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Original Article

Application of autologous blood transfusion in spine surgery and its influence on hemorheology and inflammatory factors

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Abstract: Objective: The goal of this study was to evaluate application of autologous blood transfusion in spine surgery and its influence on hemorheology and inflammatory factors. Methods: A total of 160 patients undergoing spinal internal fixation were recruited and randomized into two groups, group A receiving autologous blood transfusion and group B receiving allogeneic blood transfusion, with 80 patients in each group. The concentrations of serum tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) before and after blood transfusion as well as day 1, day 3, and day 7 after surgery were determined by enzyme-linked immunosorbent assay. The hemorheological indices of the two groups were determined by automated hematology analyzer. The postoperative coagulation function and incidence of complications of the two groups were observed. Results: There were no significant differences in prothrombin time, thrombin time, activated partial thromboplastin time and level of fibrinogen between the two groups (all P>0.05). No significant differences existed in hemorheological indices between the two groups at different time points (all P>0.05). Serum TNF-α concentration in group A was significantly lower than that in group B at day 1, day 3 and day 7 after surgery (all P<0.001). Serum IL-6 concentration in group A was significantly lower than that in group B respectively at day 1 and day 3 after surgery (both P<0.001). The incidence of postoperative complications in group A was significantly lower than that in group B (P<0.001). Conclusion: Autologous blood transfusion in spine surgery showed no effect on coagulation function of the patients, and patients maintained sufficient blood supply along with a reduced incidence of complications. Stabilizing hemorheology with inhabitation of inflammatory reactions may be a therapeutic mechanisms of autologous blood transfusion.

Keywords: Spine surgery, autologous blood transfusion, hemorheology, inflammatory factor

Introduction

Spine surgery is a common procedure in orthopedics with intraoperative traumatic hemorrhage and time-consuming operations. Such patients often suffer from a decrease in hemoglobin count with certain reduction in oxygen-carrying capacity of the hematological system during and after the surgery, thus leading to a series of complications [1]. Blood transfusion is a must in most cases of spine surgery. Autologous blood transfusion and allogeneic blood transfusion are common choices for clinical blood transfusion. Allogeneic blood transfusion is widely used in clinical practice, but antibodies in allogeneic blood could induce various adverse reactions like fever, hemolysis, and even systemic inflammatory reactions with multiple organ failure in a serious condition [2, 3]. Autologous blood transfusion refers to a blood donation with certain process to blood components for use by the donor. It is a relatively reliable blood transfusion method, and can effectively avoid the problems related to allogeneic antigen [4]. Compared with allogeneic blood transfusion, autologous blood transfusion can obtain concentrated red blood cells by collecting the blood loss during surgery and removing harmful substances in blood through a filter in autologous blood re-transfusion system, thus reducing the amount of intraoperative blood loss and the input amount of allogeneic blood, with relief in the clinical blood supply strain and reduction in incidence rate of complications caused by allogeneic blood transfusion [5].

Autologous blood transfusion is an effective blood-saving method with good blood conservation, and has been widely put in service in various clinical fields [6-8]. Many previous studies have reported the application of autologous blood transfusion in spine surgery, but few have focused on the effects of autologous blood transfusion on hemodynamics and inflammatory response of the patients with spine surgery. In order to improve the effect of blood reinfusion in spine surgery, reduce usage of blood and avoid the potential complications from allogeneic blood transfusion to the greatest extent, this study adopted autologous blood transfusion in spine surgery to observe its influence on hemorheology and inflammatory factors of the patients, aiming to provide a scientific basis for the effectiveness and safety of autologous blood transfusion.

Materials and methods

Patients

A total of 160 patients undergoing spinal internal fixation in Shanxi Dayi Hospital from February 2014 to March 2016 were recruited and randomized into two groups by random number table method, group A receiving autologous blood transfusion and group B receiving allogeneic blood transfusion, with 80 patients in each group. In group A, there were 35 males and 45 females aged from 25 to 73 years old with a median age of 51.8 years old (SD=9.1); the weight ranged from 41.1 to 89.6 kg with a median weight of 58.41 kg (SD=9.34). While there were 37 males and 43 females in group B, aged from 21 to 69 years old with a median age of 50.9 years old (SD=8.2); the weight ranged from 42.0 to 87.5 kg with a median weight of 59.06 kg (SD=10.75). All the included patients were confirmed as class I to II according to American Society of Anesthesiologists (ASA) Physical Status Classification [9]; and aged 20 years old or more and lower than 76 years old; all of them were initially treated with spine surgery. Exclusion criteria: Severe abnormal liver or renal function, hematological diseases, coagulation disorder, respiratory tract injury, malignant tumor, infectious fever or bacteremia, hypertension, or diabetes; intraoperative blood loss of ≥600 mL; hemoglobin level of <70 g/L; anemia or hematocrit (Hct) of <20%; poor physical endurance; adverse reactions during blood transfusion; blood pressure of ≤90/60 mmHg; autoimmune dysfunction; and mental disorders. The study was approved by the Medical Ethics Committee of the hospital, and informed consents were obtained from all the patients or their families.

Anesthesia

The patients were all given general anesthesia through tracheal intubation, with mechanical ventilation, establishment of venous access, intravenous infusion of sodium lactate Ringer injection, close monitoring of electrocardiograph, invasive blood pressure and central venous pressure. The patients were premeditated with injection of atropine 0.01 mg/kg at 20 minutes before general anesthesia, followed by co-administration of midazolam 2 mg and fentanyl 3 μg/kg for induction of general anesthesia, with connection of anesthesia machine, mechanical ventilation, adjustment of respiratory frequency and tidal volume, and maintenance of end-tidal carbon dioxide partial pressure to 30-35 mmHg. Under complete anesthesia, the right internal jugular vein and radial artery were punctured and cannulated. Remifentanil and propofol were given intravenously by computerized infusion pump to maintain anesthesia. Stability of circulatory dynamics and depth of anesthesia were closely monitored as well.

Blood transfusion

An autologous blood salvage and cell saver machine (3000P, Beijing Jingjing Medical Equipment Co., Ltd., China) was applied in group A to recycle autologous blood. The collection was carried out before the surgery. The pre-transfusion treatment was conducted when the recycling amount reached 600-800 mL. If the recycled blood was relatively thin, the treatment was given when the recycling amount reached 1,000 mL. For recycling blood, the amount of saline for one washing was 1,000 mL, and the pump speed was >500 mL/minute. After filtration, centrifugation and washing, fat, plasma, cracked red blood cells, hemoglobin, etc. were removed and concentrated red blood cells were obtained and stored in a blood bag at room temperature for later reinfusion. Through blood gas analysis, the recycled and treated autologous blood was infused to the patient to ensure Hct of >42% when the patient presented low hemoglobin level (<80 g/L) and low Hct (<24%).

In group B, patients received allogeneic blood transfusion during spine surgery. Allogeneic concentrated red blood cells were infused when hemoglobin level was lower than 80 g/L and Hct lower than 24%. The blood transfusion volume was determined according to the blood flow and central venous pressure.

Outcome measures

Primary outcome measures: Venous blood of all the included patients before and after blood transfusion as well as day 1, day 3 and day 7 after surgery were obtained and placed in anticoagulant tubes. A total of 5mL venous blood was collected each time. The concentrations of serum tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) of the blood samples were determined by enzyme-linked immunosorbent assay (ELISA) [10]. The assay was conducted according to instructions of the kits for TNF-α and IL-6 (Shenzhen Neobioscience Technology Co., Ltd., China). The optical density value of each well was detected at wavelength of 450 nm using Model 680 microplate reader (Bio-Rad, Hercules, CA, USA), and the concentrations of TNF-α and IL-6 were calculated.

A total of 5 mL venous blood each time was obtained from the included patients and placed in anticoagulant tubes at week 1, week 2, week 4, and week 8 after surgery. Hemorheological indices including plasma viscosity (PV), fibrinogen (FIB), Hct, erythrocyte sedimentation rate (ESR), whole blood low-shear viscosity (WBLSV) and whole blood high-shear viscosity (WBHSV) were determined by XN-9000 automatic hematology analyzer (Sysmex, Kobe, Japan). The operations were carried out according to the instruction manual of the analyzer.

Secondary outcome measures: Coagulation function indices including prothrombin time (PT; normal range: 11.0-13.0 s), thrombin time (TT; normal range: 16.0-18.0 seconds), activated partial thromboplastin time (APTT; normal range: 26.0-36.0 seconds) and FIB level (normal range: 2.0-4.0 g/L) at the end of the surgery were determined by CA-1500 automatic coagulation analyzer (Sysmex, Kobe, Japan). The operations were carried out according to the instruction manual of the analyzer. The incidence of postoperative complications in the two groups was observed, mainly including neural tube injury, adverse cardiac events, hypoproteinemia and incision infection.

Statistical analysis

The data obtained in this study were analyzed using the SPSS software version 18.0 (IBM Corp, Armonk, NY, USA). GraphPad Prism 7 was used to draw the data picture. Enumeration data were expressed as the number of cases/percentage (n/%). Between-group comparison of enumeration data was conducted by Chi-square test. Comparison of postoperative complications results was conducted by Chi-square test with continuity correction. The measurement data conformed to normal distribution are expressed as mean ± standard deviation ($\overline{x}$ ± sd). Between-group comparison of measurement data was conducted by independent-samples t test. The comparison of data at multiple time points was conducted by repeated measures analysis of variance, and Within-group pairwise comparison different time points was conducted using the Bonferroni method. For all analyses, $P<0.05$ was considered statistically significant.

Results

Baseline characteristics

There were no significant differences in sex, age, weight, smoking history, alcohol intake history, place of residence, ASA Physical Status Classification, platelet (PLT) count, hemoglobin (Hb) level, fasting blood glucose (GLU), aspartate aminotransferase (AST) level, alanine aminotransferase (ALT) level, glutamyl transpeptidase (GGT) level, blood loss, blood transfusion volume and operation time between group A and group B (all $P>0.05$) as shown in **Table 1**.

Coagulation function

There were no significant differences in coagulation indices including PT, TT, APTT and FIB level between group A and group B (all $P>0.05$) as shown in **Table 2** and **Figure 1**.

Hemorheological indices

There were no significant differences in hemorheological indices including PV, FIB, Hct, ESR, WBLSV and WBHSV between different time points after surgery (all $P>0.05$). Furthermore, no significant differences were found between group A and group B in the aforementioned hemorheological indices (all $P>0.05$) as shown in **Table 3**.

Table 1. Baseline characteristics of the two groups (n, %)

	Group A (n=80)	Group B (n=80)	t/χ^2	P
Sex (male/female)	35/45	37/43	0.101	0.751
Age (year)	51.8±9.1	50.9±8.2	0.657	0.512
Weight (kg)	58.41±9.34	59.06±10.75	0.408	0.684
Smoking history			0.447	0.504
Yes	29 (36.25)	25 (31.25)		
No	51 (63.75)	55 (68.75)		
Alcohol intake history			0.989	0.320
Yes	25 (31.25)	31 (38.75)		
No	55 (68.75)	49 (61.25)		
Place of residence			2.066	0.151
Urban	55 (68.75)	63 (78.75)		
Rural	25 (31.25)	17 (21.25)		
ASA classification			0.427	0.514
Class I	66 (82.50)	69 (86.25)		
Class II	14 (17.50)	11 (13.75)		
PLT ($10*10^9$/L)	178.68±57.29	183.75±64.58	0.525	0.600
Hb (g/L)	134.57±29.73	138.59±31.09	0.836	0.404
GLU (mmol/L)	5.91±0.42	6.01±0.37	1.598	0.112
AST (U/L)	22.18±13.52	23.07±10.76	0.461	0.646
ALT (U/L)	28.37±8.94	27.65±11.18	0.450	0.653
GGT (U/L)	10.61±3.29	10.57±5.46	0.056	0.955
Blood loss volume (mL)	973.61±193.28	965.08±179.83	0.289	0.773
Blood transfusion volume (mL)	1937.25±416.74	1952.58±537.21	0.202	0.840
Operation time (min)	145.28±26.37	139.64±21.75	1.476	0.142

Note: PLT, platelet; Hb, hemoglobin; GLU, fasting blood glucose; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, glutamyl transpeptidase.

Table 2. Comparison of coagulation function between the two groups ($\overline{x}$ ± sd)

	PT (s)	TT (s)	APTT (s)	FIB (g/L)
Group A (n=80)	13.74±1.16	11.51±2.43	22.61±1.39	1.93±0.16
Group B (n=80)	14.08±1.57	12.37±3.19	23.18±2.43	1.89±0.19
t	1.558	1.918	1.821	1.440
P	0.121	0.057	0.070	0.152

Note: PT, prothrombin time; TT, thrombin time; APTT, activated partial thromboplastin time; FIB, fibrinogen.

Inflammatory factor

There were statistical differences in serum TNF-α and IL-6 concentrations at different time points after surgery. The serum TNF-α concentrations after blood transfusion and at day 1 and 3 after surgery in group A and group B were significantly higher than those before transfusion and at day 7 after surgery (all P<0.001), while the serum TNF-α concentrations at day 1, 3 and 7 after blood transfusion in group A were significantly lower than those in group B (all P<0.001). The serum IL-6 concentrations at day 1 and 3 after surgery in group A and group B were significantly higher than those before blood transfusion and at day 7 after surgery (all P<0.001), while the IL-6 concentration in group A was significantly lower than that in group B at day 1 and 3 after surgery (both P<0.001) as shown in **Tables 4** and **5**, and **Figure 2**.

Postoperative complications

The postoperative complication rate of group A was 7.50%. Of the six cases with complication, there were 2 cases (2.50%) of nerve canal injury, 1 case (1.25%) of adverse cardiac event, 2 cases (2.50%) of hypoproteinemia, and 1 case

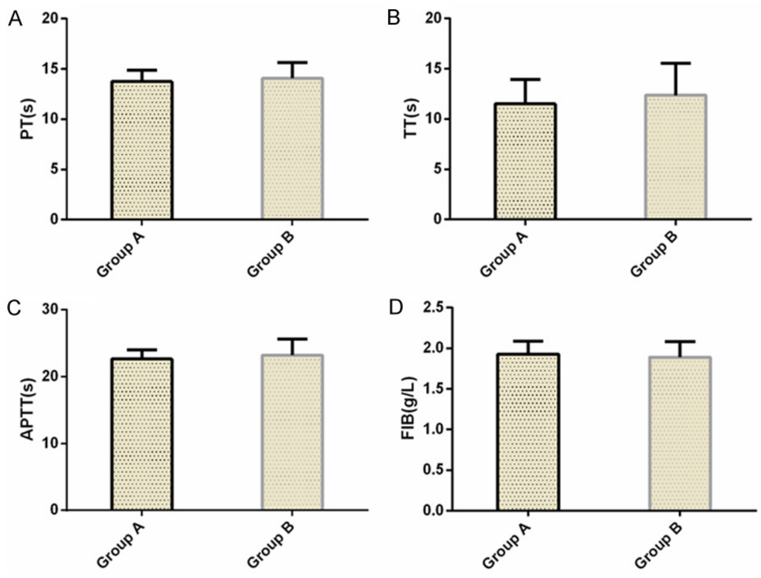

Figure 1. Comparison of coagulation indices between two groups after surgery. PT, prothrombin time; TT, thrombin time; APTT, activated partial thromboplastin time; FIB, fibrinogen.

(1.25%) of incision infection. In group B, complication rate was 33.75%, with 5 cases (6.25%) of nerve canal injury, 5 cases (6.25%) of adverse cardiac events, 10 cases (12.50%) of hypoproteinemia, and 7 cases (8.75%) of incision infection. The incidence of postoperative hypoproteinemia in group A was significantly lower than that in group B (P<0.05). The postoperative complication rate in group A was significantly lower than that in group B (P<0.001) as shown in **Table 6**.

Discussion

Blood loss in relatively large volume presents as a major concern in spine surgery. Timely supplement of the patient's blood volume is frequently needed to ensure the stability of vital signs during surgery [11]. A series of complications often occur in the process of allogeneic blood transfusion, along with relatively large economic burden to the patients [12]. Autologous blood transfusion is to recover the patient's blood bleeding during the operation, and then infuse it into the patient after certain treatment, which avoids transfusion reactions such as fever and allergy from allogeneic blood transfusion, controls the occurrence of various blood-borne diseases, and relieves the clinically blood supply tension, thus being widely used in clinical practice [13-16].

Hemorheology can effectively manifest the characteristics of blood flow of the body under pathological conditions, and can well reflect the microcirculation of the body [17]. Whether the hemorheological function of human body presents pathological changes mainly depends on the blood viscosity, and Hct is the main determinant of blood viscosity [18]. Increased blood viscosity will cause tissue ischemia and hypoxia, thus slowing down the microcirculation of the body and further delaying wound healing [19]. Red blood cells are the main component of the recovered blood. Hemolysis happens easily due to the high negative suction pressure during surgery when conducting blood salvage. The autologous blood salvage and cell saver machine can process the blood by removing harmful substances such as tissue fragments, fragments of red blood cell and inflammatory mediators, thus ensuring that red blood cells infused are in normal condition [20]. In this study, there were no significant differences in PT, TT, APTT, and FIB level between group A and group B after spine surgery, indicating that neither allogeneic nor autologous blood transfusion during surgery would affect the coagulation function of the patients. Moreover, the hemorheological indices showed no significant changes at week 1, 2, 4, and 8 after spine surgery, which indicated that no significant influence existed on the hemorheological indices of the red blood cells of the patients with autologous blood transfusion, but still with adequate microcirculation of the body and normal blood supply and oxygen transportation. XU et al. found that hemorheological indices of the patients with spinal cord injury were negatively affected, with significant increases at week 1 and 2 after surgery, and recovery to normal level at week 4 after surgery [21]. Accordingly, autologous blood transfusion plays a certain stabilizing role on hemorheology of patients after spine surgery.

A relevant study reported that interleukin released after surgery in acute phase and func-

Table 3. Comparisons of hemorheological indices at different time points after surgery between the two groups ($\overline{x}$ ± sd)

	Time	PV (mPa·s)	FIB (g/L)	Hct (%)	ESR (mm/h)	WBLSV (mPa·s)	WBHSV (mPa·s)
Group A (n=80)	Week 1 after surgery	1.31±0.39	3.41±0.32	30.12±5.84	17.63±1.24	8.17±0.63	4.11±0.52
	Week 2 after surgery	1.37±0.33	3.38±0.39	30.67±6.07	17.51±2.08	7.99±0.32	4.08±0.46
	Week 4 after surgery	1.36±0.28	3.46±0.28	30.28±5.42	17.66±1.37	8.16±0.49	3.97±0.31
	Week 8 after surgery	1.29±0.43	3.40±0.31	32.21±5.83	17.39±1.59	8.07±0.57	4.12±0.29
F		0.910	0.864	2.173	0.474	2.152	2.287
P		0.436	0.460	0.091	0.700	0.094	0.079
Group B (n=80)	Week 1 after surgery	1.35±0.37	3.37±0.41	30.05±6.73	17.24±1.16	8.11±0.45	4.06±0.41
	Week 2 after surgery	1.30±0.51	3.42±0.54	31.68±6.64	17.38±1.29	8.03±0.51	4.17±0.38
	Week 4 after surgery	1.34±0.37	3.39±0.47	29.56±7.41	17.53±1.37	8.13±0.53	4.02±0.57
	Week 8 after surgery	1.27±0.42	3.35±0.58	30.62±7.03	17.35±1.29	8.16±0.38	4.13±0.45
F		0.616	0.281	1.372	0.699	1.114	1.740
P		0.605	0.839	0.251	0.554	0.343	0.159

Note: PV, plasma viscosity; FIB, fibrinogen; Hct, hematocrit; ESR, erythrocyte sedimentation rate; WBLSV, whole blood low-shear viscosity; WBHSV, whole blood high-shear viscosity.

Table 4. Comparison of serum TNF-α concentration at different time points after surgery between the two groups ($\overline{x}$ ± sd)

		Group A (n=80)	Group B (n=80)	t	P
TNF-α (ng/mL)	Before blood transfusion	91.66±13.58	90.28±15.93	0.590	0.556
	After blood transfusion	108.28±17.09***	107.59±16.65***	0.259	0.796
	Day 1 after surgery	122.56±22.67$^{***,\#\#\#}$	143.07±23.73$^{***,\#\#\#}$	5.590	<0.001
	Day 3 after surgery	134.58±23.49$^{***,\#\#\#,\&\&\&}$	156.73±24.98$^{***,\#\#\#,\&\&\&}$	5.778	<0.001
	Day 7 after surgery	92.37±18.54	104.27±13.69	4.618	<0.001
F		74.870	166.100		
P		<0.001	<0.001		

Note: TNF-α, tumor necrosis factor-α; compared with serum TNF-α concentration before blood transfusion and at day 7 after surgery, ***P<0.001; compared with serum TNF-α concentration after blood transfusion, $^{\#\#\#}$P<0.001; compared with serum TNF-α concentration day 1 after surgery, $^{\&\&\&}$P<0.001.

Table 5. Comparison of serum IL-6 concentration at different time points after surgery between the two groups ($\overline{x}$ ± sd)

		Group A (n=80)	Group B (n=80)	t	P
IL-6 (ng/mL)	Before blood transfusion	28.62±7.45	27.61±7.08	0.879	0.381
	After blood transfusion	31.54±13.86	35.18±14.67	1.613	0.109
	Day 1 after surgery	42.68±14.59***	53.37±15.53***	4.487	<0.001
	Day 3 after surgery	57.55±27.49$^{***,\#\#\#}$	81.64±31.28$^{***,\#\#\#}$	5.174	<0.001
	Day 7 after surgery	31.69±12.83	34.61±12.96	1.432	0.154
F		41.100	115.500		
P		<0.001	<0.001		

Note: IL-6, interleukin-6; compared with serum IL-6 concentration before and after blood transfusion and at day 7 after surgery, ***P<0.001; compared with serum IL-6 concentration at day 1 after surgery, $^{\#\#\#}$P<0.001.

tional changes in blood properties could change physiological state and induce systemic inflammatory reaction, which may damage vascular function and stabilization of hemodynamic system [22]. Systemic inflammatory reaction syndrome is the major blood transfusion-related

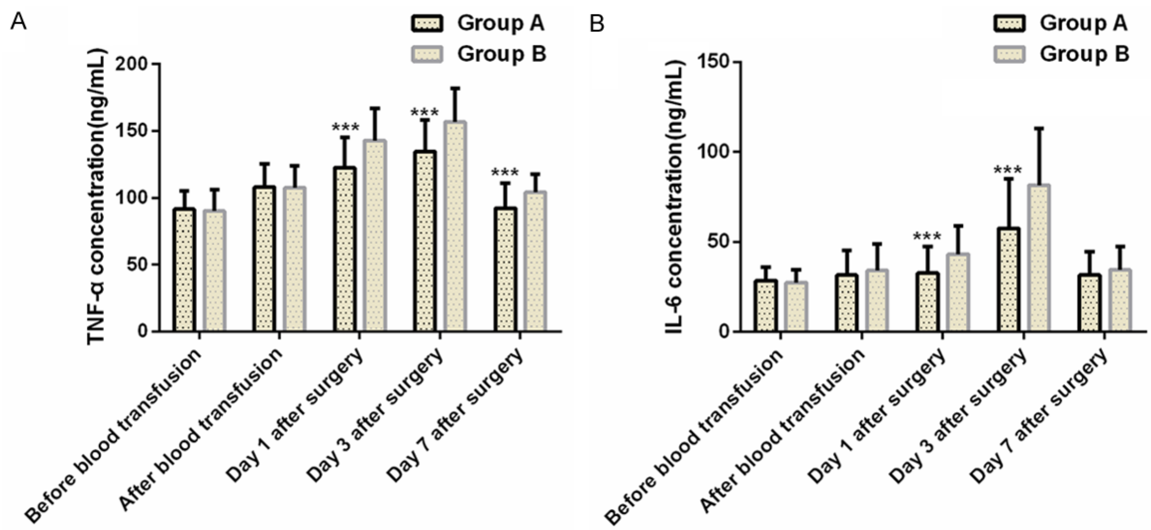

Figure 2. Comparison of serum TNF-α and IL-6 concentrations at different time points after surgery between two groups. A: Comparison of serum TNF-α concentration at different time points after surgery between two groups; B: Comparison of serum IL-6 concentration at different time points after surgery between two groups; compared with group B, ***$P<0.001$.

Table 6. Comparison of postoperative complications between the two groups (n (%))

	Nerve canal injury	Adverse cardiac event	Hypoproteinemia	Incision infection	Total incidence (%)
Group A (n=80)	2 (2.50)	1 (1.25)	2 (2.50)	1 (1.25)	7.50
Group B (n=80)	5 (6.25)	5 (6.25)	10 (12.50)	7 (8.75)	33.75
χ^2	0.598	1.558	4.414	3.289	16.840
P	0.440	0.212	0.036	0.070	<0.001

inflammatory reaction clinically, which can cause transfusion-related lung injury, severe septicemia and multiple organ failure [23]. TNF-α, a polypeptide factor secreted by monocyte/macrophage after stimulation of the body, presents an important role in immune regulation and anti-infection, and can promote production of IL-6. As a result, overexpression of TNF-α and IL-6 can continue inflammatory reactions, thus damaging multiple organs of the body [24, 25]. This study showed that the levels of serum TNF-α and IL-6 after blood transfusion in group A and group B presented an upward trend and returned to normal at day 7 after surgery, indicating that surgery with blood transfusion could lead to the imbalance of patients' immune system and induce the production of inflammatory factors. While the upward trend of levels of serum TNF-α and IL-6 in group A was significantly lower than that in group B, indicating that autologous blood transfusion could effectively in some degree lower down the level of inflammatory factors in patients with spine surgery and could strengthen the anti-inflammatory ability of the body. The incidence of postoperative complications in group A was found to be significantly lower than that in group B, which indicated that autologous blood transfusion presented higher safety and could reduce the incidence of complications. Newman et al. found that perioperative allogeneic blood transfusion was related to the higher reoperation rate of suspected acute infection, and risk factors for infection in patient's exposure to allogeneic blood increased [26]. Therefore, autologous blood transfusion may maintain stable hemodynamics, avoid excessive inflammatory reactions, and improve the anti-infection ability of the body after surgery, and could be conducive to the balance of anti-inflammatory and pro-inflammatory reactions.

Although this study has confirmed that autologous blood transfusion can maintain hemorheology and inhibit inflammatory reaction in patients with spine surgery, the experimental design still has certain defects. In this study, oxygenation function and fracture healing of the patients was not observed. Moreover, in-depth analysis of factors affecting the therapeutic effect during the perioperative period of spine surgery were not conducted. Those deficiencies need to be supplemented in future studies with further corroborative evidence for the conclusion of this study.

In conclusion, autologous blood transfusion in spine surgery presents no effect on coagulation function of the patients, and it can maintain sufficient blood supply as well as reduce the incidence of complications. Stabilizing hemorheology with inhabitation of inflammatory reactions may be one of the therapeutic mechanisms of autologous blood transfusion.

Disclosure of conflict of interest

None.

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