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REVIEW

Red blood cell abnormalities and the pathogenesis of anemia in end-stage renal disease

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Anemia is the most common hematologic complication in end-stage renal disease (ESRD). It is ascribed to decreased erythropoietin production, shortened red blood cell (RBC) lifespan, and inflammation. Uremic toxins severely affect RBC lifespan; however, the implicated molecular pathways are poorly understood. Moreover, current management of anemia in ESRD is controversial due to the "anemia paradox" phenomenon, which underlines the need for a more individualized approach to therapy. RBCs imprint the adverse effects of uremic, inflammatory, and oxidative stresses in a context of structural and functional deterioration that is associated with RBC removal signaling and morbidity risk. RBCs circulate in hostile plasma by raising elegant homeostatic defenses. Variability in primary defect, co-morbidity, and therapeutic approaches add complexity to the pathophysiological background of the anemic ESRD patient. Several blood components have been suggested as biomarkers of anemia-related morbidity and mortality risk in ESRD. However, a holistic view of blood cell and plasma modifications through integrated omics approaches and high-throughput studies might assist the development of new diagnostic tests and therapies that will target the underlying pathophysiologic processes of ESRD anemia.

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1 Introduction

Chronic kidney disease (CKD) is a major public health problem. The increasing incidence of CKD in combination with population aging, age-associated comorbidities, and greater access to care, resulted in the explosion of the number of dialysis patients during the past decade, with an annual growth rate about six to sevenfold higher than that of the world population [1]. End-stage renal disease (ESRD) is a state of severe kidney damage. It refers to patients exhibiting glomerular filtration rate <15 mL/min/1.73m^2 and/or those treated by dialysis, irrespective of the filtration rate levels. Diabetes and arterial hypertension represent the leading causes of ESRD, although infections and genetic or autoimmune disorders may also result in advanced kidney failure. Anemia, cardiovascular risk, mineral and bone disorders constitute common complications of ESRD [2].

Anemia is ascribed to decreased erythropoietin production by the kidney, shortened red blood cell (RBC) lifespan [3], and inflammation [4]. Erythropoiesis stimulating agents (ESAs) in combination with iron supplementation is the primary treatment strategy for the management of anemia in CKD patients, although anemia is resistant to ESAs in approximately 10–20% of the cases. Iron deficiency, resulting from decreased iron absorption and chronic inflammation, leads to poor ESA response [5]. Hemodialysis (HD) aims to remove accumulated metabolic waste from blood and is the therapy of choice for the vast majority of dialysis patients compared to peritoneal dialysis.

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Abbreviations: **ATP**, adenosine 5′-triphosphate; **CKD**, chronic kidney disease; **CRP**, C-reactive protein; **ESAs**, erythropoiesis stimulating agents; **ESRD**, end-stage renal disease; **Hb**, hemoglobin; **HD**, hemodialysis; **IL**, interleukin; **MPs**, microparticles; **NO**, nitric oxide; **PS**, phosphatidylserine; **RBC**, red blood cell; **rhEpO**, recombinant human erythropoietin; **SOD**, superoxide dismutase

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Anemia associated with reduced lifespan of uremic RBCs is poorly understood. This review summarizes the profile of RBC and plasma modifications in ESRD patients and their probable association with anemia. The critical reading of the miscellaneous and often contradictory data on uremic anemia, intends to suggest a more integrated approach, which could reveal hidden pieces of the puzzle of the underlying pathophysiology.

2 Uremic plasma: The natural environment of the erythrocytes in ESRD

2.1 Redox status

The total antioxidant capacity of the uremic plasma is higher than in controls but significantly decreases post-HD following the removal of uric acid [6–8]. At the same time, elimination of water during the HD procedure leads to increased concentration of endogenous antioxidants compared to the pre-HD levels [9]. In general, uremic plasma contains low levels of antioxidant vitamins, like C (ascorbate) and E [6,8,10], usually in response to the inflammatory status of the patients [11]. HD negatively affects the concentration of ascorbate [6, 8], while variable effects have been reported for other vitamin levels [8, 10].

Regarding the enzymatic antioxidant capacity in ESRD plasma, glutathione reductase (GR) activity has been found increased [12, 13] and glutathione peroxidase (GPx) levels decreased [13–15], compared to controls. Low activity may be the result of protein modifications by oxidation, carbamylation, or glycosylation reactions [16], inhibition by uremic toxins [17], low synthesis by the nephron, [18] or plasma deficiency in micronutrients that are critical cofactors [19]. Indeed, dietary restrictions, inflammatory responses [20], and removal by dialysis, lead to low levels of trace elements, including selenium [13, 19, 21], zinc [19–21], and copper [19], that are partially elevated after HD [22].

As a result of reduced or overwhelmed antioxidant systems, several oxidative modifications have been reported in plasma lipid and protein components, including lipoperoxidation [10, 19], oxidized LDL [23], and protein carbonylation [24]. They usually increase by HD [25] and by the number of years the patients have been on HD treatment [26] (Table 1). Although HD-related issues, such as hemoincompatibility, might trigger ROS release by activated neutrophils, accumulated evidence confirms the hypothesis of Inagi et al. [27], that uremia is per se a state of carbonyl overload and oxidative stress. In this context, biologically active advanced glycation end products produced by oxidative and nonoxidative reactions on plasma components are now considered uremic toxins whose effective removal is related to the quality of HD [28].

Albumin is one of the main scavengers of carbonyl species and the major protein target of oxidative stress in plasma [29]. However, malnutrition and chronic inflammation cause reduced synthesis or increased catabolism of albumin in ESRD [30, 31]. In several studies, uremic albumin in combination with total antioxidant capacity has been positively correlated with hemoglobin (Hb) [31, 32] and negatively with the β2 microglobulin levels [33] (Table 1).

Finally, plasma nitric oxide (NO) concentration is usually higher in ESRD patients than in control samples [6], probably as a result of either the shear stress imposed on the endothelium by the HD [34] or the cytokine-mediated increased expression of NO synthase [35]. These conditions pose a high risk of cell and tissue injury, thus contributing to anemia, cardiovascular diseases, and other uremia-associated complications.

2.2 Inflammation status

HD and CKD, independently and in combination, trigger an inflammatory response, as revealed by the overexpression of acute phase proteins C-reactive protein (CRP) [36, 37] and ferritin [38], of pro-inflammatory cytokines interleukin 6 (IL-6), tumor necrosis factor alpha, and soluble IL2R and of monocyte/neutrophil activation markers [36, 37, 39]. Infections, bacterial contamination and bioincompatible membranes [40], are HD-related causes of monocyte and neutrophil activation [39]. However, HD is only partially responsible for the inflammatory response, as CKD patients who are not on dialysis exhibit inflammatory markers too [41], showing that uremia constitutes an inflammatory state per se. Inflammation markers exhibit positive correlation with elastase and recombinant human erythropoietin (rhEpO) dose [42] but negative correlation with Hb [32] and antioxidant components of uremic plasma [32, 43] (Table 1), highlighting the functional connection of inflammation with anemia and oxidative stress.

In fact, inflammatory cytokines trigger anemia in several ways. They inhibit erythropoiesis by suppressing the expression of EpO [44] and by inducing the expression of the soluble EpO receptor [45], the apoptotic death of erythroid progenitors, and interferon gamma stimulation [46]. Moreover, they may accelerate the destruction of RBCs through macrophage activation. Finally, inflammatory cytokines affect iron metabolism and availability by regulating the expression of transferrin and lactoferrin receptors in erythroid cells and the expression of ferroportin—and thus hepcidin levels—in macrophages [47].

2.3 Circulating microparticles (MPs)

Extracellular vesicles or microparticles (MPs) are small membrane particles ubiquitously released by cells under physiological or pathological conditions [48]. They participate in processes such as immune regulation, coagulation, and inflammation [49]. Elevated levels of circulating MPs have been reported in many diseases, including ESRD [50], as a result of

Table 1. Statistically significant (positive + or negative −) correlations between blood and uremia parameters

Parameter	Correlation with	References
Duration on HD	(+) Spectrin fragmentation	[61]
	(+) Plasma lipid peroxidation	[26]
Duration on rhEpO treatment	(−) Serum and RBC-lipid peroxidation	[31]
	(+) TAC	[31]
rhEpO dose	(−) Hb, albumin, serum iron	[5]
	(−) RBC-SOD	[84]
	(−) Serum albumin and iron	[127]
	(−) Hb	[128]
	(+) CRP	[5, 129]
	(+) CRP, IL-6, TNF-α	[130]
Blood		
Hct	(−) Endothelial MPs	[54]
	(+) Albumin	[131]
Hb	(−) plasma lipid peroxidation	[31]
	(+) albumin	[32]
	(−) RBC-lipid peroxidation	[31, 132]
	(+) TAC	[31]
	(+) RBC-SOD	[84]
	(−) CRP	[32]
RDW	(+) RBC-ROS	[61]
	(−) RBC-spectrin	[62]
Mean cell Hb	(−) RBC-Hsp70	[61]
Mean cell Hb concentration	(−) RBC-Hsp70	[61]
Echinocytes	RBC spectrin fragmentation	[61]
Stomatocytes	RBC membrane stomatin and Peroxiredoxin-2	[61, 62, 71]
CRP	(−) Serum iron	[127]
	(−) Plasma vitamin C	[11]
	(−) TAC	[31]
	(−) Plasma α-tocopherol	[43]
	(−) Serum albumin	[127]
	(+) Plasma elastase	[42]
	(+) IL-6, IL-10	[36]
	(−) Albumin	[32, 43, 127, 129]
Serum ferritin	(+) Plasma lipid peroxidation	[133]
	(+) CRP	[32]
	(−) RBC-GSH-Px	[133]
	(+) Serum calcium	[7]
	(−) Serum uric acid	[7]
Creatinine	(+) RBC membrane protein carbonylation	[61]
	(+) RBC membrane peroxiredoxin-2	[61]
	(−) Total blood GPx	[134]
	(+) RBC-PS exposure	[97]
β2 microglobulin	(−) Plasma TAC	[33]
Albumin	(−) Ferritin	[131]
	(−) Serum bicarbonate	[131]
	(+) Plasma TAC	[31]
Uric acid	(+) Albumin, creatinine	[135]
	(−) IL-6	[135]
	(+) TAC	[31, 33]
Urea	(+) RBC membrane protein carbonylation	[61]
	(+) RBC membrane Hsp70 (post-HD)	[61]
	(−) Total blood GPx	[134]

GPx, glutathione peroxidase; RDW, red blood cell distribution width; SOD, superoxide dismutase; TAC, total antioxidant capacity; TNF-α, tumor necrosis factor alpha.

inflammation, oxidative stress, and cellular activation [51,52]. HD exacerbates vesiculation [50, 52], probably as a result of the repetitive mechanical stress imposed on cells [53]. However, lower levels of MPs have also been reported post-HD, following the removal of uremic toxins [50,54]. p-cresol toxin triggers vesiculation of endothelial cells [50] but restricts MPs generation from neutrophils by preventing their calcium-independent activation [55,56]. This might explain why MPs levels do not always correspond to the severity of renal failure [52].

Although MPs, and especially the platelet-derived ones, in ESRD appear to be less procoagulant than in other diseases [57], they might promote atherosclerotic events [54,58]. Furthermore, the endothelium-derived MPs may decrease the endothelial NO release, thus contributing to endothelial dysfunction [59].

3 RBCs in ESRD

3.1 Physiological characteristics

Uremic patients are characterized by low RBC count and Hb concentration that are partially improved by HD [60,61]. The high RBC distribution width index [60–63] is associated with increased reticulocyte count, anisocytosis, and iron deficiency. In a context of iron sufficiency, the same index reflects inflammatory response and malnutrition issues, being positively correlated with serum CRP and ESA hyporesponsiveness, but inversely with serum albumin levels [63].

Uremic RBCs exhibit increased rigidity but reduced surface charge [64] and deformability compared to healthy controls [65,66]. Their susceptibility to osmotic lysis is pathologically increased [65, 67, 68] or decreased [69], as a result of aberrations in RBC membrane lipid content, antioxidant capacity, ATPases activity (where ATP is adenosine 5'-triphosphate) and plasma parathormone levels. Removal of uremic toxins and normalization of serum osmolarity by HD seem to improve osmotic fragility [65, 68]. RBC membrane vesiculation has been reported to be either normal [57] or increased [59]. Release of sialoglycoproteins-containing MPs is associated with reduced surface charge [64]. As a result of ion homeostasis disturbance, cellular volume regulation, oxygen delivery, and calcium-driven signaling are also affected. Indeed, decreased rate of anion transport [69], down-regulation of Na^+/K^+ pump and lower activity of Na^+/K^+, Mg^{2+}, and Ca^{2+} ATPases have been reported in uremic RBCs [69,70].

Plasma osmolarity, uremic toxins, and structural/functional modifications in RBC membrane pave the way for RBC shape perturbations to stomatocytes, knizocytes, spherocytes, echinocytes, and target cells [61, 64, 69, 71] that may influence blood rheology in ESRD [72, 73]. Regarding echinocytosis, reconstitution experiments with healthy blood have suggested a plasmatic factor as a probable contributor [72], although elevated levels of membrane cholesterol [67,69], lipid peroxidation [74], and calcium influx [75, 76] may also promote the echinocytic transformation of RBCs. HD induces a reversible increase in echinocytosis [72], however, final removal of uremic toxins lowers echinocyte percentage and improves membrane deformability compared to the pre-HD status [65, 72]. Stomatocytosis might be the result of an increased antioxidant activity [77], since it has been related with intracellular ROS levels and membrane-binding of protein markers of oxidative stress [61] (Table 1).

3.2 Redox homeostasis in ESRD RBCs

Once again, contradictory findings have been reported on the intracellular antioxidant capacity in ESRD. Several studies have reported insufficient antioxidant status in uremic RBCs, reflected in low GSH/GSSG (oxidized glutathione) ratio [78], low vitamin E [79], and antioxidant enzymes activity [13, 19, 79] but high, potentially pro-oxidant, vitamin A levels [10]. In these cases, the antioxidant factors are supposed to be severely affected by the uremia or overwhelmed by the sustained oxidative stress.

On the other side, overexpression or increased activity of antioxidant factors has also been reported in ESRD RBCs [13, 80], probably as a homeostatic response to the sustained oxidative and toxicity stresses, or as a result of reticulocytosis in rhEpO-treated patients. Elevated activity of GST is thought to be as a response of erythroid progenitors to the progressive inactivation of GST in circulating RBCs by uremic toxins [80].

The effect of dialysis on the activity of the RBC antioxidant enzymes is also ambiguous. The reported findings depend on whether HD triggers or not ROS generation in RBCs. GSH and ascorbate levels usually decrease [81] but eGST activity remains increased post-HD, probably due to the usage of undialyzable toxic substances as substrates [82].

Despite conflicting reports on antioxidant activity, there is a consensus regarding the oxidative damage of uremic RBCs. Although not common in all patients [61,71], pathologic accumulation of ROS and oxidative modifications in membrane proteins [61, 75] and lipids [83], have been reported in uremic RBCs. Uremic plasma, before and after HD, enhances ROS accumulation in healthy RBCs [75], while HD does not significantly affect it [61], highlighting the adverse effect of renal pathology on redox homeostasis [71]. Conversely, HD has been reported to increase membrane lipid peroxidation [83] and the susceptibility to ROS accumulation following oxidant stimuli [61]. Notably, deterioration of RBC protein carbonylation after HD has been related to cardiovascular mortality risk in retrospective studies [71].

The close relation between anemia and redox status in ESRD is highlighted in patients receiving antioxidant factors. According to various reports, vitamin E, ascorbate, GSH, or L-carnitine administration not only attenuate oxidative stress and promote antioxidant activity [79] but also improve anemia [84], rhEpO dose requirement and RBC functional properties, including membrane fragility and deformability [85,86].

Ascorbate and *N*-acetyl cysteine supplementation further improve the inflammatory response [87]. Notably, rhEpO that mainly targets anemia, exerts additional antioxidant effects on RBCs as shown in normal or thalassemic erythrocytes [88,89]. However, EpO regulates NO synthase activity in RBCs or facilitates GSH oxidation, thus acting either as a pro-oxidant or as an antioxidant factor, depending on L-arginine availability [90]. In fact, this dual effect is true for most of the antioxidant supplements offered to ESRD patients [91,92], indicating the need for further investigation of antioxidant therapies.

These findings in association with the previously reported increased levels of oxidative stress in nondialysis CKD patients, suggest that oxidative stress is an intrinsic state in uremia, and the uremic RBCs have successfully adapted to it. HD does not induce oxidative stress, although it might exacerbate it [93]. This kind of stress, which is very clearly reflected in RBC remodeling, may contribute to anemia through well characterized molecular pathways involving removal signaling and eryptosis.

3.3 RBC membrane composition and properties

The erythrocyte membrane plays a key role in RBC survival. However, uremia itself and the therapeutic strategies (rhEpO, HD), may affect certain RBC membrane proteins and lipids. Membrane remodeling in ESRD RBCs consists of protein deficiencies, aberrant lipid levels [64, 69], binding of cytosolic components (e.g. peroxiredoxin-2), protein aggregation/complexation (e.g. spectrin–Hb complex [94], fragmentation, oxidation /carbonylation (e.g. ankyrin [94]) and activation (e.g. of calpain following increase in cytosolic calcium) (Table 2). This kind of remodeling is the final outcome of elevated oxidative/calcium stress and premature aging.

The frequently reported deficiency in band-3 [61, 69, 95] and skeletal proteins [60,71] may affect the mechanical properties of the membrane and weaken the adhesion of cytoskeleton to the lipid bilayer, thus promoting vesiculation. Probably due to the fact that the rhEPO-treated patients have a younger RBC population compared to healthy subjects, modifications associated with the band-3-based model of RBC aging are not usually observed [69]. However, aberrations in CD47 marker-of-self protein and IgGs membrane binding have been found. Fluctuation in membrane expression of the water channel protein aquaporin-1 is probably related to the osmolarity of the uremic plasma, while higher expression of β-adducin [96], has been interpreted as a compensatory response to the increased RBC osmotic fragility and disturbed calcium homeostasis.

Removal of uremic toxins and damaged RBCs, restoration of plasma osmolarity, and the increased rate of erythrophagocytosis [97] probably account for the improved expression of some proteins (e.g. spectrin) [61] post-HD (Table 2). MPs release during HD may further assist the selective removal of stress markers. Duration of HD treatment has been associated with overexpression of glucose transporter-1, stomatin, and aquaporin-1 [71]. Interestingly, these proteins contribute to structural integrity [98], metabolism [99], vesiculation [100], and antioxidant activity of the RBCs [81]. In other cases, however, HD had a negative effect on the protein composition and the mechanical properties of the membrane [65].

The membrane proteome of the ESRD erythrocyte exhibits substantial differences compared to that of healthy subjects, but there is a high degree of interpatient variability, probably associated with different primary defects, comorbidities, and treatments. These complex associations indicate that all the factors which, either directly or indirectly, affect anemia, also impact the remodeling of the RBC membrane, with molecular events of defeat or defense in the battlefield of uremia [61, 62, 71, 96].

3.4 Removal signaling

Despite high variation among patients, distorted calcium homeostasis has been repeatedly reported in ESRD RBCs [76]. Extracellularly, the uremic plasma negatively affects the activity of erythrocyte Ca-pump [101] and Ca^{2+}-ATPase [102], resulting in calcium excess [75, 102, 103]. Intracellularly, the defective band-3 [95] inhibits the activity of voltage-dependent Ca^{2+} channels [103] and the ATP depletion inhibits the activity of Ca^{2+}/Mg^{2+}-dependent ATPase [104]. Deficiency of uremic RBCs in Ca^{2+} regulatory proteins [104], in addition to increased serum levels of parathormone have been associated with cytosolic excess and increased membrane permeability of Ca^{2+} [103], respectively, although the contribution of parathormone has not been verified by others [101, 104]. HD has been reported to ameliorate [104] or have no effect [75, 102, 103] on intracellular calcium levels. Notably, even in cases exhibiting similar intracellular calcium concentration pre- and post-HD, post-HD plasma causes significantly lower calcium accumulation in control RBCs compared to pre-HD plasma [75], supporting the Gafter's hypothesis that a dialyzable component of ESRD plasma triggers Ca^{2+} accumulation in uremic RBCs [102].

Increased activity of this universal signaling molecule is expected to activate Ca^{2+}-sensitive proteins (calpain, synexin, Gardos channel, scramblase, etc.) and pathways in uremic RBCs [105], such as the release of Hb-containing MPs, cytoskeleton stability, elastic deformation, osmotic resistance, ATP depletion, band-3 cleavage, NO production, shape, volume, and redox state homeostasis. For example, intracellular calcium concentration has been positively correlated with the incidence of echinocytosis and spherocytosis in ESRD [102].

Increased cytosolic Ca^{2+} activity has been further associated with the apoptotic death of uremic erythrocytes [64, 75, 106]. Eryptotic RBCs are characterized by shrinkage, membrane blebbing, ceramide formation, and phosphatidylserine (PS) exposure, and thus, by a rapid clearance from circulation. Although eryptosis may precede and protect against hemolysis, it is a part of anemia and aberrant

Table 2. Modifications in the expression of membrane proteins in ESRD erythrocytes compared to healthy subjects

Protein	Patients vs. control	RBC pathway	Ref.
Actin	Lower	Structural integrity	[61]
α-Adducin	Lower	Structural integrity	[71]
β-Adducin	Higher (pre-HD)		[96]
Ankyrin	Lower (pre-HD)	Structural integrity	[136]
Aquaporin-1	Lower (pre-HD)	Volume and shape regulation	[137]
	Higher (pre-HD)		[61, 71]
Band-3	Lower (pre-HD)	Aging, RBC removal, oxidative stress	[69, 95]
	Lower		[61]
	Lower (pre-HD)		[136]
	Higher (pre-HD)		[60]
Calpain-1	Higher	Calcium stress, eryptosis	[71]
CD47	Lower (post-HD)	Aging, RBC removal	[61, 71]
Clusterin	Lower	RBC aging, oxidative stress, MPs release	[71]
GAPDH	Higher	Energy metabolism	[60, 62]
	Lower		[71]
Glucose Transporter-1	Higher	Energy metabolism, structural integrity, redox homeostasis	[81]
	Lower		[71]
Hsp 71/72	Lower (pre-HD)	Proteome stress	[96]
Pallidin	Lower (post-HD)	Structural integrity	[61]
	Lower		[71]
Peroxiredoxin-2	Higher	Oxidative stress, calcium stress	[61]
	Higher		[71]
Stomatin	Higher (pre-HD)	MPs release	[62]
	Lower (post-HD)		[61]
Spectrin	Lower (pre-HD)	Structural integrity	[60, 62]
Spectrin fragments	Higher (pre-HD)	Oxidative stress, MPs release	[61]
			[71]
Spectrin-Hb complex	Higher	Aging, oxidative stress	[61]
Tropomodulin-1	Higher (pre-HD)	Structural integrity calcium stress	[96]
Ubiquitinylated proteins	Higher (post-HD)	Proteome stress, structural integrity	[61]

microcirculation. There is evidence that HD, in addition to dialyzable plasma components [75] and uremic toxins [107], triggers eryptosis in uremia, in part by increasing intracellular Ca^{2+}, ROS, and ceramide formation. It has been reported that EpO is not able to completely inhibit eryptosis since it favors intracellular calcium influx [88].

Either as a part of an activated program for eryptotic removal or not, uremic state has been related with increased PS exposure on RBCs, regardless of the dialysis treatment [97]. Although the identity of the uremic contributors remains unclear, serum creatinine has been suggested as candidate trigger in undialyzed patients [97]. rhEpO administration ameliorates PS exposure through preventing RBC oxidative imbalance, but it seems unable to inhibit PS externalization in calcium-stressed RBCs [88]. In other studies, a decrease in the number of PS exposing RBCs after EpO supplementation has been reported, resulting from partial inhibition of RBC cation channels [108]. PS exposure is expected to promote erythrophagocytosis [109], procoagulant phenotype [110] (in association with the release of PS-exposing MPs [64]) and increased adhesion to endothelium [111], thus decreasing NO release by endothelial NO synthase, an effect that has been associated with cardiovascular mortality risk in ESRD.

4 Integrated omics tools as a promising approach toward understanding uremic anemia

As analyzed before, uremic anemia is a multifactorial outcome. It is cited to be at the crossroad of inhibited erythropoiesis, shortened RBC survival, uremia, inflammation, and distorted redox and iron homeostasis (Fig. 1), in close interaction with many pathologies and risks for hospitalization, cardiovascular morbidity, and mortality [112, 113]. Although the correlations between the above-mentioned interacting parameters (Table 1) do not prove causality, they reflect the severity of ESRD, the severity of anemia, and other clinical outcomes.

Pathogenesis of anemia in ESRD remains terra incognita, since EpO deficiency is definitely not the sole cause. In the modern era of ESRD management, characterized by technological advances in HD methods and ESAs formulations, uremic RBCs still exhibit half of their normal lifespan. The cause of this detrimental discount is largely unknown, and so are the element(s) of the uremic milieu that contribute to it [3]. Even the management of anemia in ESRD patients is currently controversial due to the "anemia paradox" phenomenon. According to this, targeting Hb to >13g/dL is

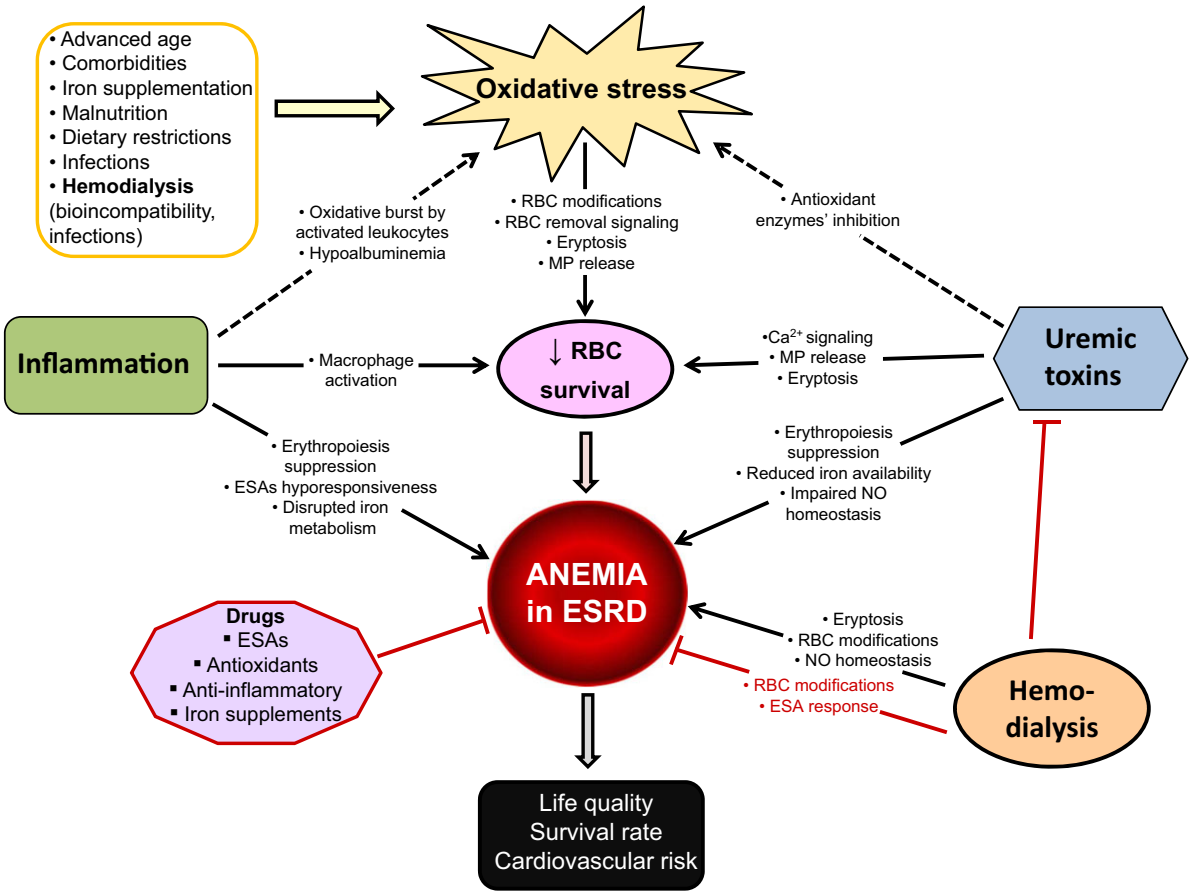

Figure 1. The currently established network of contributing (black arrows) and inhibiting (red arrows) factors in ESRD-associated anemia. Indirect effects of inflammation and uremic toxins toward reduced RBC survival (through oxidative stress) are shown by dotted arrows.

associated with increased cardiovascular events but paradoxically, if this level is achieved mortality is lower [114], suggesting that ESAs, especially at very high doses, have off-target effects in other tissues [112]. These findings highlight the need for a better understanding of the molecular mechanisms of anemia in ESRD and for a more individualized approach to anemia therapy, with the hope of developing new schemes that more closely target the underlying pathophysiology of low Hb, reducing in parallel the therapy-related adverse outcomes [96].

Notably, the primary and secondary contributors to anemia are imprinted in blood modifications observed in both cellular and soluble components [61,62,64,71,96]. Integrated omics approaches and high-throughput studies in uremic blood and RBCs may reveal proteins, metabolites, and other factors that mediate anemia and anemia-related morbidity in ESRD. In this direction, proteins differentially expressed between dialyzed and nondialyzed patients [96] and alterations in the lipid pattern [115] have been recently detected in the ESRD RBC membrane by omics analyses. Clinical laboratory markers, including alkaline phosphatase, IL-6 and CRP have already been associated with the clinical manifestations of the disease [116]. These approaches may target specific subpopulations of dialysis patients, e.g. with or without diabetes, responsive or not to rhEpO therapy etc. and thus might be more relevant to the underlying mechanisms involved in any specific pathophysiological sub-background.

There is a yet unexplored network of interactions between causal and contributing factors of anemia in dialyzed patients. Omics represent very sensitive tools for characterizing molecular changes in all the implicating parts: cells, MPs, plasma, urine, and dialysis fluids. They hold the potential for simultaneous identification and quantification of proteins and metabolites involved in oxidative stress, intercellular communication, transport of ions, cellular shape modification, energy metabolism, inflammation signaling, premature aging, cellular clearance, and apoptotic death, in addition to evaluating how they are modified in response to different primary defect backgrounds or ESAs therapy. Refinement of those data by modern bioinformatics tools is an effective way for maximizing the synergy between the current and future knowledge, to advance our understanding of the etiology of anemia and other complications in ESRD and thus to proceed toward the identification of novel uremia and risk

markers in high-risk ESRD patients. The pioneer proteomic [116], metabolomic [117–119], and lipidomic [115, 117, 120] studies on CKD plasma have paved the way to this ultimate goal.

Indeed, novel metabolic markers of ESRD (dicarboxylic acids, amino acid, and nucleotide derivatives, etc.) [117] have been identified in uremic plasma, serum, and urine by targeted and nontargeted metabolomics analyses. The pathophysiologically relevant alterations in glucose, steroid hormone, purine, NO, tryptophan, and lipid metabolism, in patients at different stages of CKD [119, 121] might be used to detect CKD earlier than traditional clinical methods [122]. Arginine derivatives for example, have been considered as promising candidates to identify individuals at risk of renal impairment and mechanisms of kidney disease progression [119]. HD has been associated with unexpected increases in several metabolites that suggest cellular hypoxia and activation of a broad catabolic program, including glycolysis, lipolysis, ketosis, and nucleotide breakdown [117, 118]. In the same context, proteomics approaches resulted in the detection of protein indicators of early CKD, such as α1-microglobulin [123], and of HD effects [124, 125]. Moreover, protein risk factors and mechanisms that promote cardiovascular disease in CKD have been proposed, based on proteomics data that suggest differential pathophysiological pathways involving coagulation, lipoprotein homeostasis, and inflammation in initial and advanced stages of kidney failure [124]. Disturbed triglyceride catabolism, beta-oxidation, and LDL lipidomic profile have been confirmed in advanced CKD [117, 126]. Extending the application of metabolite, proteome, and lipidome profiling to RBCs is a challenging task in the research field of ESRD that might be used to broaden our understanding on uremic anemia.

5 Conclusions

Anemia is associated with poor physical and clinical outcomes in ESRD patients undergoing HD and ESAs therapy. Despite being well described at the clinical laboratory level, its multifactorial nature in combination with the variable disease and therapy profiles among the patients, complicate the elucidation of the underlying molecular mechanisms and consequently, the efficacy of treatment. On the other side, all aspects of uremic anemia, from the multiple contributing factors to the relevant morbidity, are well imprinted in RBC and plasma modifications that are characteristic of the disease. Besides, reduced RBC lifespan is a major contributor to uremic anemia. Close examination of these data has revealed that anemia in ESRD is developed around a complex network of interacting pro-inflammatory and pro-oxidant factors under the orchestration of a highly toxic uremic environment. The therapeutic manipulations, namely ESAs, dialysis and pharmaceutical supplements, do not exhibit uniform effects on any of the variable factors which determine the severity of uremic anemia. Omics approach of uremic blood components, both cellular and soluble, is probably the most appropriate tool for characterizing molecular changes associated with the uremic anemia and their fluctuation in relation to the variables of the disease. These insights hold promise for the development of new diagnostic tests and therapies that directly target the pathophysiologic processes underlying this specific form of anemia.

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