# Haemolysis in Patients with End-Stage Renal Disease Receiving Haemodialysis: A Cross-Sectional Study

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# Abstract

**Background:** Haemolysis due to mechanical stress on red blood cells (RBCs) during extracorporeal circulation has been reported in patients receiving haemodialysis. However, little is known about the incidence of, and the mechanisms underlying haemolysis in patients receiving maintenance haemodialysis. We sought to investigate the incidence and underlying mechanisms of haemolysis in patients with end-stage renal disease undergoing maintenance dialysis by measuring erythrocyte creatine.

**Method:** In a cross-sectional study, we evaluated 69 patients with end-stage renal disease receiving haemodialysis (n = 55) or peritoneal dialysis (n = 14). Erythrocyte creatine, a quantitative marker of mean RBC age, was measured with other haemolytic markers including haptoglobin, reticulocyte count and lactate dehydrogenase.

**Results:** RBC age was significantly shorter [47.7 (43.2–52.8) days vs. 59.8 (56.0–66.2) days, $p<0.0001$], and haptoglobin was significantly lower in haemodialysis patients than in those with peritoneal dialysis, although haemoglobin levels were comparable in the two groups. When haemodialysis patients were stratified by median RBC age (47.7 days), patients with shortened RBC age had higher intradialytic ultrafiltration rates, larger erythropoiesis-stimulating agent doses, and a higher erythropoietin resistance index compared to that of those with preserved RBC age.

**Conclusions:** Haemolysis exists in patients receiving maintenance haemodialysis, especially in those with greater intradialytic fluid extraction.


# Introduction

Anaemia is a common complication in patients with end-stage renal disease and it is widely known that the deficiency of erythropoietin production is the major cause of anaemia [1]. Anaemia and increased erythropoiesis-stimulating agent (ESA) doses are associated with poor long-term survival in patients receiving maintenance haemodialysis [2, 3]. Although technological advancements in dialysis membrane and equipment have reduced the risk of massive haemolysis during maintenance haemodialysis, mechanical stress on red blood cells (RBCs) through the extracorporeal circulation leads to potential haemolytic conditions, resulting in adverse clinical conditions including anaemia and an increase in the ESA dose. However, haemolysis is unrecognized in clinical settings because of a lack of an easily detectable method. Labelling erythrocytes with radioactive chromium ($^{51}Cr$) is the standard method to estimate RBC age, which requires exclusive equipment for radioactive materials and a prolonged examination period with serial blood withdrawals from patients [4]. Compared to the $^{51}Cr$-labelling method, erythrocyte creatine is a simple, rapid, and economically favourable marker that uses a single blood sample examination. Erythrocyte creatine is deemed as a quantitative marker to determine mean RBC age, because young RBCs contain substantially higher creatine levels than older RBCs, and creatine contents in RBCs decrease gradually with advancing cell age; an elevation of erythrocyte creatine thus

reflects shortened RBC age [5–7]. Moreover, in contrast to reticulocyte levels, which reflects present erythropoiesis, erythrocyte creatine levels reflects average or cumulative erythropoiesis up to the present [6, 8]. Accordingly, we utilized erythrocyte creatine with other haemolytic markers to elucidate the incidence and underlying mechanisms associated with haemolysis in patients with end-stage renal disease on maintenance haemodialysis or peritoneal dialysis.

## Materials And Methods

# Study design

We assessed haemolysis in out-patients with end-stage renal disease recruited from the dialysis unit at the Kansai Medical University Hospital from May to November 2019. Patients aged over 20 years who had been established on maintenance haemodialysis 3 times a week or peritoneal dialysis therapy for at least 6 months were included in this cross-sectional study. The exclusion criteria were as follows: patients undergoing both haemodialysis and peritoneal dialysis, patients with a bleeding event within the last 3 months, blood transfusion within the last 3 months, concurrent malignancy, haemolytic disease, mechanical heart valves. The study protocol was approved by the ethics committee of Kansai Medical University (No.2018233) and was registered in the University Hospital Medical Information Network (UMIN) clinical trial registry (URL: https://www.umin.ac.jp/ctr/, Unique Identifier: UMIN000036418). All patients gave written informed consent and the investigation conforms to the principles outlined in the Declaration of Helsinki.

# Measurements

Body weight was obtained pre- and post-dialysis in haemodialysis patients. In peritoneal dialysis patients, body weight was measured after discarding dialysate from the peritoneal cavity. After enrolment, blood samples were drawn from all patients to examine erythrocyte creatine, haemolytic markers (reticulocyte count, haptoglobin and lactate dehydrogenase) and other laboratory parameters (haemoglobin, haematocrit, albumin, transferrin saturation and ferritin). Patients with haemodialysis were obtained blood sample immediately before haemodialysis received. A weekly dose of erythropoiesis-stimulating agents (ESA) was administered as a darbepoetin alfa equivalent dose. ESA was converted using the following formula: darbepoetin alfa (μg) = epoetin beta pegol (μg) × 0.8 = epoetin (U) × 200, based on previous reports [9–11]. ESA responsiveness was assessed using an erythropoietin resistance index, which was calculated using the following formula: erythropoietin resistance index (U/kg/week/g/dL) = weekly dose of epoetin (U/week)/(Body weight (kg) × Haemoglobin (g/dL)) [11]. Post-haemodialysis weight was extracted as a body weight in patients receiving haemodialysis.

# Haemodialysis and peritoneal dialysis

Haemodialysis was performed via native arterio-venous fistulas utilizing a dual plastic needle with a 16-gauge cannula size. Haemodialysis patients were uniformly administered with a dialysate (D-dry, Nikkiso Co., Ltd, Tokyo, Japan) and an anticoagulant with heparin sodium. Dialysate temperature of extracorporeal circulation was strictly maintained at 36 to 38 °C. Nocturnal intermittent peritoneal dialysis (Baxter Healthcare, Tokyo, Japan) was performed in all patients with peritoneal dialysis. Evaluation and treatment of anaemia, including ESA and iron therapy, were prescribed according to the KDIGO Clinical Practice Guideline 2012 [12]. Utilized ESA therapy employed darbepoetin alfa in haemodialysis patients and epoetin beta pegol in peritoneal dialysis. Quantity of blood flow (mL/min) and intradialytic ultrafiltration rate (mL/h/kg) were collected to assess haemodialysis conditions, which were calculated by an average of 3 consecutive haemodialysis sessions.

# Erythrocyte creatine

Creatine in human packed erythrocytes indicate the mean age of an RBC population [5]. Erythrocyte creatine was assayed enzymatically in accordance with previous reports [13]. Briefly, blood was collected in ethylenediamine tetra-acetic acid-containing tubes and centrifuged to remove the plasma and the buffy coat. After lysis and deproteinization of packed erythrocytes, the supernatant was obtained by centrifugation and filtration. Creatine concentration in the supernatant was measured using the enzymatic assay method. Mean RBC age (days) was obtained by $-22.84 \times \log_e(\text{erythrocyte creatine}) + 65.83$ [7]. Erythrocyte creatine levels represent average or cumulative erythropoiesis up to the present. Therefore, erythrocyte creatine levels are indicative of a chronic rather than an acute haemolytic condition. RBC age in 305 normal subjects was extracted as a healthy control from our previous report [6, 7].

# Statistical analysis

Continuous variables are presented as medians and interquartile ranges, and categorical variables are presented as numbers and percentages. Differences between the two groups were analysed using the Wilcoxon rank-sum tests for continuous variables and the chi-square tests for categorical variables. The relationship between the clinical covariates and erythrocyte creatine was explored through Spearman correlation analysis. A p-value $< 0.05$ was considered significant. The JMP 14.2.0 software (SAS Institute Inc., Cary, NC, USA) was used for all statistical analyses.

## Results

A total of 80 outpatients aged over 20 years were considered for this study. Of these, patients undergoing both haemodialysis and peritoneal dialysis (n = 6) and patients with a bleeding event within the last 3 months (n = 1), mechanical heart valves (n = 2) and was not written informed consent (n = 2) were excluded. Finally, 55 patients receiving haemodialysis and 14 patients receiving peritoneal dialysis were included for the final analysis.

There were no significant differences in patient characteristics between the two groups (Table 1). Although there was no significant difference in haemoglobin level between the two groups, patients receiving haemodialysis had significantly lower transferrin saturation and ferritin than those receiving peritoneal dialysis. The weekly dose of ESA, and the erythropoietin resistance index, were significantly higher in patients receiving haemodialysis compared to those receiving peritoneal dialysis.

Table 1
Patient profiles

| | **Haemodialysis (n = 55)** | **Peritoneal dialysis (n = 14)** | **p value** |
|---|---|---|---|
| Age (years) | 71 (63–79) | 70 (60–79) | 0.834 |
| Male | 33 (60) | 7 (50) | 0.499 |
| Body mass index (kg/m$^2$) | 21.3 (19.8–24.3) | 23.4 (21.3–24.5) | 0.124 |
| Hypertension | 47 (85) | 13 (93) | 0.463 |
| Diabetes mellitus | 32 (58) | 5 (36) | 0.132 |
| Prior cardiovascular disease | 14 (25) | 3 (21) | 0.755 |
| Laboratory data | | | |
| Haemoglobin (g/dL) | 11.0 (10.5–11.7) | 11.0 (10.5–11.8) | 0.782 |
| Haematocrit (%) | 33.9 (32.1–36.5) | 33.8 (33.1–35.9) | 0.704 |
| Albumin (g/dL) | 3.5 (3.3–3.8) | 3.5 (3.1–3.7) | 0.349 |
| Transferrin saturation (%) | 22 (16–28) | 37 (24–43) | 0.008 |
| Ferritin (ng/mL) | 50 (29–71) | 119 (105–161) | < 0.0001 |
| Erythropoiesis-stimulating agent (μg/wk) | 30 (10–45) | 12 (10–20) | 0.018 |
| Erythropoietin resistance index | 9.4 (3.4–14.1) | 3.9 (2.7–7.5) | 0.024 |
| Data presented as median (25th to 75th percentiles), or number (%). | | | |

RBC age is shown in Fig. 1. Patients receiving haemodialysis had significantly shorter RBC age than in patients with peritoneal dialysis [47.7 (43.2–52.8) days vs. 59.8 (56.0–66.2) days, p < 0.0001]. RBC age in healthy control was 60.5 days which was comparable in patients with peritoneal dialysis. Comparison of haemolytic markers between the 2 groups are shown in Fig. 2. Haptoglobin was significantly lower in patients receiving haemodialysis (haemodialysis: 78 (38–121) mg/dL vs. peritoneal dialysis: 96 (76–184) mg/dL, p = 0.040), whereas there was no significant difference in reticulocyte (haemodialysis: 1.7 (1.4–2.1) % vs. peritoneal dialysis: 1.3 (1.0–1.7) % p = 0.055) and lactate dehydrogenase (haemodialysis: 194 (176–212) U/L vs. peritoneal dialysis: 184 (156–225) U/L, p = 0.497) between the 2 groups.

When haemodialysis patients were stratified by median RBC age (47.7 days), haemodialysis patients with shortened RBC age had lower transferrin saturation, ferritin, and haptoglobin compared to those with preserved RBC age (Table 2). The weekly dose of ESA, the ESA resistance index, and the intradialytic ultrafiltration rate were all significantly higher in haemodialysis patients with shortened RBC age than in those with preserved RBC age. To investigate clinical covariates to correlate mean RBC age, univariate Spearman correlation coefficient analysis was conducted (Table 3). Transferrin saturation, weekly dose of ESA and erythropoietin resistance index were strongly associated with mean RBC age. Additionally, intradialytic ultrafiltration rate was also associated with mean RBC age.

Table 2
Comparison of clinical characteristics in patients with haemodialysis according to median RBC age

| | **Preserved RBC age (n = 27)** | **Shortened RBC age (n = 28)** | **p value** |
|---|---|---|---|
| **Age (years)** | 72 (67–79) | 71 (61–78) | 0.316 |
| **Male** | 16 (59) | 17 (61) | 0.912 |
| **Body mass index (kg/m$^2$)** | 21.3 (19.8–23.6) | 21.3 (19.5–25.2) | 0.893 |
| **Laboratory data** | | | |
| **Haemoglobin (g/dL)** | 10.9 (10.6–11.4) | 11.1 (10.3–11.9) | 0.906 |
| **Hematocrit (%)** | 33 (31–35) | 35 (33–37) | 0.09 |
| **Albumin (g/dL)** | 3.6 (3.3–3.8) | 3.5 (3.3–3.8) | 0.973 |
| **Transferrin saturation (%)** | 26 (19–39) | 18 (14–26) | 0.007 |
| **Ferritin (ng/mL)** | 57 (36–122) | 43 (20–65) | 0.045 |
| **Reticulocyte (%)** | 1.7 (1.2–2.1) | 1.7 (1.4–2.1) | 0.717 |
| **Haptoglobin (mg/dL)** | 91 (61–139) | 49 (25–114) | 0.009 |
| **Lactate dehydrogenase (U/L)** | 194 (176–207) | 198 (177–214) | 0.717 |
| **Erythropoiesis-stimulating agent (μg/week)** | 20 (10–30) | 33 (20–60) | 0.004 |
| **Erythropoietin resistance index** | 5.0 (2.8–11.8) | 11.4 (6.7–20.8) | 0.011 |
| **Quantity of blood flow (mL/min)** | 220 (200–250) | 200 (200–245) | 0.530 |
| **Intradialytic ultrafiltration rate (mL/h/kg)** | 7.3 (6.0–9.3) | 9.3 (7.0–12.7) | 0.016 |
| Data presented as median (25th to 75th percentiles), or number (%). | | | |
| RBC: red blood cell. | | | |

Table 3
Univariate correlations of clinical covariates for RBC age

| | Spearman correlation coefficient (r) | p value |
|---|---|---|
| Age (years) | 0.02 | 0.87 |
| Body mass index (kg/m$^2$) | -0.01 | 0.93 |
| Hemoglobin (g/dL) | -0.06 | 0.61 |
| Transferrin saturation (%) | -0.46 | < 0.0001 |
| Reticulocyte (%) | 0.30 | 0.01 |
| Haptoglobin (mg/dL) | -0.34 | 0.004 |
| Erythropoiesis-stimulating agent (μg/week) | 0.71 | < 0.0001 |
| Erythropoietin resistance index | 0.74 | < 0.0001 |
| Quantity of blood flow (mL/min) | -0.09 | 0.52 |
| Intradialytic ultrafiltration rate (mL/h/kg) | 0.36 | 0.007 |
| RBC: red blood cell. | | |

# Discussion

In the present study, RBC age was measured with other haemolytic markers in patients with end-stage renal disease receiving dialysis therapy, and we found significant shortening of RBC age in patients receiving haemodialysis compared to those receiving peritoneal dialysis, despite no significant differences in haemoglobin levels between the two groups. When haemodialysis patients were stratified into two groups according to the median RBC age, those with shortened RBC age had greater haptoglobin consumption, iron deficiency, higher ESA requirements, and poor ESA responsiveness compared to those with preserved RBC age. Moreover, intradialytic ultrafiltration rate was greater in haemodialysis patients with shortened RBC age.

A prospective small study using radioactive chromium ($^{51}$Cr), investigated shortening of RBC age in end-stage renal disease patients receiving haemodialysis and peritoneal dialysis, and in healthy volunteers with preserved glomerular filtration rate (> 60 mL/min/1.73 m$^2$) [14]. RBC age was shortened by 20% in end-stage renal disease compared with healthy volunteers, but there was no significant difference in RBC age between haemodialysis and peritoneal dialysis. However, due to a small number of patients in each group and a lack of haemolytic markers, they did not analyse the mechanisms of RBC age shortening in patients receiving dialysis. In our study, shortening of RBC age was observed in patients receiving haemodialysis compared to those receiving peritoneal dialysis. These data indicate that mechanical stress caused by flow resistance and turbulence during extracorporeal circuit contributed to haemolysis in patients receiving haemodialysis. Interestingly, a greater intradialytic ultrafiltration rate, not an increased

quantity of blood flow, was observed in haemodialysis patients with shortened RBC age, suggesting that greater ultrafiltration volume through the dialysis membrane rather than intraluminal blood velocity was the underlying mechanism associated with increased shear stress of the circulating erythrocyte causing haemolysis.

A single centre prospective study indicated that erythropoietin dosage is related to the degree of erythrocyte deformability in haemodialysis patients [15]. In our study, haemodialysis patients with shortened RBC age received significantly higher ESA doses and had poor ESA responsiveness compared to those with preserved RBC age. Moreover, RBC age had a strong correlation with the weekly ESA dose and erythropoietin resistance index. Therefore, an increase in the ESA dose and poor ESA responsiveness may be suggestive clinical signs of haemolysis in patients on maintenance haemodialysis.

In addition to a high prevalence of known risk factors for cardiovascular disease, haemolysis-associated endothelial dysfunction has also been reported [16–18]. Release of free haemoglobin induced by haemolysis scavenges and reduces the bioavailability of nitric oxide, which leads to impaired vascular endothelial function. Impaired endothelial function due to haemodialysis-induced haemolysis could lead to an increased risk of cardiovascular complications. Therefore, prompt identification of clinically unrecognized haemolysis is important to reduce the risk of future unfavourable cardiovascular events in patients receiving maintenance haemodialysis.

## Limitation

Several limitations of the present study should be noted. First, this study includes relatively small sample size, and there is a discrepancy in the number of patients in the haemodialysis and peritoneal dialysis group. Therefore, investigations using a larger sample size are needed to verify this result. Second, although the difference in dialytic membranes may be associated with the degree of haemolysis, we did not perform analyses comparing the use of different dialytic membranes because, in this study, many types of dialytic membranes were used in patients receiving haemodialysis.

## Conclusions

Haemolysis exists in patients receiving maintenance haemodialysis, especially in those with greater intradialytic fluid extraction.

## Abbreviations

RBC: red blood cell.

## Declarations

## Ethics approval and consent to participate:

The study protocol was approved by the ethics committee of Kansai Medical University (No.2018233).

# Consent for publication:

Patients signed informed consent regarding publishing their data.

# Availability of data and material:

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

# Competing interests:

The authors declare that they have no competing interests.

# Funding:

This study was funded by the Nakatani foundation. The funder of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report.

# Authors' contributions:

K.M., T.O. and T.S.: study design and conduction, data collection, analysis and interpretation, and manuscript writing. N.T., Y.Y., S.K., K.F. and M.O.: study conduction, data analysis and interpretation. M.O. and I.S.: study conduction, data analysis and interpretation. All authors read and approved the final manuscript.

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# Figures

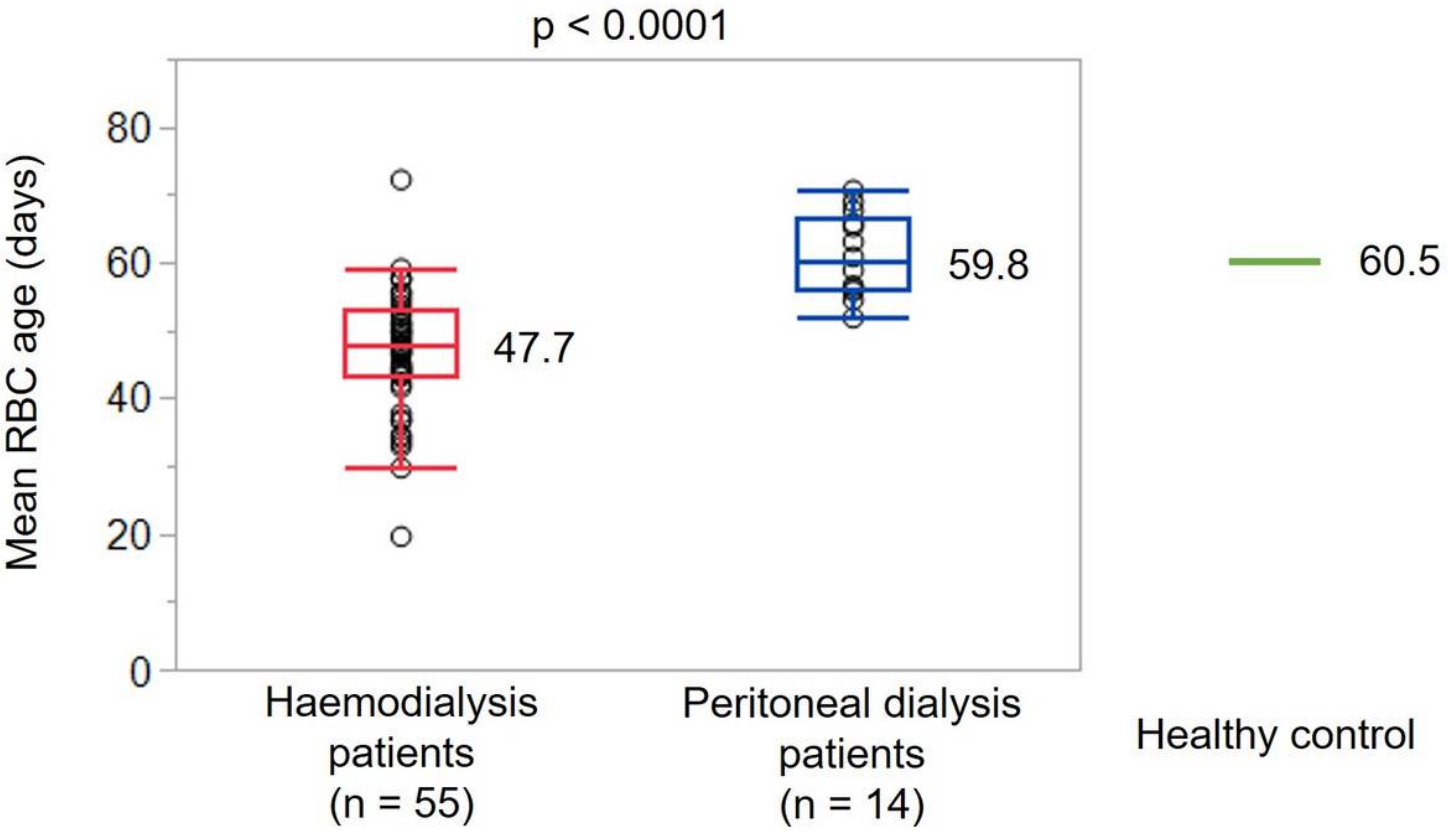
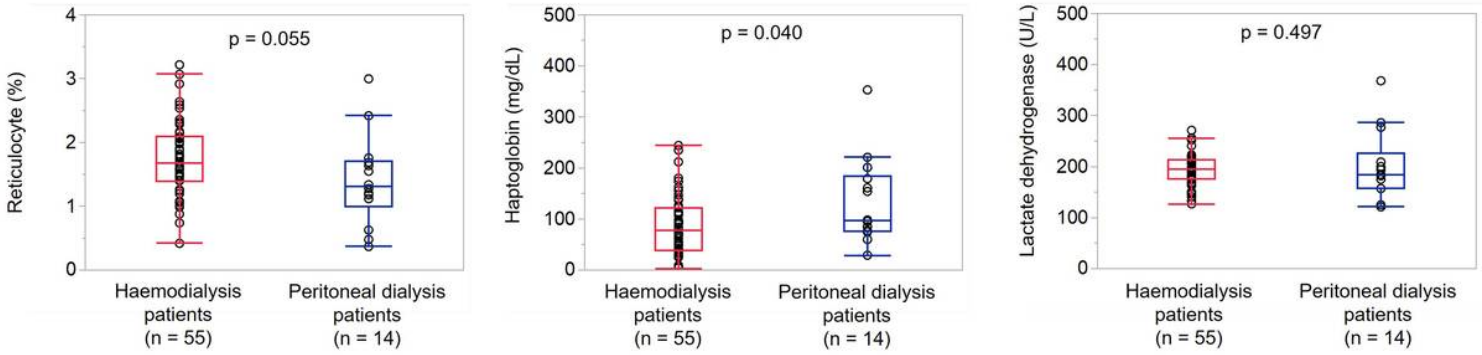



**Figure 1**

Comparison of mean RBC age. Mean RBC age in healthy control is shown as a green line. The box for two groups represents the interquartile range (25th–75th percentile), and the horizontal line in each box represents the median value. RBC: red blood cell.




## Figure 2

Comparison of haemolytic markers in patients receiving haemodialysis and peritoneal dialysis. The box for each group represents the interquartile range (25th–75th percentile), and the horizontal line in each box represents the median value.