

# Influence of autologous blood transfusion in liver transplantation in patients with hepatitis B on the function and hemorheology of red blood cells

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**Abstract.** The present study aimed to characterize the function and hemorheology of red blood cells (RBCs) recovered during liver transplantation surgery in patients with hepatitis B and decompensation. A total of 15 hepatitis B patients with decompensation who underwent liver transplantation surgery were included in the present study. Blood samples were recovered during the liver transplantation surgery using an Autologous Blood Recovery System. The morphology and structure of RBCs were characterized and compared between pre-operative and recovered blood samples. In addition, the physiological functions of RBCs were measured and compared between pre-operative and recovered blood samples. No significant differences in the morphological score, 2,3-diphosphoglycerate, Na<sup>+</sup>K<sup>+</sup>-ATPase, Ca<sup>2+</sup>-ATPase, Mg<sup>2+</sup>-ATPase, malondialdehyde and osmotic fragility were identified between RBCs in the pre-operative and recovered blood samples. The level of free hemoglobin in RBCs of

the recovered blood samples was significantly higher than in the pre-operative blood samples ( $P<0.05$ ). Medium- and high-shear blood viscosities in the recovered blood samples were significantly lower than those observed in the pre-operative blood samples ( $P<0.05$ ). Casson viscosity in the recovered blood samples was significantly higher compared with the pre-operative blood samples. However, no significant differences ( $P>0.05$ ) in the low-shear blood viscosity, plasma viscosity, relative blood viscosity, erythrocyte aggregation index or Casson yield stress were identified between recovered and pre-operative blood samples. These findings suggested that autologous blood transfusion in liver transplantation surgery in patients with hepatitis B and decompensation had no significant influence on the morphology, structure, function and hemorheology of RBCs.

## Introduction

Liver transplantation is an effective approach for the treatment of end-stage liver diseases, including hepatic carcinoma and hepatic cirrhosis (1,2), and currently accounts for 4% of liver transplants annually (3). With the progress of medical science, liver transplantation has been widely used in patients with hepatitis B virus (HBV) infection, specifically patients with chronic and severe HBV infection (4,5). However, liver transplantation is a complicated surgery that takes 5-10 h on average. In addition, patients with end-stage liver diseases typically have blood coagulation dysfunction, which may lead to substantial blood loss during liver transplantation surgery. Therefore, perioperative blood transfusion is critically important for the success of liver transplantation surgery (6).

Intraoperative autologous blood transfusion is a medical procedure involving the recovery of blood lost during surgery and re-infusing it into the patient after rinsing. Intraoperative autologous blood transfusion may resolve the problem of limited blood supply sources and reduce the economic burden on patients. In addition, intraoperative autologous blood transfusion may prevent transfusion-transmitted infections and avoid allergic, hemolytic and graft-versus-host reactions caused by allogeneic blood transfusion (7,8). As a

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**Abbreviations:** RBC, red blood cells; HBV, hepatitis B virus; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red cell distribution width; SEM, scanning electron microscope; 2,3-DPG, 2,3-diphosphoglycerate; Fhb, free hemoglobin; MDA, malondialdehyde; ATP, adenosine triphosphate

**Key words:** autologous blood transfusion, red blood cells, hemorheology, physiological function, hepatitis B, liver transplantation

major form of autotransfusion, intraoperative autologous blood transfusion has been widely used in clinical practice. Sankarankutty *et al* (9) demonstrated that autologous blood transfusion significantly reduced blood usage in liver transplantation surgery by recovering >90% of red blood cells (RBCs) in the surgical field, the recovered RBCs accounted for >50% of the total amount of RBCs used in the liver transplantation surgery. Therefore, autologous blood transfusion may markedly relieve the limitation of blood supply source in clinical settings.

Autologous blood transfusion may cause morphological and functional changes of RBCs. Different autologous blood transfusion methods may have distinct influences on the morphology and function of recovered RBCs (10). A study conducted by Wan *et al* (11) suggests that autologous blood transfusion had no significant adverse effects on the morphology and deformability of RBCs during coronary artery bypass surgery. Ling (12) reported that autologous transfusion of rinsed RBCs in orthopaedic surgeries had no evidently negative impacts on the overall hemorheology in the patients. Another study also reported a limited influence of intraoperative autologous blood transfusion on the function of RBCs in patients with liver and kidney diseases (13).

In end-stage HBV patients, the membrane structure and function of RBCs have been altered by the accumulation of toxic substances due to decompensation and liver dysfunction (14). It remains unclear whether autologous blood transfusion has an influence on the morphology and function of RBCs in HBV patients with decompensation. The present study examined the morphological and functional changes of RBCs and evaluated the alternation of hemorheology caused by intraoperative autologous blood transfusion in chronic HBV patients at the end-stage. The results of the current study provide a theoretical basis for the application of autologous blood transfusion in liver transplantation surgery.

## Materials and methods

**Patients.** All human studies have been approved by The Institute Research Medical Ethics Committee of the Third Affiliated Hospital, Sun Yat-Sen University (Guangdong, China). All human studies have been performed in accordance with the ethical standards outlined in the 1964 Declaration of Helsinki and its later amendments. All patients provided their informed consent prior to their inclusion in the study.

From January 2014 to June 2015, a total of 15 male patients with HBV at the end-stage underwent liver transplantation for HBV-related liver disease in our center, were included in the present study. Inclusion criteria for participating in the study were: Patients (only male) were aged 18-70 years, were hepatitis B surface antigen-positive for at least 6 months, HBV DNA-positive, accepted to sign the informed consent paper and were expected to survive >6 months after surgery. Exclusion criteria for the present study were: hepatitis B surface antigen-negative, expected to survive <6 months after surgery, were positive for hepatitis C virus or HIV co-infection, hepatocellular carcinoma and exhibited drug allergies. Due to liver decompensation, the 15 patients received orthotopic liver transplantation under general anesthesia with midazolam (0.1 mg/kg), propofol (1.0 mg/kg), fentanyl

(4 µg/kg) and vecuronium (0.1 mg/kg) (all from Yangtze River Pharmaceutical Group, Jiangsu, China) at the Third Affiliated Hospital, Sun Yat-Sen University. The patients (age, 18-70 years; weight, 50-75 kg) were classified as ASA III-IV according to the ASA physical status classification system (15). Whole blood, serum, plasma and urine from each patient were tested, and no blood, endocrine or autoimmune diseases were identified in the 15 patients. No pus, bile or cancer cells were identified in the blood from the patients. Informed consent was obtained from the patients and their families prior to the liver transplantation surgery.

**Reagents and instruments.** All reagents used in the present study were provided by Nanjing Jiancheng Bioengineering Institute (Nanjing, China). An Autologous Blood Recovery System (Autologous 2000) was purchased from Wandong Medical Equipment Company (Beijing, China). Automatic blood analyzer (Sysmex XT 2000i) was purchased from Sysmex Corp. (Kobe, Japan). A blood rheometer (LVDV-III+) was purchased from the Brookfield Co. (San Francisco, CA, USA).

Vital signs of the patients were monitored during surgery under anesthesia induced with midazolam (0.1 mg/kg), propofol (1 mg/kg), fentanyl (4 µg/kg) and vecuronium (0.1 mg/kg), and maintained with sufentanil (0.5 mg/kg), propofol (0.5 mg/kg), heptafluoro ether (1 mg/kg), and vecuronium (0.08 mg/kg) (all from Yangtze River Pharmaceutical Group, Jiangsu, China). Each patient underwent invasive arterial pressure measurement, deep vein puncture and floating catheter manometry. Anticoagulation of pre-operative blood (15 ml) collected from the deep veins of 15 patients (n=15) was conducted using EDTA. Blood collected during the surgery using the autologous blood recovery machine was mixed with anticoagulants and filtrated (filter diameter, 20-40 µm) in the blood storage. Subsequently, the filtrated blood was centrifuged at 6,000 x g for 15 min at 4°C and rinsed twice with PBS (Beijing Dingguo Changsheng Biotechnology Co., Ltd., Beijing, China) to remove cell debris, free hemoglobin (FHb) and anticoagulants. The hematocrit of recovered RBCs was between 30 and 50%. Following this, 15 ml of the recovered blood was used in subsequent experiments to compare with the pre-operative blood samples (n=15).

**Morphological and structural evaluation of RBCs.** To evaluate the morphology of RBCs, EDTA-anticoagulated blood samples (2 ml) were analyzed in an automatic blood analyzer to determine the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and red cell distribution width (RDW). A drop of blood (~50 µl) was spread on a glass slide and left for 2 min at room temperature. Following Wright staining, the slide was examined under an inverted optical microscope (IX80; Olympus Corporation of the Americas, Center Valley PA, USA) with an oil lens at a magnification of x100 to evaluate the morphology of 100 RBCs according to the morphological scoring system (16). In this scoring system, 1 point was assigned to RBCs of biconcave-discoid shape. Points 2, 3, 4 and 5 were assigned to spiny RBCs of disc shape, spiny RBCs, spherical and spiny RBCs and spherical RBCs, respectively. Imaging was conducted under an inverted microscope.

To evaluate the structure of RBCs using a scanning electron microscope (SEM), blood samples were washed using 10X normal saline and centrifuged twice at  $2,000 \times g$  at  $4^{\circ}\text{C}$  for 5 min. Subsequently, the precipitate was fixed in 2.5% glutaraldehyde for 1 h at  $4^{\circ}\text{C}$  and dehydrated in phosphate-buffered saline (0.1 M), graded ethanol solution (50, 70, 90 and 100%), and 100% iso-amyl acetate. Following critical point drying using a Hitachi HCP-2 type critical point dryer (Tokyo, Japan) and metal coating using E-1045 ion-beam sputtering apparatus (Techcomp Ltd., Beijing, China) (17), the samples were examined under a SEM to evaluate the ultrastructure of the surface of RBCs.

**Analysis of RBC membrane proteins.** Following centrifugation of the blood samples in a heparinized tube at  $2,000 \times g$  for 10 min at  $4^{\circ}\text{C}$ , the supernatant and upper white fluffy layer (primarily leukocytes) were removed using a pipette. Packed RBCs were obtained by washing the precipitate with normal saline for 5 min and centrifuged at  $6,000 \times g$  for 10 min at  $4^{\circ}\text{C}$  three times. Packed RBCs were suspended in hypotonic buffer and incubated at  $4^{\circ}\text{C}$  for 20 min. Following centrifugation at  $20,000 \times g$  for 30 min at  $4^{\circ}\text{C}$ , a pink precipitate was observed in the bottom. The precipitate was washed with hypotonic buffer three times for 10 min to collect the 'spectrin layer' that primarily contains the RBC membrane proteins. A total of  $2 \mu\text{g}$  of RBC membrane proteins were stained with Coomassie brilliant blue to measure the protein content. Other RBC membrane proteins were stored at  $30^{\circ}\text{C}$  for subsequent experiments.

RBC membrane proteins were appropriately homogenized using an ultrasonic homogenizer and mixed with 5X loading buffer. Following boiling for 5 min, the proteins ( $15 \mu\text{l}$ ) were separated by 10% SDS-PAGE. Following electrophoresis, the discolored gel was scanned and imaged on a LiCor Odyssey scanner (Li-Cor Biosciences, Lincoln, NE, USA). The electrophoresis pattern of RBC membrane proteins was analyzed using Quantity One software version 4.5.2 (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

**Evaluation of RBCs physiological functions.** Levels of 2,3-diphosphoglycerate (2,3-DPG), FHB, erythrocyte membrane ATPase and malondialdehyde (MDA) in pre-operative and recovered blood samples were measured using different kits, including the 2,3 DPG kit (HY-60073; eBioscience; Thermo Fisher Scientific, Inc., Waltham, MA, USA), FHB kit (HY-60068; eBioscience; Thermo Fisher Scientific, Inc.), ATPase assay kit (A016-1; Nanjing Jiancheng Bioengineering Institute) and MDA assay kit (MAK085; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) according to the manufacturer's protocol.

Pre-operative or recovered blood samples (2 ml) were diluted with normal saline in a 10:1 ratio and centrifuged at  $2,000 \times g$  for 10 min at  $4^{\circ}\text{C}$  to prepare packed RBCs. Graded NaCl solution (1-8.5 g/l) was added to the packed RBCs (0.05 ml) following incubation at  $37^{\circ}\text{C}$  for 5 min. To compare the osmotic fragility between pre-operative and recovered RBCs, the RBC hemolysis rate at different concentrations of NaCl was calculated according to a previous study (18).

**Hemorheology.** Pre-operative or recovered blood samples (3 ml) were treated with lithium heparin anticoagulant.

Anticoagulated blood samples were analyzed in a blood rheometer to evaluate low-shear blood viscosity, high-shear blood viscosity, plasma viscosity, blood relative viscosity, erythrocyte aggregation index, Casson viscosity and yield stress.

**Statistical analysis.** Measurement data were presented as the mean  $\pm$  standard deviation. Statistical analyses were performed using SPSS statistical software version 13.0 (SPSS, Inc., Chicago, IL, USA). Comparison of values between the pre-operative and recovered blood samples was conducted using paired t-tests.  $P < 0.05$  was considered to indicate a statistically significant difference.

## Results

**Morphological and structural changes in RBCs recovered during liver transplantation surgery.** As presented in Fig. 1A, the majority of RBCs in the pre-operative blood samples were a normal biconcave-discoid shape. However, the number of RBCs exhibiting a normal biconcave-discoid shape decreased in the recovered blood samples. Furthermore, spherical and spiny RBCs were observed in the recovered blood samples (Fig. 1B). As presented in Fig. 1C, RBCs in the pre-operative blood samples were observed to have a smooth surface without abnormal protrusions, based on SEM examination. Protrusions were observed on the surface of RBCs in the recovered blood samples (Fig. 1D).

While the morphological scores of RBCs in the recovered blood samples were higher than that of RBCs in the pre-operative blood samples, the difference was not statistically significant. In addition, no significant differences in the MCV, MCH, MCHC, red cell distribution width-coefficient of variation (RDW-CV) and red cell distribution standard deviation (RDW-SD) values were identified between RBCs in the pre-operative blood samples and recovered blood samples (Table I).

**Membrane protein changes in RBCs recovered during liver transplantation surgery.** As presented in Fig. 2, no marked changes in the molecular weight of membrane proteins were observed between pre-operative and recovered RBCs.

**Comparison of physiological functions between RBCs in the pre-operative and recovered blood samples.** Tables II and III demonstrate no significant differences for 2,3-DPG,  $\text{Na}^+$   $\text{K}^+$ -ATPase,  $\text{Ca}^{2+}$ -ATPase,  $\text{Mg}^{2+}$ -ATPase, MDA and osmotic fragility were identified between RBCs in the pre-operative blood samples and recovered blood samples ( $P > 0.05$ ). However, FHB in recovered RBCs ( $50.7 \pm 12.6 \text{ mg/l}$ ) was significantly higher than that in pre-operative RBCs ( $37.1 \pm 5.7 \text{ mg/l}$ ;  $P < 0.05$ ; Table II).

**Influence of autologous blood transfusion in liver transplantation surgery on hemorheology.** Medium- ( $3.56 \pm 1.35 \text{ mpa.s}$ ) and high-shear ( $2.33 \pm 0.7 \text{ mpa.s}$ ) blood viscosities in the recovered blood samples were significantly lower than those in the pre-operative blood samples ( $5.64 \pm 2.35$  and  $3.64 \pm 1.06 \text{ mpa.s}$ , respectively;  $P < 0.05$ ). Casson viscosity ( $1.7 \pm 0.62 \text{ mpa.s}$ ) in the recovered blood samples was significantly higher compared



Table I. Comparison between the morphological scores of MCV, MCH, MCHC and RDW between red blood cells in the pre-operative blood samples and recovered blood samples.

Variable	Pre-operative blood samples (n=15)	Recovered blood samples (n=15)	P-value
Morphological score	121±16	130±19	P>0.05
MCV	94±11	95±10	P>0.05
MCH	33±4	31±5	P>0.05
MCHC	350±13	333±38	P>0.05
RDW-SD	47±9	48±9	P>0.05
RDW-CV	0.14±0.02	0.14±0.03	P>0.05

Data are presented as mean ± standard deviation. MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red cell distribution width; RDW-SD, red cell distribution width standard deviation; RDW-CV, red cell distribution width-coefficient of variation.

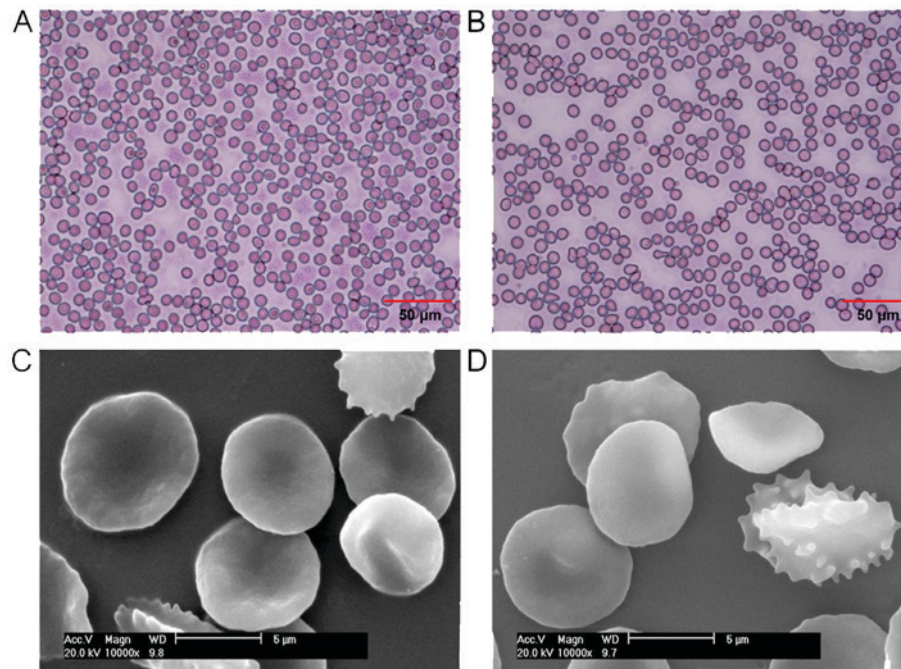


Figure 1. Morphological changes to red blood cells following recovery (A). Red blood cells in the pre-operative blood samples are of the normal biconcave-discoid shape. Scale bar =50 μm. Magnification, x100. (B) Spiny red blood cells of disc shape, spiny and spherical red blood cells were observed in the recovered blood samples. (C) Red blood cells in the pre-operative blood samples had a smooth surface without abnormal protrusions and (D) protrusions were observed on the surface of red blood cells in the recovered blood samples, observed using a scanning electron microscope.

with the pre-operative blood samples ( $0.9\pm0.03$  mpa.s;  $P<0.05$ ). However, no significant differences in the low-shear blood viscosity, plasma viscosity, relative blood viscosity, erythrocyte aggregation index and Casson yield stress were identified between recovered and pre-operative blood samples (Table IV).

## Discussion

A biconcave disc shape is an important feature of RBCs, essential for the deformability, suspension stability and osmotic fragility of RBCs (19). In the present study, the majority of RBCs in the pre-operative blood samples are of the normal biconcave-discoid shape. However, the numbers of spiny RBCs

of disc shape and spiny red blood cells in the recovered blood samples increased. In addition, spherical and spiny RBCs and spherical RBCs in the recovered blood samples observed. While the scores of RBCs in the recovered blood samples were higher than that of RBCs in the pre-operative blood samples, the difference was not statistically significant. In addition, no significant differences in the levels of MCV, MCH, MCHC and RDW-CV were identified between RBCs from pre-operative blood samples and RBCs in the recovered blood samples in the liver transplantation surgery. These results suggest that autologous blood transfusion during liver transplantation in patients with hepatitis B with decompensation had a limited influence on the morphology of RBCs. Deformability and membrane fluidity are necessary for the normal physiological

Table II. Comparison of physiological functions between red blood cells in the pre-operative blood samples and recovered blood samples.

Variable	Pre-operative blood samples (n=15)	Recovered blood samples (n=15)	P-value
2, 3-DPG, $\mu\text{mol/gHb}$	15.9 $\pm$ 4.5	19.4 $\pm$ 4.0	P>0.05
Na <sup>+</sup> K <sup>+</sup> -ATPase, U/mg protein	5.91 $\pm$ 1.93	5.29 $\pm$ 1.88	P>0.05
Ca <sup>2+</sup> -ATPase, U/mg protein	8.23 $\pm$ 2.46	7.98 $\pm$ 2.01	P>0.05
Mg <sup>2+</sup> -ATPase, U/mg protein	4.99 $\pm$ 1.32	4.78 $\pm$ 1.01	P>0.05
MDA, nmol/ml	4.3 $\pm$ 0.1	5.7 $\pm$ 0.5	P>0.05
FHb, mg/l	37.1 $\pm$ 5.7	50.7 $\pm$ 12.6 <sup>a</sup>	P<0.05

<sup>a</sup>P<0.05 vs. pre-operative red blood cells, t=6.01. Data are presented as mean  $\pm$  standard deviation. 2,3-DPG, 2,3-diphosphoglycerate; ATP, adenosine triphosphate; MDA, malondialdehyde; FHb, free hemoglobin.

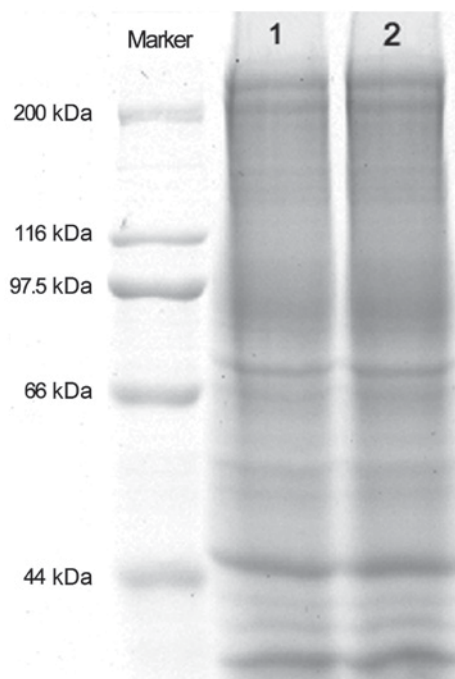


Figure 2. SDS-PAGE analysis of membrane proteins from red blood cells. Lane 1: Membrane proteins from pre-operative red blood cells. Lane 2: Membrane proteins from recovered red blood cells.

function of RBCs. The composition and structure of membrane proteins in RBCs are of importance in the function of RBCs. The majority (~60%) of membrane proteins in RBCs form a continuous network in the cytoplasm to support and control cell shapes (20). Any changes in the quality and quantity of membrane cytoskeletal proteins in RBCs may cause abnormal morphology and function of RBCs. In the present study, SDS-PAGE analysis demonstrated no significant difference in the molecular weight of membrane proteins in RBCs between pre-operative blood samples and recovered blood samples. This suggests that autologous blood transfusion in liver transplantation in patients with hepatitis B and decompensation had no significant influence on the structure of RBCs.

Membrane-binding adenosine triphosphate (ATP) enzymes have important roles in the maintenance of RBC

Table III. Comparison of the hemolysis rates between red blood cells in the pre-operative blood samples and recovered blood samples in all 15 patients.

NaCl concentration, g/l	Pre-operative blood samples, %	Recovered blood samples, %
8.5	100	100
7.5	85 $\pm$ 9	80 $\pm$ 13
7.0	77 $\pm$ 6	65 $\pm$ 13
6.5	61 $\pm$ 22	56 $\pm$ 21
6.0	47 $\pm$ 33	45 $\pm$ 27
5.5	30 $\pm$ 34	41 $\pm$ 21
5.0	34 $\pm$ 46	20 $\pm$ 11
4.5	36 $\pm$ 46	20 $\pm$ 13
4.0	32 $\pm$ 12	22 $\pm$ 14
3.5	14 $\pm$ 26	10 $\pm$ 3
3.0	14 $\pm$ 20	9 $\pm$ 2
2.0	13 $\pm$ 22	8 $\pm$ 2
1.0	10 $\pm$ 11	7 $\pm$ 10

Data are presented as mean  $\pm$  standard deviation. No significant differences were observed (P>0.05).

morphology, structure and functions. As an intermediate product in the metabolism of RBCs, 2,3-DPG is an important indicator of the oxygen-carrying capacity (21). In the human body, the oxygen supply in different tissues is regulated by the concentration of 2,3-DPG in RBCs. Under the same conditions, an increased concentration of 2,3-DPG in RBCs promotes O<sub>2</sub> release from oxyhemoglobin. It has been reported that the levels of 2,3-DPG and ATP in recovered RBCs was higher than that in stored RBCs (22). In addition, it has been demonstrated that recovered blood cells exhibited an improved oxygen carrying capacity and stronger anti-infiltration capacity than stored blood cells, which reduce the incidence of metabolic acidosis and electrolyte imbalance caused by the infusion of a large number of stored blood cells (23). Other studies that compared intraoperative cell salvage with allogeneic blood transfusion have demonstrated an increased

Table IV. Comparison of the hemorheology between recovered and pre-operative blood samples.

Variable	Pre-operative blood samples (n=15)	Recovered blood samples (n=15)	P-value
Low-shear blood viscosity	15.5±10.5	15.3±8.73	P>0.05
Medium-shear blood viscosity	5.64±2.35	3.56±1.35 <sup>a</sup>	P<0.05
High-shear blood viscosity	3.64±1.06	2.33±0.70 <sup>a</sup>	P<0.05
Plasma viscosity	2.1±1.5	1.3±0.6	P>0.05
Relative blood viscosity	13.5±8.19	12.2±6.27	P>0.05
Erythrocyte aggregation index	4.75±0.89	7.00±3.21	P>0.05
Casson viscosity	0.90±0.03	1.7±0.62 <sup>a</sup>	P<0.05
Yield stress	6.2±6.5	7.8±6.1	P>0.05

<sup>a</sup>P<0.05 vs. pre-operative blood samples. The t value of the comparisons of medium-shear blood viscosity, medium-shear blood viscosity and Casson viscosity between recovered and pre-operative blood samples were 3.23, 3.11 and 4.39. Data are presented as mean ± standard deviation.

mean erythrocyte viability, increased 2,3-DPG (24,25) and increased ATP levels (26,27) in salvaged blood. In the present study, no significant difference in the levels of Na<sup>+</sup>K<sup>+</sup>-ATPase, Ca<sup>2+</sup>-ATPase, Mg<sup>2+</sup>-ATPase and 2,3-DPG were observed between pre-operative and recovered RBCs, suggesting that autologous blood transfusion in liver transplantation in patients with hepatitis B and decompensation had no significant influence on the functions of RBCs.

FHb is plasma hemoglobin associated with the damage of RBCs. In the present study, the concentration of FHb in the recovered blood samples from liver transplantation in patients with hepatitis B and decompensation was significantly higher than that in the pre-operative blood samples in the same patients, suggesting hemolysis in the process of blood recovery. The use of a high-speed centrifugal machine, tubing extrusion, suction, mechanical damage and other factors in the blood recovery process may cause hemolysis (28). A number of measures may be considered to reduce the chance of hemolysis. For example, the negative suction pressure used in blood recovery may cause major damage to RBCs, which has a large influence on the quality of recovered RBCs. To minimize damage to RBCs, the recommended suction pressure is typically <0.02 MPa (150 mmHg) (29). In addition, dilution of recovered blood using saline may also markedly inhibit the damage on RBCs (30). Osmotic fragility, which is measured by the tensile capacity of the cell membrane, is an important mechanical property of RBCs. Resistance to the hypotonic solution of RBCs is associated with it membrane thickness. The thicker membrane is associated with a higher osmotic fragility due to a lower ratio of the membrane area to volume (18). Spain *et al* (31) revealed that recovered and rinsed RBCs still have a normal volume, hemoglobin content, hemoglobin concentration and osmotic fragility. In the present study, no significant difference in the NaCl concentration between recovered blood samples and pre-operative blood samples was identified. This suggests that autologous blood transfusion during liver transplantation in patients of hepatitis B with decompensation had no significant influence on the osmotic fragility of RBCs.

Rheological properties of RBCs, primarily determined by the aggregation and deformation of RBCs, are critically important for normal blood circulation in blood vessels (5). Casson viscosity is associated with RBC deformability. Low-shear and high-shear whole blood viscosities reflect the aggregation and deformation of RBCs. RBCs of low-shear viscosity tend to aggregate and RBCs of high-shear viscosity have poor deformability. In the present study, Casson viscosity and low- and high-shear whole blood viscosities in the recovered blood were significantly higher than those in the pre-operative blood samples. This suggests an increased deformation ability of recovered RBCs. Suction and high-speed centrifugation may damage aging RBCs and RBCs of poor deformability, which are removed by hemolysis to cause increased deformation ability of recovered RBCs. In the present study, no significant difference in the low-shear whole blood viscosity and aggregation index was identified between recovered blood and pre-operative blood samples. Therefore suggesting that the autologous blood transfusion in liver transplantation of patients with hepatitis B and decompensation had no significant influence on the aggregation of RBCs.

In conclusion, the results of the current study demonstrated that autologous blood transfusion in liver transplantation in patients with hepatitis B and decompensation had no significant influence on the morphology, structure, function and hemorheology of recovered RBCs. Therefore, autologous blood transfusion in liver transplantation may be widely applied. However, the process of blood recovery and transfusion remains to be further investigated and improved to keep the morphology, function and hemorheology of recovered RBCs.

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