, RAAS and antidiuretic hormone activation, sodium and water retention, appearance of edema, increase in venous return to the heart, and eccentric LVH.

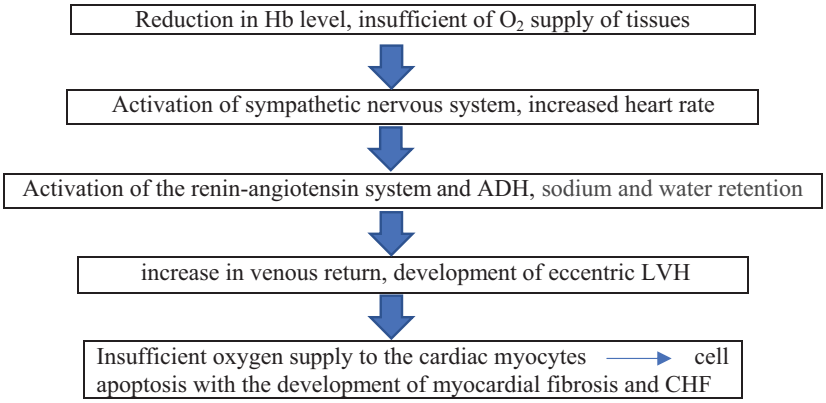

Figure 1. The impact of anemia on the LVH and CHF development in patients with CKD and anemia in the absence of its timely correction.

In addition, changing levels of recently identified new cardiovascular CKD markers such as sKlotho and FGF-23 progressively contribute to the cardiac remodeling in CKD especially in anemia persisting due to CKD progression. Increased FGF-23 and insufficient supply of oxygen to cardiomyocytes lead to cell apoptosis, myocardial fibrosis, and CHF [17–19].

5. Klotho and anemia in chronic kidney disease

It was found that circulating form of Klotho (sKlotho) protein has pleiotropic effects as a humoral factor that protects CV system [13, 30]. Adequate sKlotho expression provides both renal and CV protection. According to our date [31], patients from third stage of CKD have shown a direct linear relation between GFR fall and decreasing in sKlotho level. As it was detected firstly by M. Kuro-o, Klotho is synthesized in kidney tubules. This marker is very

sensitive to anemia, oxidative stress, intrarenal increasing of angiotensin II, and inflammation that all take part in reduction of Klotho expression in kidneys [30, 32, 33].

Decrease in serum Klotho may be also a result of inhibition of its extrarenal production. In this regard, it is interesting to know findings of Takeshita et al. [34] that indicate the presence of Klotho gene expression in sinoatrial node and high frequency of sudden cardiac death of mice due to cardiac arrhythmias caused by sinoatrial node's dysfunction with blocked Klotho gene. It has been also recently found the strong correlation of the sKlotho low level with advancing of vascular calcification and CHF in CKD [35, 36]. In addition, it was shown in mice that loss of Klotho results in reduced MCV and MCH in circulating red blood cells (RBC) as well as the expression of HIF-1α and HIF-2α was significantly attenuated in Klotho−/− mice, resulting in suppression of EPO [37]. At the same time, recent studies have shown that administration of recombinant EPO can induce Klotho expression in the rat nephropathy model [37] and in the preliminary clinic studies [32]. Hb levels ≥ 110 g/l were associated with higher serum sKlotho levels in anemia management by epoetin and iron, in patients with anemia at CKD 3B-4 stages ($p < 0.001$) [32]. Moreover, transmembrane Klotho is a co-receptor for FGF-23. FGF-23 is considered now as a new uremic toxin in CKD advancing because of its pathogenic action predominantly on the heart in CKD. In a normal state, FGF-23 produced by osteocytes regulates phosphorus, vitamin D, and PTH metabolism. However, as was later shown, an elevated FGF-23 is associated with a high risk of death from cardiovascular events (CVE) [18]. It is believed, the effects of FGF-23 to the heart may be caused by its nonselective binding to FGF-4-receptors in the myocardium due to the significant increasing of serum FGF-23 along with progressive Klotho deficiency (as co-receptor for FGF-23) during CKD progression, especially if anemia persists [18, 19]. The data, obtained in our clinical center, indicate association between FGF-23 and Troponin-I, which may be a result of FGF-23 cardiotoxic effect on the myocardium, leading to the appearance of the detectable serum Troponin-I levels [19].

So, taking into account that hypoxia is an independent factor in reducing sKlotho synthesis in CKD and an urgent need of Klotho for CV protection, CKD patients with anemia who are managed to achieve the target Hb and to keep it within the reference range by EPO and iron therapy would have been expected to save sKlotho production and accompanied significantly lower CV risk [13, 32, 36].

6. The main practical approaches to the treatment of anemia

6.1. Treatment of anemia at predialysis stages of chronic kidney disease

According to the International Guidelines of Kidney Disease Improving Global Outcomes (KDIGO, 2012), treatment of renal anemia with EPO should be begin if Hb level is decreased under 100 g/l in adults and children 12–14 years old [1].

In patients with comorbid diseases (CV diseases, DM, advanced atherosclerosis, malignant tumors) as well as in case of notes on stroke, malignancies in past medical history, the decision

about EPO treatment should be made individually and started if Hb level <100 g/l, firstly by iron medication (intravenous or per os) [1, 20, 38]. Preliminary correction of iron deficiency is obligatory requirement for the EPO therapy [1, 9, 20]. The velocity of Hb level decrement, the need for transfusions, response to the previous iron treatment, the severity of anemia symptoms, as well as the patient's lifestyle (active professional life, intensive school classes in children) should be taken into account at determining time for starting EPO therapy [20].

The upper limit of Hb reference range is determined as 115 g/l for most of the patients. Hb target level should be reached during 4 months [1, 20].

At predialysis CKD stages, short-acting EPO (epoetin-alfa or beta) is administered to patients subcutaneously at a dose of 50–100 IU/kg per week, it is about 6 thousand IU/week subcutaneously [1, 20, 39, 40] (**Figure 2**). Subcutaneous administration way of short-acting EPO is considered as a choice because it allows using lower doses and reducing the treatment cost [21]. The studies of pharmacokinetic of EPO show that subcutaneous administration prolongs half-life period of elimination of short-acting EPO (epoetin alfa and epoetin beta) [40].

At starting therapy, the rate of Hb increasing should be maintained at 10–20 g/l a month. Changes of hemoglobin level less than 10 g/L or more than 20 g/L require to gradual EPO dose correction by 25% weekly up (but not more than 720 IU/kg/week) or downward (**Figure 2**).

The rate of Hb increase >20 g/l per month is undesirable because of increase in risk of thrombosis. In this case, it is necessary to titrate down a total weekly EPO dose by 25–50%. Monitoring Hb level at starting treatment period should be performed every 2 weeks, then once a month. Hb's concentration change less than 10 g/L requires to gradual EPO dose correction by 25% upward [1, 9, 20, 39].

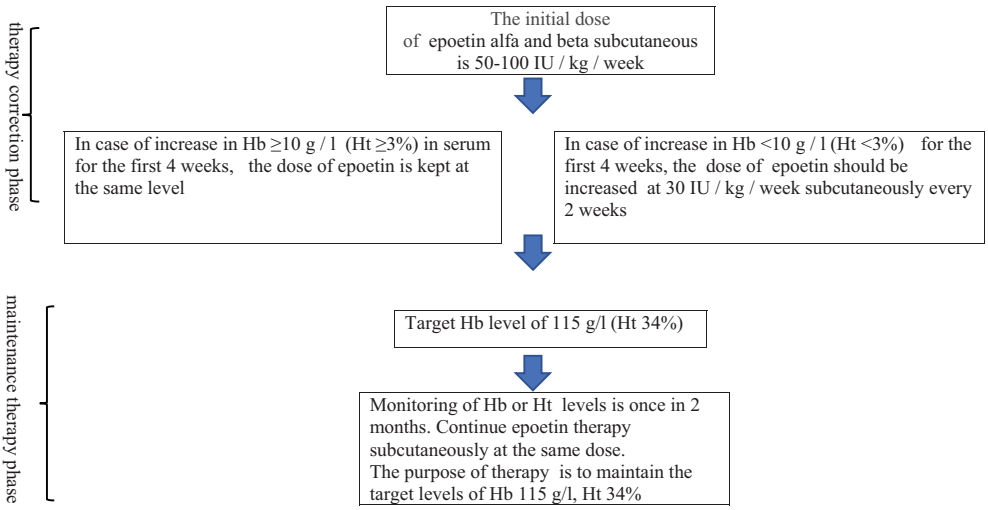

Figure 2. Algorithm for the treatment of anemia with epoetin α or β by subcutaneous administration of the drug at predialysis stages of CKD.

In CKD patients at stages 3–4, iron supplementation is necessary for prevention of iron deficiency, due to increased needs in iron due to EPO treatment, to achieve and maintain Hb level minimum at 115 g/l during EPO treatment. At the CKD stages 3, iron serum balance might be maintained by iron administration per os, for example, ferrum hydroxides (III) polymaltosate, wherein the dose of dietary iron should be at least 200 mg/day [1, 20, 41].

However, in patients with anemia of chronic diseases, it is a good practice to inject iron intravenously only because of insufficient intestinal absorption and iron retention by RES [1, 23, 26].

In addition, it is necessary to control during treatment the residual renal function (GFR and creatinine blood level in the dynamics), blood pressure (ambulatory blood pressure monitoring), hydration (blood volume), and cardiac hemodynamics. Therefore, low-protein diet with sodium restriction that combined with EPO and antihypertensive therapy may also play an important role in Hb maintenance [1, 9, 20]. The lower increasing in Hb level (less than 10 g/l a month or Hct level less than 0.5% a month) is a sign of insufficient effect of EPO treatment.

The most common cause of insufficient response to EPO is an iron deficiency [1, 20, 26, 42]. Adequate correction of iron deficiency is an important part of anemia treatment in patients at predialysis CKD stages and on dialysis [1, 9, 20]. In case of early successful anemia treatment onset in CKD patients, they are required less EPO doses to achieve the target Hb level at hemodialysis reaching as well as a lower incidence of CV complications [9, 39, 43].

6.2. Anemia treatment in CKD patients on maintenance hemodialysis (HD) or continuous ambulatory peritoneal dialysis (CAPD)

Short-acting EPO can be administered to dialysis patients subcutaneously and intravenously as well, but subcutaneous administration way requires lower doses of EPO [1, 20, 44]. Target Hb level is considered as 100–110 g/l (Hct 30–33%) for the majority of hemodialysis (HD) patients [1, 2, 9, 20]. Dose of 100 IU/kg/week of EPO alfa or beta intravenously or of 60 IU/kg/week of EPO alfa or beta subcutaneously is required to achieve and maintain this target Hb level. Subcutaneous EPO administration 1–2 times a week is a way of choice for anemia correction in patients receiving CAPD treatment because it helps to keep conditions for the formation of a fistula in case of further switching to HD treatment[1, 9, 20]. If patients are undergoing CAPD, and neither subcutaneous nor intravenous EPO administration ways can be used, for example, in children, intraperitoneal administration way to the “dry” abdominal cavity is applied, particularly when higher EPO doses are needed than in case of subcutaneous or intravenous injections. Hb level should be monitored every 4–6 weeks in patients on HD and CAPD [1, 9, 20]. Dialysis patients receiving EPO are required iron supplementation intravenously to achieve and maintain target Hb level, for example iron (III) sucrose, low molecular iron dextran or ferric III-hydroxide olygomaltosate intravenously slowly during the last 2 hours of the dialysis session (200 mg once every two weeks) under control of ferritin blood level [1, 45].

Iron deficiency developing during the EPO treatment requires fast correction, which is possible only in case of intravenous administration way of iron. Optimal and tolerance levels of iron metabolism parameters are presented in the **Table 1** (1, 9, 20).

Marker	Optimal level	Tolerance level
Ferritin, mcg/l	200–500	100–800
Transferrin saturation, %	30–40	20–50
Number of hypochromic red blood cells, %	<2.5	<10

Table 1. Optimal and tolerance levels of the iron metabolism parameters.

Iron deficiency is the most definitive for HD patients [1, 22, 23, 26, 45]. The main reason of its development is blood losses during medical manipulations that could be about 3–4 liters, equivalent to 2 g of iron a year: the blood that remains in the extracorporeal circuit (dialyzer, blood tubing line), blood loss from the puncturing locus, blood loss during catheter use, concealed hemorrhage in the gastrointestinal tract, and blood loss during laboratory studies. Young women require more iron supply than men [1, 20, 28].

Efficiency and safety of intravenous iron medications depend on its molecular weight, dose, and ingredients. Complexes of a low molecular weight, such as a ferrum gluconate, are less stable and faster release iron into plasma. Free iron may catalyze reactive oxygen forms generation, causing of lipid peroxidation and tissue damage. The significant portion of a dose of the such drugs is excreted by kidney in the first 4 hours after drug administration and not used for erythropoiesis [1, 45]. Despite of large molecular weight and strength of iron dextran drugs, its disadvantage is the possibility to increase the risk of allergic reactions. Ferric III—hydroxide olygomaltosate [100 mg/ml, 2 ml №5] is not accompanied by free iron release and does not cause antibodies formation and therefore has a good safety profile [1, 9, 20, 45].

The problem of variability of Hb levels during the period of EPO maintenance therapy is important in clinical practice of anemia treatment in CKD patients [4, 28]. According to retrospective analysis of the results of therapy of 281 dialysis patients by short-acting EPO, 90% of those have shown a cyclical variability of Hb levels by more than 15 g/l during 8 weeks. There were three such fluctuations in average during the 1 year observation. These cycles were characterized by increase in average Hb level up to 130 g/l and going down to 103 g/l, and the most common cause (84% of episodes) of it was the changes of EPO dose, 6 times a year in average [46].

One of approaches of solving this problem may be the administration of long-acting EPOs for renal anemia treatment, in particular, darbepoetin alfa and constant erythropoietin receptor activator—methoxypolyethylene glycol epoetin-beta [1, 20, 47, 48]. The starting dose of darbepoetin alfa is 0.45 mcg/kg/weekly in case of subcutaneous or intravenous administration way. It is allowed subcutaneous administration way of a dose of 0.75 mg/kg biweekly [1, 47]. If the increase in Hb levels is less than 10 g/l in 4 weeks, dose of darbepoetin-alfa should be up-titrated by 25%, while the up-titration should not be more often than once every 4 weeks. During maintenance therapy phase, the EPO administration can be continued once a week or biweekly. Darbepoetin alfa could be introduced subcutaneously once a month of a double maintenance dose once biweekly [1, 9, 20].

Starting dose of methoxypolyethylene glycol epoetin-beta is 1.2 mcg/kg once in 4 weeks subcutaneously (baseline Hb level is not taken into account). In case of increase in Hb level less than 10 g/l in 4 weeks, it is necessary titrating dose up by 50%, in case of increase in Hb level more than 20 g/l the dose should be titrated down by 50%. It is important to note that half life of methoxypolyethylene glycol epoetin beta is 139 hours in case of subcutaneous administration way, that is, 7 times longer than half life of epoetin alfa and 3 times longer than half life of darbepoetin alfa [1, 48].

Studies performed in dialysis centers of Germany and the UK have shown that in case of short-acting EPO switching to the long-acting erythropoiesis stimulating agent-methoxypolyethylene glycol epoetin beta (1 injection once a month) or darbepoetin alfa, medical expenses for anemia treatment have been reduced by 58 and 35%, respectively [21].

New classes of erythropoietin-stimulating agents are currently approved. The phase III study of efficacy and safety of Rosksadustat—an inhibitor of prolyl-4-hydroxylase—enzyme, that accelerates HIF deterioration, is carried out. The drug is administered per os. It was shown in preclinical studies that Roksadustat-activated alfa-subunit of HIF and initiated endogenous dose-related EPO secretion, improved iron utilization, and resolved anti-erythropoetin effects of inflammatory cytokines in renal anemia.

7. Anemia after kidney allotransplantation

Despite the impressive successes of transplantation over the last 30 years, 60% of all recipients' deaths occur with a functioning kidney transplant [49, 50]. The cause of death more than a half of these cases is CV complications. In renal transplant recipients, CHF risk factors include DM, age over 65 years, increase in systolic blood pressure, hypoalbuminemia, cytomegalovirus infection, and post-transplant anemia (PTA). There has been an increase in the prevalence of PTA over the recent decades [51]. In addition to the general factors for renal anemia (iron and erythropoietin deficiency), other reasons of PTA are considered [49, 50]:

- widening of indications for renal transplantation for high-risks patients (with diabetic nephropathy, RPGN and etc.)
- elderly age
- renal transplantation from marginal donors
- modification of recipient's immunosuppressive treatment when increased using mycophenolate mofetil, calcineurin inhibitors (tacrolimus, cyclosporine A) and rapamycin that associated with high risk of anemia development.

7.1. The forms of post-transplant anemia

There are relapsing and permanent course of PTA. PTA is also could be of early and late forms, depending on the time after transplantation [50]. Sixty percent of renal transplant recipients show recurrent PTA episodes associated with decreasing of transplant function (GFR < 60 ml/min, serum creatinine more than 190 mmol/l) as well as with recurrent of acute

transplant rejection, re-transplantation, that more common in females, in case of donor's age is older than 60 years, in iron deficiency (ferritin <100 mg/l), in patients with DM, secondary hyperparathyroidism, obstinate infections accompanying by increased CRP levels more than 50 mg/ml, in cases of combined administration of rapamycin and mycophenolate mofetil (MMF) or in combined administration of RAAS blockers and azathioprine or allopurinol [1, 9, 50].

Permanent PTA is found in 30–40% of adults and in 60–80% of children as renal transplant recipients [51, 52]. Early PTA develops in the first 6 months after transplantation; it occurs in 2–3 times more often than the late form of PTA that develops after the sixth month from transplantation [51, 53]. The incidence of the early severe PTA form is currently high despite the effective correction of renal anemia with EPO drugs during preparation for transplantation (at regular HD) and rapid increase in EPO synthesis in transplant. This is due to a post-operative blood loss, inflammatory complications, and hyperparathyroid osteodystrophy. Three months later after surgical intervention, PTA severity decreases as erythropoiesis in transplant increases. However, 4–5 years later after transplantation, the risk of anemia development increases (late PTA) because of the progressive dysfunction of transplant (due to chronic rejection, sandimmune nephropathy, nephropathy recurrence in the transplant, nephritis de novo in transplant), as well as appearance of the late transplant complications such as persistent infections, cancerous diseases [54]. There is an inverse relationship between creatinine and Hb levels in the most of recipients with late PTA that is typical for CKD in general [1, 20, 53].

7.2. Causes of post-transplant anemia

Causes of PTA are multifactorial (**Table 2**) [51], but the basic mechanism of either early or late PTA forms is the fall of endogenous EPO synthesis often associated with the absolute or relative iron deficiency. EPO level increases to the normal values after renal transplantation in case of immediate transplant functioning and decreases in case of its delayed start of function. The frequent reason of PTA is a resistance to EPO appears in case of rejection crisis of transplant as well as in severe secondary hyperparathyroidism and particularly often in iron deficiency [50–53]. The absolute or relative iron deficiency is found in 40–50% of recipients, and it is usually associated with PTA. Post-operative blood loss, chronic infections with increased CRP level, absence of iron stores in recipient, and small-scale blood loss may result in iron deficiency [51].

Important causes of PTA are the iatrogenic factors that could impair erythropoiesis, iron metabolism, and aggravate resistance to EPO [51, 52]. Decreased response to EPO may be due to the iron deficiency that could be a result of gastrointestinal erosions, accompanying treatment with non-steroid anti-inflammatory drugs, direct and indirect anticoagulants, and corticosteroids [1, 51]. On the other hand, corticosteroids oppose to myelotoxicity of cytostatics, including iatrogenic hypoplastic PTA, due to both a direct stimulating effect on erythropoiesis and granulocytopoiesis and influence on pharmacodynamic of cytostatics [1, 51]. A number of studies have shown the role of RAAS blockers in the PTA pathogenesis. RAAS blockers diminish EPO synthesis due to blocking renin-angiotensin-aldosterone system and increasing serum level of tetrapeptide AcSDKP as endogenous inhibitor of erythropoiesis. The negative

Anemia pathogenetic mechanism	Causes of the PTA
Iron deficiency	Post-operative blood loss, attenuation of iron stores in case of chronic inflammation, drug-induced erosions of gastrointestinal tract (NSAIDs, glucocorticosteroids)
Erythropoetin deficiency	Acute injury of transplant due to a long period of thermic ischemia, chronic transplant rejection, ciclosporin nehpropathy
Resistance to erythropoetin	Recurrent acute transplant rejection crises, severe hyperparathyroidism, protein-energy malnutrition, pure red-cell aplasia (PRCA)
Disease activity	Systemic disease, bacterial and viral infections
Malignant tumors	B-cell lymphoma, Kaposi sarcoma, hepatocellular carcinoma
Drug-induced disorders of erythrogenesis	Cytostatics (azathioprine, MMF, Sirolimus), RAAS blockers, anti-viral medication (ribavirin, ganciclovir)

Table 2. Causes of anemia prevalence after kidney transplantation.

effect of RAAS blockers on erythropoiesis is a dose-dependent one. Combined administration of RAAS blocker with azathioprine or allopurinol is the most dangerous for erythropoiesis [1, 20, 51, 53].

High doses of sandimmune that are administered in the first few weeks after transplantation enhance EPO synthesis in tardive functioning transplant due to vasoconstriction and activation of renin-angiotensin-aldosterone system, but it does not lead to increase in hemoglobin level. Long term using of calcineurin inhibitors at late stages of transplantation is often accompanied by such complications as progressive tubulointerstitial fibrosis of transplant (sandimmune nephropathy) with EPO deficiency and PTA progression. Myelotoxic effect of sirolimus (rapamycin) has been found in the majority of studies, and it has been more significant that in case of MMF administration. Upon joining the MMF and sirolimus, PTA develops in 30–57% of cases. The degree of decreased in Hb level was correlated with sirolimus dose. Many of new cytostatics used in transplantation require significant increase in EPO dose due to its aggravation of PTA [1, 9, 51, 53].

Rare PTA forms include an autoimmune PTA, a pure red-cell aplasia (PRCA) with autoantibodies to EPO or to its receptor [54]. PRCA appearance as complication of cytostatic therapy (azathioprine, MMF, tacrolimus) with replication of myelotropic parvovirus B19 manifests severe progressive anemia with absolute resistance to EPO and total dependence on blood transfusions.

In recipients, long-term treated by immunodepressants (including polyclonal and monoclonal anti-lymphocyte antibody and anti-cytokine drugs), iatrogenic PTA may be due to manifestation of infectious (tuberculosis, CMV, HBV, HCV replication) or cancer complications of transplantation: malignant lymphoma, hepatocellular carcinoma, Kaposi's sarcoma, melanoma, bladder cancer, parathyroid cancer, and cervical cancer [1, 20, 51]. Iatrogenic PTA (with an increase in the requirement of EPO) often develops in the case of treatment of viral hepatitis by ribavirin, ganciclovir.

7.3. Risk factors of post-transplant anemia

Risk factors of PTA include depression of transplant function (GFR <60 ml/min, blood creatinine > 190 mmol/l), recurrent relapses of acute transplant rejection, retransplantation, female gender, donor age over 60 years, iron deficiency (ferritin < 100 mcg/l), diabetes mellitus, uremic hyperparathyroidism, persistent infection with increase in CRP levels > 50 mg/mL, combined administration of rapamycin and mycophenolate mofetil (MMF), as well as RAAS blockers and azathioprine or allopurinol [1, 51, 53].

On the other hand, PTA is a risk factor of the acute transplant dysfunction and long-term mortality of recipients [49]. Early anemia may aggravate transplant dysfunction immediately after transplantation [50]. So, early PTA aggravates hypoxia of transplant's medullary zone, and ischemia-reperfusion syndrome thereby increases in risk of ischemic acute tubular necrosis, acute transplant abruption reaction, acute pyelonephritis and also slows down regeneration of the epithelium of the convoluted tubules in recipients with delayed transplant function [49]. Extra renal manifestations of early PTA include exacerbation of ischemic heart disease, progressive CHF, arrhythmia, prolonged immobilization with a poor exercise activity, blood transfusion complications, and viral infections [50].

Despite of the fact that the late PTA has a moderate degree in 90% of recipients (Hb level is of 110–115 g/l), it could worsen prognosis by increasing risk of long-term cardiac mortality [53]. A number of studies have shown correlation between PTA severity and survival of recipients [15, 49, 50, 53]. The decrement in hemoglobin level by 10 g/l significantly increases the risk of CHF, cardiovascular, and overall mortality. The data have shown that iron deficiency affect the survival rate of recipients. Recipients with diabetic nephropathy and Hct level of more than 30% have significantly lower risk of CV complications than when Hct < 30% [1, 20, 50].

7.4. Epoetin drugs in post-transplant anemia treatment

The observational studies over the last years did not confirm a detrimental effect of EPO on transplant function and an increase in risk of thrombosis as suggested in 1980–90s. [1, 2, 9, 20]. In the case of early PTA form, despite of presence of factors that may induce resistance to EPO, there is usually an adequate response to EPO treatment with rapid normalization of Hb levels and improving quality of life. In recent years, more preliminary data have been obtained regarding positive effects of early PTA correction with EPO on transplant's function and survival, cardiovascular, immune, and endocrine system of recipient [1, 20, 51].

Response to the EPO treatment of PTA may be enhanced significantly by the modification of drug therapy (**Table 3**): in the case of PRCA, EPO therapy should be cancelled until antibodies to EPO in blood will disappear; in the case of severe hemolysis, the doses of corticosteroids should be increased and plasmapheresis to be carried out [54].

Precedent data have been received are positive about cardioprotective effect of EPO on regression of LVH according to echocardiography and on improving quality of life that was observed in the case of EPO treatment in recipients with blood creatinine less than 190 mmol/l

Drugs	Form of anemia	Therapy approach
NSAIDs, Glucocorticosteroids	Iron-deficiency	The cancel of NSAID, dose decline of glucocorticosteroids, up-titration of iron agents in the presence of the same epoetin dose
Rapamycin, MMF	Hypoplastic	Dose decline of Rapamycin, up-titration of epoetins dose
Azathioprine, MMF, tacrolimus	PRCA	The cancel of cytostatic agents, epoetin and iron agents, high dosage of glucocorticosteroids, plasmapheresis, blood transfusion
RAAS blockers and Azathioprine, Allopurinol	Hypoplastic	The cancel of Azathioprine, Allopurinol, dose decline of ACE inhibitors, its substitution for Angiotensin II Receptor Blockers, calcium antagonists, β-blockers
Ribavirin	Hemolytic	Dose decline of Ribavirin, up-titration of epoetin

Table 3. The principals of the corrections of iatrogenic PTA.

and hematocrit rising (to the level of 33–36%). Cardioprotective effects of EPO are associated as considered with both antianemic and pleiotropic effects: activation of stem endotheliocytes and myocardial neoangiogenesis, as well as with inhibition of myocardiocytes apoptosis and maintenance of sKlotho levels [1, 16, 32, 43].

8. Conclusion

In recent years, renal anemia is considered as not only a risk factor for CKD progression but mainly as a separate independent powerful risk factor for CVD that is the main cause of death in CKD patients. It is recognized not only direct pathogenic effects of anemia on CV system due to its association with increased cardiac output, left ventricular hypertrophy, heart failure but also due to contribution to the reduction of cardiorenoprotective factor's synthesis such as the soluble form of Klotho protein in CKD.

Anemia accompanying CKD in diabetic nephropathy as well as due to systemic diseases occurs earlier than in others patients of CKD populations and requires close attention and control at CKD 2–3a stages.

In dialysis patients along with decreased EPO production and anti-proliferative effects of uremic toxins, chronic immune activation due to the contact of the immune system cells with a dialysis membrane as well as due to frequent episodes of infection, leading to the typical chronic disease anemia, changes of iron metabolism may play a role in the genesis of anemia.

In recent decades, an increase in the prevalence of anemia in patients after kidney transplantation has been due to: expanding indications for renal transplantation for high-risk patients, elderly age, renal transplantation from marginal donors («unideal» donors, a donor over 60 years old or over 50 years old and the cause of death whom was cardiovascular disease), modification of recipient's immunosuppressive treatment when mycophenolate mofetil, calcineurin inhibitors (tacrolimus, cyclosporine A) and rapamycin, associated with high risk of anemia development are used more often.

Early diagnosis of anemia, minimizing and eliminating the possible factors contributing to its development, and timely initiation of treatment are considered today as an important strategy to reduce CV and total mortality of patients, to improve their quality of life and to reduce costs of a hospital treatment of CKD patients with anemia.

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Abbreviations

CKD	Chronic Kidney Disease
CAPD	Continuous Ambulatory Peritoneal Dialysis
CRP	C-reactive protein
CHF	Congestive heart failure
CVC	Cardiovascular complications
CVE	cardiovascular events
DM	diabetes mellitus
EPO	erythropoietin
eGFR	estimated Glomerular Filtration Rate
FGF-23	fibroblast growth factor
HD	Regular Hemodialysis
HIF	hypoxia inducible factor
HRS	hypochromic red blood cells percentage
LVH	left ventricular hypertrophy
MCV	mean corpuscular volume
MCH	mean cell Hb
MCHC	mean cell hemoglobin concentration

MMF	mycophenolate mofetil
PTH	parathyroid hormone
PTA	Post-transplant anemia
PRCA	Pure Red-Cell Aplasia
RCTs	randomized controlled trials
RAAS	renin-angiotensin-aldosterone system
RBCs	red blood cells
SLE	Systemic Lupus Erythematosus
TSAT	transferrin saturation coefficient

Author details

Yuriy S. Milovanov, Lidia V. Lysenko (Kozlovskaya), Ludmila Y. Milovanova*, Victor Fomin, Nikolay A. Mukhin, Elena I. Kozevnikova, Marina V. Taranova, Marina V. Lebedeva, Svetlana Y. Milovanova, Vasiliy V. Kozlov and Aigul Zh. Usubalieva

*Address all correspondence to: ludm.milovanova@gmail.com

I.M. Sechenov First Moscow State Medical University of the Ministry of Health, Moscow, Russian Federation

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