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Hemodiafiltration improves red blood cell lifespan in patients with end-stage renal disease

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Abstract

Introduction: Uremic toxin-induced shortening of red blood cell (RBC) lifespan is an important mechanism of anemia in end-stage renal disease (ESRD). Conventional hemodialysis does not improve RBC lifespan; the efficacy of hemodiafiltration (HDF) for alleviating RBC lifespan has not yet been evaluated in patients with ESRD.

Methods: Twenty-three patients with ESRD in maintenance hemodialysis were enrolled. Baseline data for sex, age, dialysis vintage, pre-dialysis hemoglobin (Hb), blood urea nitrogen (BUN), intact parathyroid hormone (iPTH), single pool Kt/V (spKt/V), and plasma indophenol sulfate (IS) were collected. RBC lifespans before and after one session of HDF were compared. The resultant differences were subjected to correlational analyses with baseline data.

Results: RBC lifespan increased from 73 (66, 89) days at baseline to 77 (71, 102) days after a single HDF treatment ($p = 0.034$). Meanwhile, plasma IS concentration decreased from 113.05 (80.67, 133.05) mg/L to 83.87 (62.98, 96.78) mg/L ($p < 0.001$). RBC lifespan increases correlated negatively with Hb levels.

Conclusions: A single HDF treatment improved RBC lifespan in ESRD patients on maintenance hemodialysis, with more severe pre-HDF anemia at baseline being associated with greater increases in RBC lifespan.

1 | INTRODUCTION

Anemia is a major manifestation of end-stage renal disease (ESRD) and an important prognostic factor for patients with ESRD. Uremic toxins play an important role in accelerating the destruction of red blood cells (RBCs).[1] Conventional hemodialysis, the primary life-extending treatment option for ESRD, is not effective for alleviating RBC lifespan reduction.[2] Recent large-sample observational studies and multicenter randomized controlled trials have shown that online hemodiafiltration (HDF), which removes medium and large uremic toxins from plasma to a greater extent than conventional hemodialysis, can reduce the need for erythropoietin therapy and support RBC replenishment.[3,4] In vitro data showing that medium- and large-molecule uremic toxins, such as indophenol sulfate (IS) and acrolein, among others, stimulate eryptosis and RBC death[5,6] suggest that HDF-mediated removal of uremic toxins may reduce RBC destruction.

RBC lifespan refers to the duration that RBCs survive in circulation after they are released from bone marrow. The average RBC lifespan in healthy people is about 120 days. A shortened RBC lifespan is a reliable marker of accelerated RBC destruction. However, classical RBC markers, such as ^{51}Cr, ^{15}N-glycine, and biotin, cannot be

used for immediate monitoring of HDF effects on RBC destruction because they take weeks or months to measure. Fortunately, Levitt's CO breath test, based on the discovery that endogenous CO in exhaled breath originates mainly from heme oxidation associated with hemoglobin (Hb) degradation following RBC rupture, can be used to measure RBC lifespan within 15 min with results similar to those produced by classical methods.[7,8] Applying a simple, rapid, and accurate test, Medina et al.[9] found that hemodialysis with low-biocompatibility cellulose acetate membrane dialyzers resulted in a further reduction in RBC lifespan. In contrast, we found that hemodialysis with synthetic polyethersulfone membrane dialyzers with better biocompatibility did not affect RBC lifespan.[10]

Based on the above observations, the aims of the present study were twofold. Firstly, we sought to use Levitt's CO breath test to examine RBC lifespan in ESRD patients on maintenance hemodialysis. Secondly, we investigated whether a single online HDF treatment with a polyflux membrane dialyzer could prolong RBC lifespan.

2 | METHODS

2.1 | Study subjects

A group of 23 ESRD patients on maintenance hemodialysis were recruited between April 2019 and April 2020 from the outpatient dialysis clinics of Nanshan Hospital of Guangdong Medical University (Huazhong University of Science and Technology Union Shenzhen Hospital) in Shenzhen, China. All patients were on thrice-weekly hemodialysis (4 h per session). The inclusion criteria were being ≥18 years old; having been on dialysis for ≥3 months; and being a nonsmoker or having quit smoking at least 3 months prior. The exclusion criteria included malignancy; hematologic disorders; acute infection; and unwillingness to participate. ESRD was diagnosed in accordance with the 2012 KDOQI guidelines.[11] The study complied with ethical standards for human trials and was conducted ethically in accordance with the World Medical Association Declaration of Helsinki (as revised in Tokyo 2004). The protocol for the research project was approved by the ethics committee of Nanshan Hospital of Guangdong Medical University (Huazhong University of Science and Technology Union Shenzhen Hospital) (No. 2017080101). All patients provided written informed consent to participate in the study. Our study has been registered in Chinese clinical trial registry with the registration number of ChiCTR2100043150.

2.2 | Baseline data collection

General clinical data and laboratory findings at the time of patient enrollment were collected, including sex, age, dialysis history, primary disease of renal failure, pre-dialysis Hb, blood urea nitrogen (BUN), intact parathyroid hormone (iPTH), and single pool Kt/V (spKt/V) as baseline values. Routine blood analysis was performed in a Sysmex XN fully automatic hematology analyzer with accompanying reagents. Routine biochemical analysis for BUN was performed in a Hitachi 7600 automatic biochemistry analyzer with related reagents. iPTH analysis was performed in a Siemens fully automated chemiluminescence immunoassay machine with supporting reagents. Before the specimens were tested, calibration and quality curve control were performed with standards according to the operating instructions of the reagent kits.

2.3 | HDF procedure

A 4-h post-dilution HDF session was conducted in the morning for eligible patients. Fresenius 5008S dialysis machine was used with online HDF functionality and Gambro Polyflux 140H dialyzers. The blood flow rate was set on 230–280 ml/min. On average, 17 L of replacement fluid was infused.

2.4 | RBC lifespan measurement

Levitt's CO breath test was applied to determinate the RBC lifespan.[7,12] The principle of the breath test is that mean RBC lifespan can be measured as the total capacity of CO from Hb degradation divided by the quantity of CO released per day. According to the study design, RBC lifespans of pre- and post-HDF were measured, respectively. Alveolar air samples were collected 5 min before HDF and immediately after the completion of HDF. Immediately after each alveolar gas sample collection, double venous anticoagulant samples were taken, one for Hb concentration measurement by Sysmex XN fully automatic hematology analyzer and the other for plasma IS measurement (see below).

The procedures of alveolar air sample collection were as following: After a deep inspiration, each subject was instructed to hold his or her breath for 10 s and then exhale into the collection system through a mouthpiece. The system discarded the first 300 ml of volume, which was considered to contain dead space gas, and then directed subsequent alveolar air into a foil collection bag. If needed, the procedure was repeated until the collected air sample reached 1000 ml. The thus filled 1000-ml bag was detached and sealed immediately. Atmospheric samples were collected just after breath sampling. Alveolar air and atmospheric samples were stored at room temperature and analyzed within 3 days.

The instrument used to determine RBC lifespan was the ELS Tester (Seekya Biotec Co., Ltd., Shenzhen, China), which measures endogenous CO by nondispersive infrared comparison of the CO content within an alveolar air sample versus that in the accompanying atmospheric air sample according to Levitt's formula.[7] Operation of this instrument involved three steps: (1) addition of alveolar and environmental samples; (2) inputting of Hb concentration; and (3) pressing a start button. The measurements and calculations for each assessment were completed within 15 min.

2.5 | Plasma IS measurement

The sampling time of pre- and post-HDF plasma IS measurement was exactly the same as that of RBC lifespan measurement (see above). A Thermo Fisher U3000 high-performance liquid chromatograph was used for the determination of plasma IS levels.[13] Each plasma sample (precisely pipetted, 300 μl) was combined with 400 μl of acetonitrile and centrifuged at 4800 rotations/min for 30 min. Subsequently, 200 μl of supernatant was pipetted into an autosampler bottle, of which 5 μl was injected into the sample. Chromatographic separation was completed on an Ultimate XB-C18 liquid chromatographic column with the initial mobile phase A of 10 mmol/L acetic acid and 2.7 mmol/L trifluoroacetic acid aqueous solution and a phase B solution of acetonitrile (A:B = 82.5:17.5) for 10 min. The 10th minute mobile phase was changed to A:B = 55:45 and maintained for 10 min; the injection interval equilibration time was 10 min. The whole process took 30 min. The IS standard curve settings were 1.0, 5.0, 10.0, 15.0, and 20.0 mg/L.

2.6 | Statistical analysis

RBC lifespan changes after one HDF session, and the relationship of RBC lifespan changes with IS changes and with baseline clinical parameters were analyzed in SPSS 22.0 (SPSS for Windows, version 22, SPSS, Chicago, IL). Normally distributed data are reported as means with standard deviations (SDs). Non-normally distributed data are reported as medians with interquartile ranges (IQRs). Counts are reported with percentages. The Pearson and Spearman analyses were used to analyze correlations between normally and non-normally distributed datasets, respectively. Paired *t* tests and non-parametric tests were performed to compare data before versus after HDF treatment for normally and non-normally distributed data, respectively. In all cases, $p < 0.05$ was considered statistically significant.

3 | RESULTS

3.1 | Patient characteristics

A total of 23 patients with ESRD on maintenance hemodialysis were enrolled in the study. Their clinical characteristics and baseline laboratory values are listed in Table 1.

3.2 | Changes in RBC lifespan and plasma IS

As illustrated in box plots in Figure 1 and Table 2, one HDF session increased ESRD patients' median RBC lifespan significantly from 73 (66, 89) days to 77 (71, 102) days ($p = 0.034$). Meanwhile, the HDF session decreased median plasma IS concentrations from 113.05 (80.67, 133.0547) mg/L to 83.867 (62.9785, 96.7846) mg/L ($p < 0.001$).

TABLE 1 Baseline (pre-dialysis) patient characteristics ($N = 23$)

Characteristic	Median value (IQR)[a]
Male patients, *N* (%)	15 (65.2)
Mean age ± SD, years	54 ± 14
Duration of dialysis history, months	39.5 (19, 74)
RBC lifespan, days	73 (66, 89)
Primary disease, N	
Chronic glomerulonephritis	11
Diabetic nephropathy	6
Gouty kidney damage	2
Polycystic kidney	1
Autoimmune disease	1
Chronic pyelonephritis	1
Obstructive nephropathy	1
Blood variables	
Hb, g/L	110.5 (106, 126)
Kt/V	1.20 (1.14, 1.30)
BUN, mmol/L	22.5 (20.3, 28.3)
iPTH, pmol/L	37.1 (16.2, 51.8)
IS, mg/L	113.05 (80.67, 133.05)

[a]Median (IQR) unless otherwise indicated.

3.3 | Variable correlations with RBC lifespan change

Spearman's bivariate correlation analyses were conducted to examine the relationships of RBC lifespan changes following HDF with baseline characteristics, including age, sex, dialysis vintage, spKt/V, IS, Hb, BUN, and iPTH values. As shown in Figure 2, we found that increases in RBC lifespan correlated inversely with Hb ($r = -0.577$, $p = 0.004$). There was no correlation with other parameters (Figures 3–5).

4 | DISCUSSION

In contrast to our previous study where a cohort of 17 nonsmoking patients with ESRD undergoing conventional hemodialysis did not show any significant difference of RBC lifespan,[10] this within-subject control study revealed that RBC lifespan was immediately and significantly prolonged after a single HDF treatment session treatment in 23 maintenance hemodialysis patients diagnosed with ESRD. This prolongation was accompanied by a decrease in plasma IS concentrations; and a greater the degree of anemia before the treatment was associated with greater prolongation of RBC lifespan.

Loge and colleagues[14] found that infusion of RBCs from patients with chronic renal failure into healthy subjects restored a normal RBC lifespan in these cells, whereas infusion of RBCs from healthy subjects into patients with chronic renal failure resulted in a shortening of the lifespan of the transferred RBCs. These studies suggest plasma constituents, presumably uremic toxins, that have a direct toxic effect on

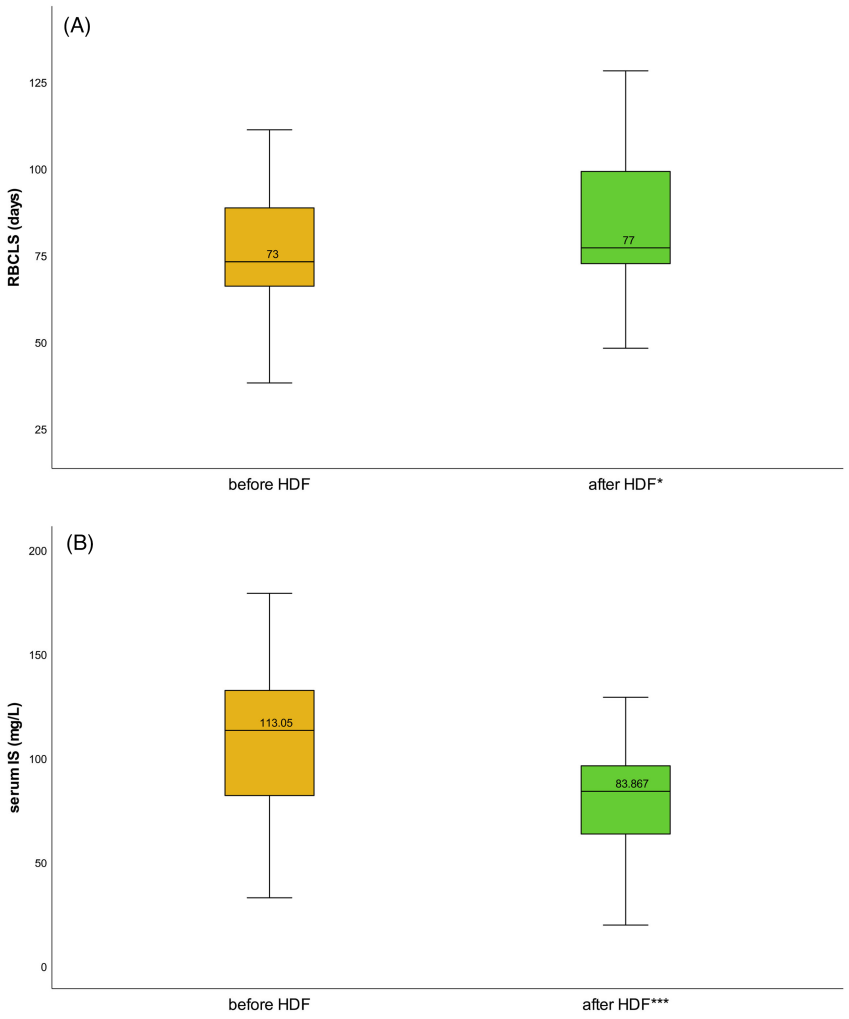

FIGURE 1 Comparison of pre-hemodiafiltration (HDF) and post-HDF red blood cell (RBC) lifespan (A) and serum indophenol sulfate (IS) levels (B) before versus after one HDF session; $^{*}p = 0.034$, $^{***}p < 0.001$ [Color figure can be viewed at wileyonlinelibrary.com]

Characteristic	Pre-HFD	Post-HDF	*p* value
RBC lifespan, days	73 (66, 89)	77 (71, 102)	0.034
Plasma IS, mg/L	113.05 (80.67, 133.0547)	83.867 (62.9785, 96.7846)	<0.001

TABLE 2 Changes in RBC lifespan and plasma IS ($N = 23$)

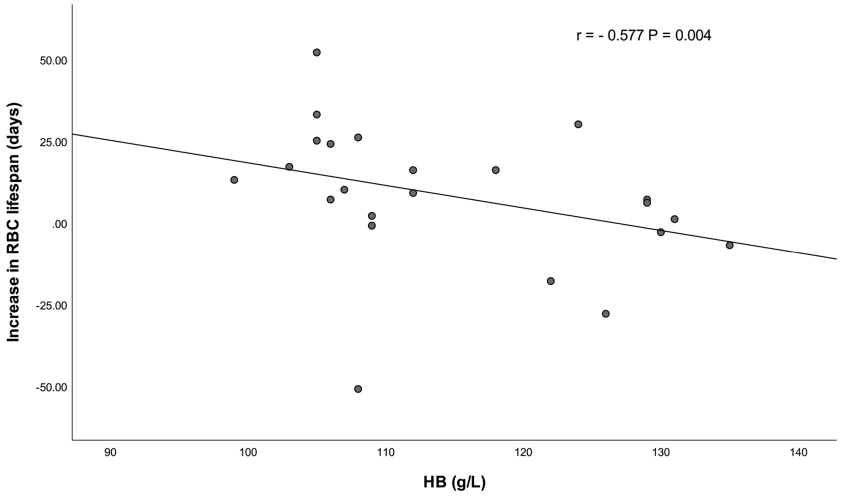

FIGURE 2 Negative correlation between increase in RBC lifespan from baseline to posttreatment and baseline Hb

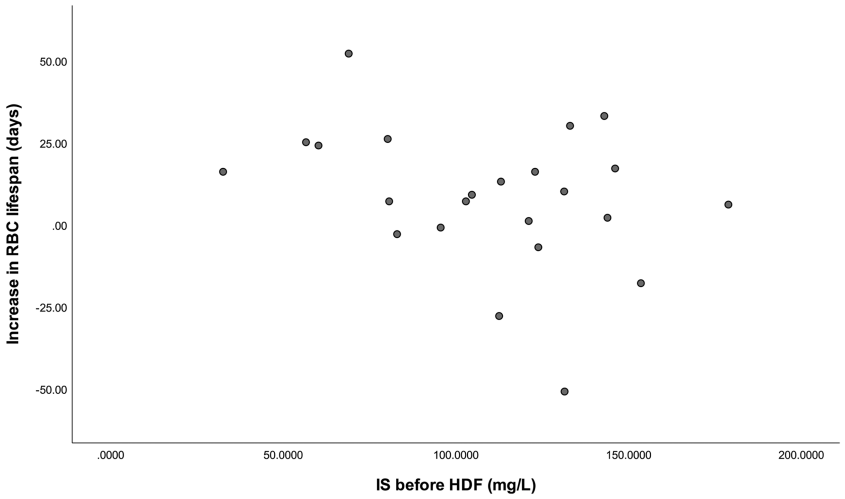

FIGURE 3 No correlation between increase in RBC lifespan from baseline to posttreatment and baseline IS

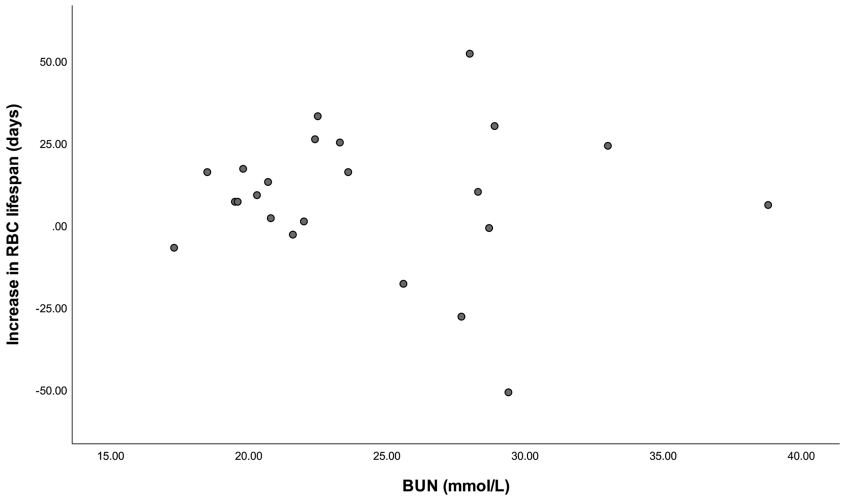

FIGURE 4 No correlation between increase in RBC lifespan from baseline to posttreatment and baseline BUN

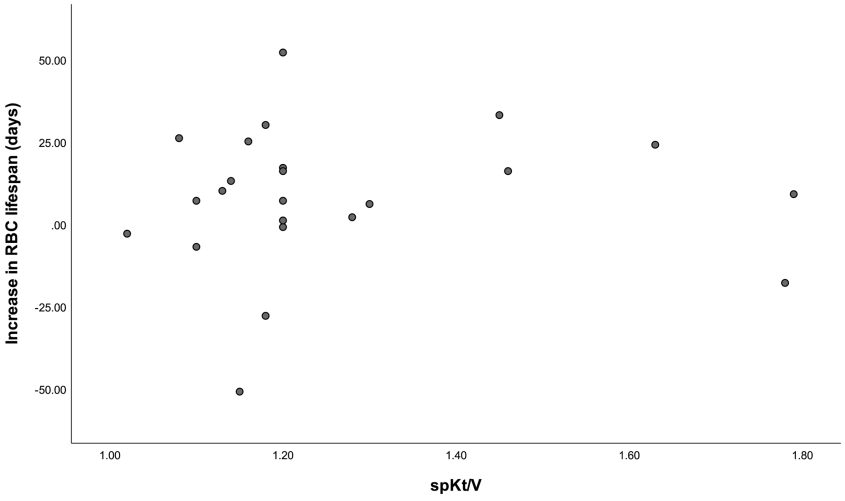

FIGURE 5 No correlation between increase in RBC lifespan from baseline to posttreatment and baseline spKt/V

RBCs cannot be removed effectively by conventional dialysis. In the present study, we observed a dramatic increase in RBC lifespan, a difference of about 5 days, following a single HDF treatment session. These results indicate that the erythrotoxic molecules in the plasma of uremic patients are medium to large uremic toxins that can be cleared by HDF, corroborating the findings of Sirolli et al.,[15] who found that peripheral RBC decay was reduced in patients treated with modified HDF (i.e., HDF with online endogenous reinfusion). Therefore, it is

reasonable to postulate that the mechanism by which HDF reduces the need for erythropoietin therapy and thus makes anemia easier to correct, compared to conventional hemodialysis, involves a prolongation of RBC lifespan owing to removal of medium to large uremic toxins.[3,4,13] Furthermore, we found that the magnitude of improvement in RBC lifespan correlated negatively with degree of anemia before HDF treatment. Consequently, the ESRD patients with the most severe anemia before HDF treatment showed the greatest improvement in RBC lifespan after HDF treatment. We thus speculate that ESRD patients with more severe anemia have higher blood concentrations of medium to large uremic toxins.

The uremic toxin IS is a well-studied protein-binding molecule. In in vitro cellular experiments, Ahmed et al.[5] demonstrated that IS (50–600 μM) increases phosphatidylserine exposure on the surface of RBCs, reduces RBC volume, and promotes eryptosis by increasing cytosolic Ca^{2+} activity and ceramide concentration. Higher IS concentrations generated more pronounced effects. It has been proved that hemofiltration can effectively remove IS from plasma. For example, Abad et al.[16] reported that a single HDF treatment could reduce IS by an average of 48.7 ± 14.1%. The immediacy of IS clearance after a single session HDF treatment observed in our study is consistent with the notion that RBC lifespan expansion after HDF may be related to the clearance of IS from plasma. However, we did not observe a significant correlation between IS concentration and RBC lifespan change after HDF. Similarly, our study had shown that BUN, BUN clearance (Kt/V), or iPTH did not correlate with RBC lifespan change either. So it is possible that another not yet to be identified toxin may have a more pronounced effect on RBC survival than these factors.[17–19]

A major limitation of this study is that it was performed at a single center with a small sample. Thus, we did not have the power to identify factors that influence changes in RBC lifespan. Future research may need an in vitro erythrocyte incubation test to compare the effects of plasma of prior- and post-HDF on the integrity of healthy donor erythrocytes to support the findings of this study. Nevertheless, the finding that one session of HDF prolongs RBC lifespan dramatically in this patient population is clinically important. We might expect a further improvement after serial HDF treatments.

5 | CONCLUSION

One HDF treatment session improved RBC lifespan in ESRD patients, with the magnitude of benefit being greatest in those patients with the most severe anemia. The mechanism by which HDF improves RBC lifespan may be related to the removal of medium- to large-molecule uremic toxins. Regular intensive HDF may improve refractory ESRD anemia.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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