KI REPORTS
KIReports.org

Prolonged Intradialytic Hypoxemia Is Associated With Functional Disturbances in Erythrocytes

Susan Hartzell[1], Meredith Haverly[1], Samira Farouk[1], Viktoriya Kuntsevich[2], Chiara Cantarelli[1,3,4] and Paolo Cravedi[1,4]

[1]Renal Division, Department of Medicine, Icahn School of Medicine at Mount Sinai, New York, New York, USA; [2]Division of Nephrology & Hypertension, Mount Sinai Beth Israel, New York, New York, USA; and [3]Dipartimento di Medicina e Chirurgia, Università di Parma, UO Nefrologia, Azienda Ospedaliero-Universitaria di Parma, Parma, Italy

Correspondence: Paolo Cravedi, Icahn School of Medicine at Mount Sinai, 1 Gustave L. Levy Place, New York, New York 10029, USA. E-mail: paolo.cravedi@mssm.edu

[4]Co-senior authors.

Received 11 May 2021; revised 30 June 2021; accepted 3 July 2021; published online 12 July 2021

Kidney Int Rep (2021) **6,** 2702–2705; https://doi.org/10.1016/j.ekir.2021.07.005

More than 10% of hemodialysis (HD) patients have prolonged intradialytic hypoxemia (PIH), a condition defined as arterial oxygen saturation below 90% for at least one-third of the treatment time.[1] This is a matter of concern, as PIH is associated with significantly increased morbidity and mortality.[1] In a large retrospective study in 983 HD patients, the risk of all-cause hospitalization was 2.2-fold higher in PIH patients compared to the nonhypoxemic group. Mortality risk was also significantly higher in HD patients with PIH, regardless from the presence of heart failure or chronic obstructive pulmonary disease (COPD).[1]

Intriguingly, patients with PIH also have higher requirement of erythropoietin (EPO) and other erythropoiesis-stimulating agents (ESAs), a finding that is counterintuitive, because hypoxia is the main stimulus for residual endogenous production of EPO.[1] To start addressing this issue, we reasoned that hypoxemia has been shown to impair resilience against reactive oxygen species (ROS) and may increase erythrocyte apoptosis (eryptosis), characterized by reduced cell dimension, bleb formation, and phosphatidylserine exposure.[2] Therefore, we hypothesized that higher ESA requirement is the result of higher red blood cell (RBC) fragility and shorter half-life in PIH patients, due to an accumulation of intracellular reactive oxygen species (iROS).

To test this idea, we designed a cross sectional study to compare RBC phenotype and function in HD patients with or without PIH and in healthy controls. In HD patients, online intradialytic arterial oxygen saturation (from arteriovenous fistula) was monitored using the Crit-Line Monitor[3] (see Supplementary Methods).

We collected blood samples and measured eryptosis and iROS production in RBCs from patients with or without PIH (see Supplementary Methods). Finally, we measured reticulocytes (RCs) and levels of the different RC subsets.

RESULTS

Patient Characteristics

From March 2019 to October 2020, we included 56 patients on chronic HD and 18 healthy controls. Of the 56 HD patients, 12 had PIH. Although PIH has been previously associated with older age and longer dialysis vintage,[1] in our cohort demographic characteristics did not significantly differ between HD patients with or without PIH. Kt/V was also similar between the 2 groups.

Consistent with prior reports,[1] PIH patients had lower hematocrit levels, despite significantly higher use of ESA (Table 1[4]). Healthy controls were significantly younger than HD patients (Table 1).

HD Patients With PIH Have High levels of Eryptosis and iROS

We started our analyses by measuring eryptosis and found that HD individuals with PIH had significantly higher levels of annexin-V−positive RBCs than healthy controls. In contrast, annexin-V−positive RBCs tended to be higher in HD patients without PIH compared to healthy controls, but this difference did not reach statistical significance (Figure 1a, b).

We next tested the hypothesis that increased eryptosis in HD patients with PIH is due to increased

Table 1. Patient characteristics and clinical parameters at sample collection

Characteristic or parameter	Controls n = 18	HD patients, total n = 58	HD hypoxemic patients n = 12	HD nonhypoxemic patients n = 46
Age, yr	**32.3 ± 7.0**	**64.4 ± 14.4**	68.2 ± 14.6	63.4 ± 14.3
Men, %	6 (33.3%)	35 (59.3%)	7 (58.3%)	28 (60.9%)
Race, %				
Asian	2 (11.1%)	3 (5.2%)	1 (8.3%)	2 (4.3%)
Black	**0 (0%)**	**29 (50.0%)**	5 (41.7%)	24 (52.2%)
White	**16 (88.9%)**	**26 (44.8%)**	6 (50.0%)	20 (43.5%)
Ethnicity, %				
Hispanic or Latino		11 (19.0%)	1 (8.3%)	10 (21.7%)
Dialysis vintage, yr		4 (1.75-8)	1 (1-3)	5 (2-9)
Dialysis adequacy, Kt/V[a]		1.60 ± 0.31	1.58 ± 0.29	1.60 ± 0.31
Comorbidities, %				
Diabetes		20 (34.4%)	2 (16.7%)	18 (39.1%)
Hypertension		45 (77.6%)	8 (66.7%)	37 (80.4%)
COPD		6 (10.3%)	3 (25.0%)	3 (6.5%)
CHF		19 (32.8%)	3 (25%)	16 (34.8%)
Systolic BP		145.1 ± 22.0	140.5 ± 22.4	146.4 ± 21.9
Diastolic BP		75.3 ± 14.5	68.8 ± 12.1	77.2 ± 14.7
Laboratory tests				
WBC count, 1000/μl		6.9 ± 2.1	7.36 ± 2.5	6.77 ± 2.0
RBC count, 1000/μl		3.8 ± 0.4	3.6 ± 0.4	3.79 ± 0.5
sAlbumin, g/dl		3.9 ± 0.3	3.8 ± 0.5	3.9 ± 0.3
HGB, g/dl		11.1 ± 1.2	10.5 ± 0.81	11.2 ± 1.3
HCT, %		35.3 ± 3.8	**33.70 ± 2.81**	**35.69 + 3.95**
MCV, fl		94.5 ± 6.7	94.4 ± 7.7	94.6 + 6.5
MCH, pg/cell		30.0 ± 2.4	30.0 ± 3.2	30.1 + 2.4
RDW, %		16.3 ± 2.0	17.12 ± 2.2	16.1 + 1.9
Ferritin, ng/ml		1002 (584.9-1256.5)	625 (583.3-1069)	1005 (594.5-1334)
PTH, pg/ml		596 (379-996.3)	485 (383-793)	632 (377-1048)
Calcium		8.9 ± 0.6	9.0 ± 0.4	8.8 ± 0.6
Potassium		5.0 ± 0.7	5.1 ± 0.5	5.0 ± 0.7
Therapy				
ESA, %		26 (44.8%)	**9 (75.0%)**	**17 (37.0%)**
Iron, %		18 (31.0%)	4 (33.3%)	14 (30.4%)
Antihypertensive, %		11 (19.0%)	2 (16.7%)	9 (19.6%)
ACEi, %		4 (6.9%)	0 (0%)	4 (8.7%)
Vitamin D, %		54 (93.1%)	10 (83.3%)	44 (95.7%)

Data are represented as mean ± standard deviation or as median (interquartile range). Numbers in boldface type are significantly different between groups. Significance set at $P < 0.05$. ACEi, angiotensin converting enzyme inhibitor; BP, blood pressure; CHF, congestive heart failure; COPD, chronic obstructive pulmonary disease; ESA, erythropoiesis stimulating agents; HCT, hematocrit; HD, hemodialysis; HGB, hemoglobin; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume; PTH, parathyroid hormone; RBC, red blood cell; RDW, red cell distribution width; WBC, white blood cell.
[a]Kt/V was calculated based on the Daugirdas formula.[4]

intracellular ROS production. The analyses were performed within 2 hours from withdrawal to prevent altered iROS production. As shown in Figure 1c, d, HD patients with PIH had significantly higher iROS levels than nonhypoxemic patients. Consistent with previously published results,[S1] there was no significant difference in the iROS levels between healthy controls and nonhypoxemic HD patients, regardless of hypoxic status.

Altogether, these data support the hypothesis that PIH increases iROS production in RBCs, leading to accelerated RBC eryptosis and loss. This mechanism explains, at least in part, the increased requirement for ESA in HD patients with PIH.

PIH Is Not Associated With Higher Levels of Inflammatory Markers

An alternative, additional mechanism responsible for higher ESA requirement in PIH patients could be an impaired bone marrow response to ESA, leading to lower reticulocyte production. One of the main reasons for resistance to ESA therapy is chronic inflammation, a condition that often associates with PIH.[1] We analyzed the levels of serum ferritin and albumin, markers of systemic inflammation.[5] Ferritin levels in HD patients were high overall, but did not significantly differ between patients with or without PIH. Similarly, we found no significant difference in serum albumin levels between the 2 HD groups (Table 1).

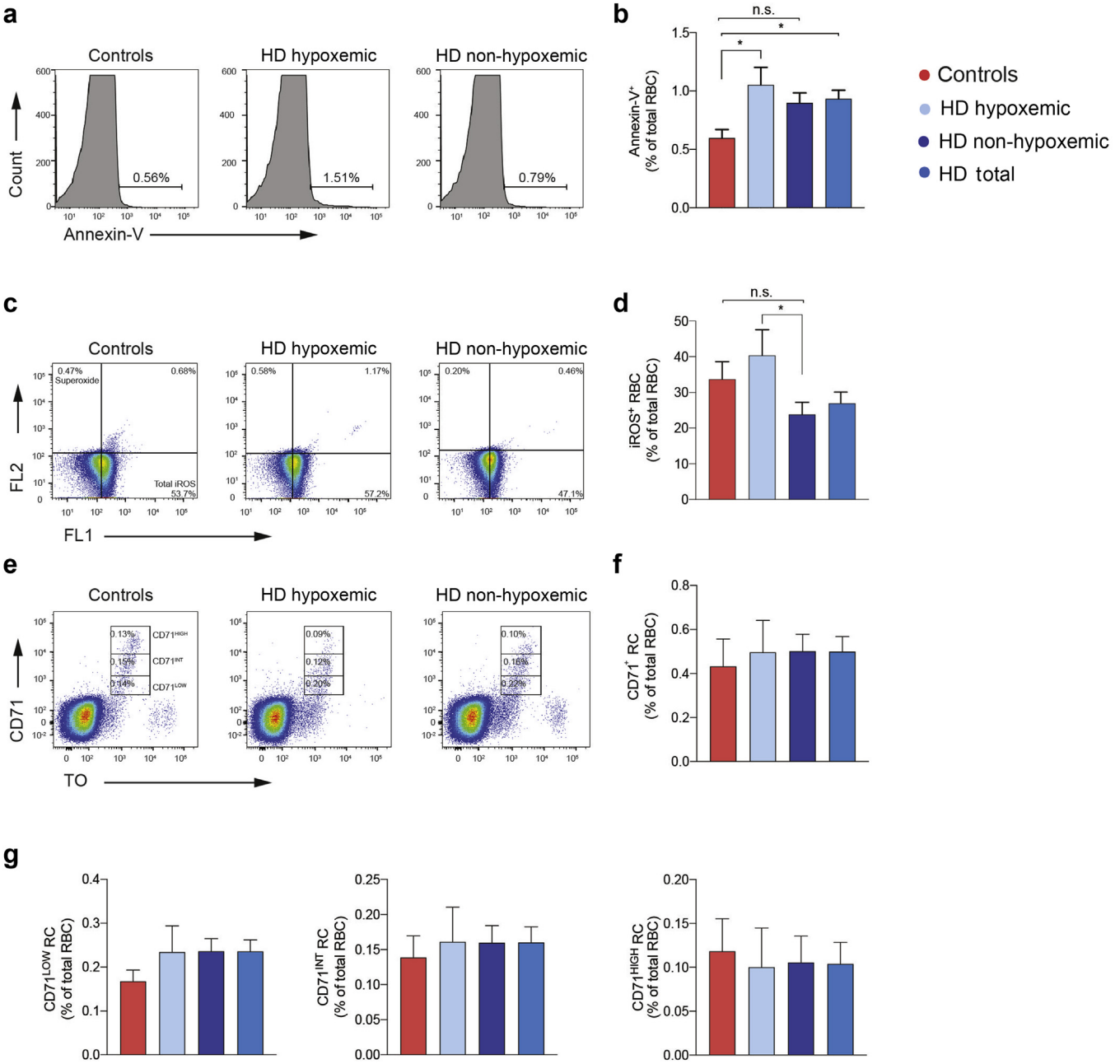

Figure 1. Red blood cell (RBC) phenotypic characterization. (a) Representative plots and (b) data quantification of annexin V^+ RBCs. (c) Representative plots and (d) quantification of $iROS^+$ RBCs. (e) Representative plots of reticulocyte populations and (f) quantification of total $CD71^+$ reticulocytes. (g) Quantification of $CD71^+$ reticulocyte subpopulations. Quantifications of flow cytometry data are shown as average ± standard error of the mean. $*P < 0.05$. iROS, intracellular reactive oxygen species; n.s., not significant.

No Significant Differences in Reticulocyte Counts Were Found Between HD Patients With or Without PIH

Next, we measured percentages of total reticulocytes and of those with high, intermediate, and low expression of CD71 (transferrin receptor), the expression of which is lost during reticulocytes maturation[6] (see Supplementary Methods).

We found no significant differences in the total reticulocyte percentages (Figure 1e, f) or in any of the 3 subpopulations (Figure 1g), suggesting no impaired response to EPO in HD patients with PIH.

DISCUSSION

Multiple causes have been implicated in the pathogenesis of PIH, including, among others, intradialytic hypotension, sleep apnea, congestive heart failure, and COPD.[7] Regardless of its etiology, PIH is a serious medical condition associated with intradialytic complications, such as hypotension and cramps,[7] but also with poor clinical outcomes in the long term.[1]

Hemodialysis patients with PIH have increased ESA requirements, and our data support the concept that this phenomenon is due to increased RBC eryptosis and

turnover, induced by iROS accumulation. The levels of total reticulocytes and the percentages of reticulocytes in different stages of maturation were not significantly different between PIH patients and non-PIH controls, suggesting that their response to EPO is not impaired.[1]

Although EPO deficiency represents a central mechanism in the pathogenesis of anemia in HD patients, the process is indeed multifactorial, and involves systemic inflammation that reduces the response to EPO by erythroid cells in the bone marrow. Although HD patients in our study showed signs of inflammation compared to healthy controls, we did not detect significant differences in ferritin or serum albumin levels between PIH and non-PIH patients, suggesting that increased ESA requirement in PIH patients was not due to more severe chronic inflammation.

Reduced RBC survival in HD patients can be promoted by different stimuli, including oxidative stress, various diseases (including diabetes, heart failure, and sepsis) and also uremic toxins[8] that promote eryptosis by increasing iROS production.[9] A recent study showed that uremic toxin indoxyl sulfate increases iROS production in a dose-dependent manner, in both hypoxic and nonhypoxic conditions, suggesting that hypoxia and uremic toxins promote eryptosis in an independent manner.[S2] We did not have information on uremic toxins in our cohort, but this is a variable that will be important to consider in future studies.

The major limitation of our report is the relatively small sample size. However, the results of our comprehensive RBC phenotypic analyses will form the basis for the sample size estimation of larger investigations.

The pathophysiology of PIH is only partially understood and our findings are important, as they support the concept that increased ESA requirement in PIH patients is due, at least in part, to increased RBC fragility. Our study provides a rationale for future studies testing the hypothesis that therapeutic strategies for anemia in PIH should aim at prolonging RBC survival (possibly by reducing ROS production), together with increasing erythrocyte production.

DISCLOSURE

All the authors declared no competing interests.

ACKNOWLEDGMENTS

This project was supported by a grant from Renal Research Institute (RRI), New York, NY.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Supplementary Methods

Supplementary References

REFERENCES

1. Meyring-Wösten A, Zhang H, Ye X, et al. Intradialytic hypoxemia and clinical outcomes in patients on hemodialysis. *Clin J Am Soc Nephrol*. 2016;11:616–625.
2. Lang F, Lang E, Foller M. Physiology and pathophysiology of eryptosis. *Transfus Med Hemother*. 2012;39:308–314.
3. Balter P, Artemyev M, Zabetakis P. Methods and challenges for the practical application of Crit-Line™ monitor utilization in patients on hemodialysis. *Blood Purif*. 2015;39:21–24.
4. Daugirdas JT. Second generation logarithmic estimates of single-pool variable volume Kt/V: an analysis of error. *J Am Soc Nephrol*. 1993;4:1205–1213.
5. Kalantar-Zadeh K, Kalantar-Zadeh K, Lee GH. The fascinating but deceptive ferritin: to measure it or not to measure it in chronic kidney disease? *Clin J Am Soc Nephrol*. 2006;1(Suppl 1):S9–S18.
6. Borrione P, Spaccamiglio A, Parisi A, et al. A biparametric flow cytometry analysis for the study of reticulocyte patterns of maturation. *Int J Lab Hematol*. 2010;32:65–73.
7. Campos I, Chan L, Zhang H, et al. Intradialytic hypoxemia in chronic hemodialysis patients. *Blood Purif*. 2016;41:177–187.
8. Lang E, Lang F. Triggers, inhibitors, mechanisms, and significance of eryptosis: the suicidal erythrocyte death. *Biomed Res Int*. 2015;2015:513518.
9. Dias GF, Bonan NB, Steiner TM, et al. Indoxyl sulfate, a uremic toxin, stimulates reactive oxygen species production and erythrocyte cell death supposedly by an organic anion transporter 2 (OAT2) and NADPH oxidase activity-dependent pathways. *Toxins (Basel)*. 2018;10.