RESEARCH ARTICLE

Impact of Allogenic and Autologous Transfusion on Immune Function in Patients with Tumors

Jian-Rong Guo[1]*, Feng Xu[2], Xiao-Ju Jin[3], Hua-Chun Shen[4], Yang Liu[1], Yi-Wei Zhang[1], Yi Shao[1]

Abstract

Objective: To observe the effects of allogeneic and autologous transfusion on cellular immunity, humoral immunity and secretion of serum inflammatory factors and perforin during the perioperative period in patients with malignant tumors. Methods: A total of 80 patients (age: 38-69 years; body weight: 40-78 kg; ASA I - II) receiving radical operation for gastro-intestinal cancer under general anesthesia were selected. All the patients were divided into four groups based on the methods of infusion and blood transfusion: blank control group (Group C), allogeneic transfusion group (group A), hemodiluted autotransfusion Group (Group H) and hemodiluted autotransfusion + allogenic transfusion Group (A+H group). Venous blood was collected when entering into the surgery room (T_0), immediately after surgery (T_1) and 24h (T_2), 3d (T_3) and 7d (T_4) after surgery, respectively. Moreover, flow cytometry was applied to assess changes of peripheral blood T cell subpopulations and NK cells. Enzyme linked immunosorbent assays were performed to determine levels of IL-2, IL-10, TNF-α and perforin. Immune turbidimetry was employed to determine the changes in serum immunoglobulin. Results: Both CD3+ and NK cells showed a decrease at T_1 and T_2 in each group, among which, in group A, CD3+ decreased significantly at T_2 ($P<0.05$) compared with other groups, and CD3+ and NK cell reduced obviously only in group A at T_3 and T_4 ($P<0.05$). CD4+ cells and the ratio of D4+/CD8+ were decreased in groups A, C and A+H at T_1 and T_2 ($P<0.05$). No significant intra- and inter-group differences were observed in CD8+ of the four groups ($P<0.05$). IL-2 declined in group C at T_1 and T_2 ($P<0.05$) and showed a decrease in group A at each time point ($P<0.05$). Moreover, IL-2 decreased in group A + H only at T_1. No significant difference was found in each group at T_1 ($P<0.05$). More significant decrease in group ?? at T_2, T_3 and T_4 compared with group A ($P<0.05$), and there were no significant differences among other groups ($P>0.05$). IL-10 increased at T_1 and T_2 in each group ($P<0.05$), in which it had an obvious increase in group A, and increase of IL-10 occurred only in group A at T_3 and T_4 ($P<0.05$). TNF-α level rose at T_1 ($P<0.05$), no inter- and intra-group difference was found in perforin in all groups ($P<0.05$). Compared with the preoperation, both IgG and IgA level decreased at T_1 in each group ($P<0.05$), and they declined only in Group A at T_2 and T_3 ($P<0.05$), and these parameters were back to the preoperative levels in other groups. No significant differences were observed between preoperative and postoperative IgG and IgA levels in each group at T_4 ($P>0.05$). No obvious inter- and intra-group changes were found in IgM in the four groups ($P>0.05$). Conclusions: Allogeneic transfusion during the perioperative period could obviously decrease the number of T cell subpopulations and NK cells and the secretion of stimulating cytokines and increase the secretion of inhibiting cytokines in patients with malignant tumors, thus causing a Th1/Th2 imbalance and transient decreasing in the content of plasma immune globulin. Autologous transfusion has little impact and may even bring about some improvement oo postoperative immune function in patients with tumors. Therefore, cancer patients should receive active autologous transfusion during the perioperative period in place of allogeneic transfusion.

Keywords: Allogeneic transfusion - autologous transfusion - malignant tumor - immunity

Asian Pac J Cancer Prev, **15 (1)**, 467-474

Introduction

Most cancer patients often need perioperative transfusion therapy because of preoperative chronic diseases, cancer complications and operative blood loss, and influence of blood transfusion on postoperative immune function of tumor patients is always the focus of clinical research. Numerous studies indicate that allogeneic blood transfusion can cause immune dysfunction in tumor patients to promote the growth and

*[1]Department of Anesthesiology, Shanghai Gongli Hospital of Pudong New District, Shanghai, [2]Department of Anesthesiology, The Second People's Hospital of Anhui Province, Hefei, [3]Department of Anesthesiology, Yijishan Hospital, Wannan Medical College, Wuhu, [4]Department of Anesthesiology, Ningbo No.2 Hospital, Ningbo, China *For correspondence: jrmail@163.com*

metastasis of tumor cells, leading to increase of malignant tumor postoperative recurrence, postoperative infection and other related complications (De Oliveira et al., 2011; Ojima et al., 2009; Bernard et al., 2009). Allogeneic blood transfusion can suppress immune function in tumor patients, but its action mechanism has not been made clear. Some studies suggest that the residual leukocyte ingredients in blood cause change of immune function (Hendrickson et al., 2009), while other studies suggest that metabolites produced during the storage of red blood cells cause change of immune function (Sparrow et al., 2010).

With the increase of blood volume for clinical use and short supply of blood, autotransfusion (autologous blood transfusion) is increasingly widely used in clinical practice. Autotransfusion can effectively reduce blood volume of perioperative allogeneic blood transfusion and prevent blood-borne diseases (Meng et al., 2013). Currently, autotransfusion is mainly divided into the following three ways: predeposit autotransfusion, dilute autotransfusion (hemodilution autotransfusion) and intraoperative autotransfusion. The use of predeposit autotransfusion in clinical practice is restricted because preoperative preparation time is relatively long and operations for tumor patients are deadline ones. Intraoperative autotransfusion recovers the blood on the surgical field. The recovered blood may contain tumor cells, leading to the risk of the spread of cancer cells after retransfusion, thus it currently belongs to the absolute contraindication. At present, the more feasible mature method for tumor patients to undergo clinical autotransfusion is only dilute autotransfusion.

This study observed the changes of perioperative humoral immunity, cellular immunity, plasma inflammatory substances and cytotoxic lymphocyte cytotoxic function after allogeneic red blood cell transfusion and dilute autotransfusion in tumor patients. Then, the study comprehensively evaluated the influence of allogeneic blood transfusion and autotransfusion on immune function in tumor patients to further investigate the clinical value and significance of blood component transfusion and blood protection in order to provide the guidance for perioperative reasonable blood use in tumor patients.

Materials and Methods

Drugs and reagents

Drugs and reagents: 6% hydroxyethyl starch 130/0.4 (batch number: 81EL172; Fresenius Kabi company, Germany), interleukin-2 (IL-2) kit, interleukin-10 (IL-10) kit, tumor necrosis factor-α (TNF-α) kit, perforin (PF/PFP) kit (Shanghai Huayi Biological Technology Co., Ltd.), supporting flow anti-human IgG1-FITC/IgG2a-PE monoclonal antibody, supporting flow anti-human CD45-FITC/CD14-PE monoclonal antibody, supporting flow anti-human CD3-FITC/CD4-PE monoclonal antibody, supporting flow anti-human CD3-FITC/CD8-PE monoclonal antibody, supporting flow anti-human CD3-FITC/CD16 + CD56-PE monoclonal antibody, supporting flow erythrocyte lysate (Becton Dickinson Company, USA), supporting immunoglobulin G biochemical reagents, supporting immunoglobulin A biochemical reagents and supporting immunoglobulin M biochemical reagents (Beckman Coulter Inc., USA).

Case selection

After approval by Ethics Management Committee of the hospital, we selected 80 patients for elective gastrointestinal tumor radical correction, including 44 males and 36 females, aged 38 to 69 years old, weighing 40 to 78 kg and ASA grade I~II. Among the patients, 26 cases had gastric cancer, 24 had colonic cancer and 30 had rectal cancer. Case selection criteria: patients had gastrointestinal malignant tumor diagnosed by gastrointestinal endoscope and pathology before operation; patients had no severe heart, lung, liver, kidney or endocrine diseases before operation; there was no history of operation, radiotherapy, chemotherapy, recent application of immunosuppressive agents, severe infection, blood-borne diseases, coagulation dysfunction, platelet dysfunction and blood transfusion; preoperative hemoglobin was greater than 110 g/L and hematocrit was not less than 0.33.

Anesthesia method

Preoperative fasting lasted 12 h and forbidding drinking lasted 6 h. No drugs were used to all patients before operation. After a patient was sent into an operating room, blood pressure (BP), heart rate (HR), electrocardiogram (ECG), oxygen saturation (SpO_2), central venous pressure (CVP) and the depth of anesthesia (BIS) were routinely monitored. An upper extremity vein was opened. Under local anesthesia, radial artery was catheterized for invasive arterial blood pressure (ABP) and blood gas detection. An internal jugular vein was catheterized for bloodletting, blood sampling and CVP monitoring. Drugs used during induction of anesthesia were: midazolam 0.05 mg/kg, fentanyl 4~5 μg/kg, propofol 1~2 mg/kg and cis-atracurium 0.2~0.4 mg/kg. After rapid induction, intubation was performed. Then Dräger primus anesthesia workstation was connected to perform mechanical ventilation. Inhalation oxygen concentration was 100%, the oxygen flow rate was 1.5~2.0 L/min, tidal volume was 8~10 mL/kg, respiratory rate was 12 bpm, inspiration to expiration ratio (I/E) was 1: 2 and end tidal CO_2 partial pressure ($P_{ET}CO_2$) maintained 35~45 mHg. Continuous injection pump of propofol 6~8 mg × kg^{-1} × h^{-1}, remifentanil 0.1~0.25 μg × kg^{-1}× h^{-1} and cis-atracurium 2~3 μg × kg^{-1} × min^{-1} maintained anesthesia and muscle relaxation, and BIS value maintained between 45 and 60. Operations in all patients were performed by the same group of surgeons, the same narcotic drugs and anesthesia methods were used, and anesthesia management was performed by the same group of skilled anesthesiologists to avoid operation and anesthesia technique difference which would impact postoperative immune function.

Blood transfusion methods

Control group (group C): conventional crystalloid solution and colloid solution were infused to replenish blood volume, but blood transfusion therapy had not been performed in any way.

Table 1. Comparison of the General Data of Patients in Each Group ($\bar{\chi}$±s, n=20)

Index	Group C	Group A	Group H	Group A+H
Gender (female/male)	9/11	8/12	10/10	9/11
Age (years)	63±7	61±8	62±7	64±6
Weight (kg)	56.6±9.3	59.7±8.6	60.4±9.5	62.1±7.8
Operation time (min)	173.1±34.5	169.0±24.7	168.7±25.5	175.7±22.3
Gastric cancer/rectum/ colon cancer (cases)	6/7/7	7/8/5	7/7/6	6/8/6

Allogeneic blood transfusion group (group A): conventional solution was infused, while according to intraoperative blood loss and Hb/Hct monitoring, as appropriate, red blood cell suspension was transfused.

Autotransfusion (Hemodilution autotransfusion or dilute autotransfusion) group (group H): dilute autotransfusion was performed during operation. After the patient was sent into an operating room, 6~8 mL/kg crystalloid solution was maintained physiological requirement. After anesthesia was steady, autologous blood was collected at 10~20 mL/min. Meanwhile, the equivalent volume of 6% hydroxyethyl starch (Voluven) was infused through a peripheral vein. Autologous blood storage duration did not exceed 6 h.

Blood collection volume reference formula: blood collection volume = EBV × 2 × (Hct_{actual}-Hct_{target}) / (Hct_{actual} + Hct_{target}). EBV was the expected blood volume in a body. Male: Weight (kg) ×70 mL/kg; Women: Weight (kg) ×60 mL/kg. Hct_{actual} and Hct_{target} were the expected hematocrit before and after dilution, respectively, and Hct_{ideal} was set from 28% to 30%.

Hemodilution + allogeneic blood transfusion group (Group A+H): During operation, after autotransfusion according to above method, if blood loss was comparatively large or the monitoring showed that Hb/Hct kept low, as appropriate, allogeneic red blood cell suspension was transfused.

During the experiment, the infusion liquid was pre-heated by an infusion and transfusion heating apparatus to 36°C before entering patients' blood in order to avoid rapid infusion of large amount of cold liquid to cause temperature fluctuation resulting in change in immune function.

Detection indexes

Specimen collection: For each patient in four groups, 7 mL venous blood was collected after arriving in an operating room (T_0), immediately after operation (T_1), 24 h after operation (T_2), 3 d after operation (T_3) and 7 d after operation (T_4). The blood sample was divided into three tubes: a tube with EDTA anticoagulant was used for T cell subsets and NK cell test by flow cytometry within 6 h, while the other two tubes without anticoagulant coagulated at room temperature for 30 min and were centrifuged at 3000 r/min for 20 min. The supernatant was collected into an ET tube and was stored at -70°C in a refrigerator for detecting serum TNF-α, IL-2, IL-10, PF and IgG, IgA, IgM concentrations.

Specimen test: a. Flow cytometry method (FCM) was used to detect T cell subsets and NK cells. Fluid dynamics focusing was used to guarantee that cells passed through a laser beam (detection zone) one by one in the same way. When a cell in the sample flow passed through the detection zone of the flow chamber, an elliptical laser beam can detect the cell signal and then the supporting software system can obtain the corresponding data; b. ELISA was used to detect the concentrations of plasma IL-2, IL-10, TNF-α and PF. The anti-human IL-2 (IL-10, TNF-α, PF) monoclonal antibody was coated onto elisa plates, and biotinylated anti-human IL-2 (IL-10, TNF-α, PF) antibody and horseradish peroxidase-labeled avidin were added. Chromogenic reagent was added. If there was IL-2 (IL-10, TNF-α, PF) in reaction wells, horseradish peroxidase would make colorless chromogenic agent become blue, and then, the liquid would become yellow when terminator was added. OD value at 450 nm was measured and the concentration of IL-2 (IL-10, TNF-α, PF) was proportional to the OD450 value, therefore the concentration of IL-2 (IL-10, TNF-α, PF) in specimens can be obtained by drawing standard curve.

Table 2. Changes of Hemodynamics in Patients with Hb/Hct during Operation ($\bar{\chi}$±s, n=20)

Index / Groups	n	immediately into the room	The operation started immediately	At the end of operation
Hb (g/L)				
Group C	20	130.7±7.8	125.9±9.9	101.3±9.5[a]
Group A	20	126.0±7.6	121.2±8.7	105.3±12.5[a]
Group H	20	129.3±7.7	102.6±8.9[abc]	102.8±9.27[a]
Group A+H	20	128.4±8.1	100.8±7.1[abc]	106.0±8.6[a]
Hct (%)				
Group C	20	40.9±3.9	38.4±4.7	29.4±4.6[a]
Group A	20	38.8±4.1	36.6±4.5	31.4±4.5[a]
Group H	20	40.1±4.4	28.7±2.8[abc]	30.2±4.6[a]
Group A+H	20	39.1±3.8	28.2±2.5[abc]	32.1±3.9[a]
HR (time/min)				
Group C	20	72.3±7.4	77.1±6.7	76.4±10.4
Group A	20	71.3±7.4	74.1±7.6	75.8±7.2
Group H	20	70.9±7.6	75.2±9.5	74.9±7.4
Group A+H	20	73.8±8.5	75.3±8.5	71.8±6.7
MAP (mmHg)				
GuoupC	20	74.8±6.7	73.5±5.4	75.7±5.1
GuoupA	20	72.3±5.7	75.1±5.6	73.9±5.3
Group H	20	75.4±5.8	73.8±5.2	74.9±5.7
Group A+H	20	73.7±5.5	72.6±5.4	75.9±4.9

Note: comparison with T_0, [a]P<0.05; compared with group C, [b]P<0.05; compared with group A, [c]P<0.05; compared with group H, [d]P<0.05. [a]P<0.05 vs. T_0; [b]P<0.05 vs. group C; [c]P<0.05 vs. group A; [d]P<0.05 vs. group H

Immunoglobulin detection: The coagulated blood was centrifuged at 3000 r/min and the supernatant was taken into biochemical analyzer to detect serum immunoglobulin IgG, IgA, and IgM levels with immunity transmission turbidity assay.

Statistical data analysis

Quantitative information of obtained test data was expressed as the mean ± standard deviation ($\bar{\chi}$±s) so that analysis of variance was used for the normal distribution data and rank sum test was used for non-normal distribution data; chi-square test was used for enumeration data. SPSS13.0 statistical analysis software was used for data processing and P<0.05 was considered statistically significant.

Table 3. Changes of NK Cell Subsets and T Cells of Patients in Each Group Before and after Operation ($\bar{\chi}\pm s\%$, n=20)

Index	Groups	n	T_0	T_1	T_2	T_3	T_4
$CD3^+$ (%)	Group C	20	70.8±9.1	64.2±7.8[a]	61.2±8.7[a]	69.05±7.4	69.8±5.9
	Group A	20	69.9±9.7	63.1±9.5[a]	53.9±10.2[ab]	53.85±10.4[ab]	53.9±8.2[ab]
	Group H	20	73.9±7.7	66.9±8.4[a]	65.9±8.7[ac]	71.30±8.5[c]	72.8±6.9[c]
	Group A+H	20	71.3±9.6	65.6±8.2[a]	62.5±8.6[ac]	67.1±7.9[c]	67.7±6.3[c]
$CD4^+$ (%)	Group C	20	36.7±7.8	30.5±8.7[a]	31.1±7.4[a]	34.3±7.8	37.8±6.1
	Group A	20	37.2±8.7	31.4±8.6[a]	26.4±6.7[ab]	28.7±6.8[ab]	28.2±5.7[ab]
	Group H	20	35.3±7.8	38.9±6.6[bc]	40.3±7.7[abc]	36.1±8.1[c]	36.7±6.1[c]
	Group A+H	20	37.1±7.4	31.8±7.1[ad]	32.3±5.8[acd]	35.3±6.7[c]	34.2±7.0[c]
$CD8^+$ (%)	Group C	20	31.4±7.9	29.5±7.7.	30.5±7.6	29.7±7.3	28.6±7.1
	Group A	20	26.7±6.9	26.4±6.3	28.2±6.9	30.5±7.5	29.1±7.2
	Group H	20	29.9±7.5	27.7±6.9	30.6±7.2	28.7±6.7	27.8±6.0
	Group A+H	20	30.5±7.6	28.1±7.4	29.0±6.1	29.6±8.0	29.4±7.4
$CD4^+/CD8^+$	Group C	20	1.21±0.16	1.07±0.17[a]	1.08±0.17[a]	1.20±0.14	1.22±0.18
	Group A	20	1.23±0.18	1.10±0.21[a]	1.09±0.23[a]	1.06±0.19[ab]	1.03±0.18[ab]
	Group H	20	1.24±0.21	1.25±0.19[bc]	1.26±0.23[bc]	1.25±0.22[c]	1.24±0.17[c]
	Group A+H	20	1.23±0.19	1.09±0.21[ad]	1.08±0.18[ad]	1.18±0.14[c]	1.18±0.17[c]
NK cells (%)	Group C	20	16.5±5.1	13.2±4.8[a]	13.6±4.4[a]	16.5±4.6	16.8±5.3
	Group A	20	15.7±4.8	12.6±4.3[a]	11.9±3.7[a]	11.8±3.4[ab]	12.0±4.6[ab]
	Group H	20	17.4±6.2	13.5±4.8[a]	13.4±3.6[a]	15.8±4.2[c]	16.9±4.9[c]
	Group A+H	20	16.1±5.2	12.9±3.7[a]	12.2±4.7[a]	15.6±3.8[c]	16.20±3.9[c]

Note: comparison with T_0, [a]P<0.05; compared with group C, [b]P<0.05; compared with group A, [c]P<0.05; compared with group H, [d]P<0.05. [a]P<0.05 vs. T_0; [b]P<0.05 vs. group C; [c]P<0.05 vs. group A; [d]P<0.05 vs. group H

Table 4. Changes in Plasma of Patients with Inflammatory Factors and Perforin Before and after Operation ($\bar{\chi}\pm s$, pg/mL)

Index	Groups	n	T_0	T_1	T_2	T_3	T_4
IL-2	GroupC	20	117.31±20.17	99.97±19.70[a]	97.58±23.25[a]	107.72±26.09	100.98±23.71
	GroupA	20	114.85±28.01	93.75±17.43[a]	82.47±14.56[ab]	80.90±15.54[ab]	80.47±17.70[ab]
	GroupH	20	106.09±22.42	100.33±21.92	96.39±16.97[c]	99.55±18.59[c]	102.48±18.31[c]
	Group A+H	20	114.54±25.47	94.60±19.99[a]	104.17±20.59[c]	112.35±22.63[c]	104.36±25.42[c]
IL-10	GroupC	20	14.54±4.11	30.95±7.14[a]	56.97±14.19[a]	17.30±4.07	15.99±4.37
	GroupA	20	15.42±3.98	45.33±7.82[ab]	68.70±8.16[ab]	31.50±7.19[ab]	20.17±5.42[ab]
	GroupH	20	13.95±3.67	31.19±8.15[ac]	57.47±14.86[ac]	17.45±4.47[c]	15.92±4.32[c]
	Group A+H	20	14.90±4.03	31.69±7.37[ac]	58.98±14.70[ac]	17.56±5.70[c]	16.15±4.57[c]
TNF-α	GroupC	20	22.28±8.68	24.94±6.37	28.09±7.37[a]	24.28±6.07	20.28±6.07
	GroupA	20	21.98±8.04	26.79±6.31	31.35±7.53[a]	23.74±7.25	21.53±6.17
	GroupH	20	22.94±7.47	24.71±8.16	28.99±6.05[a]	25.29±6.19	22.42±5.49
	Group A+H	20	23.06±8.24	25.29±7.29	29.35±5.88[a]	23.41±6.34	21.30±6.48
PF	GroupC	20	81.41±7.35	80.66±6.84	78.26±7.30	80.71±6.07	80.80±5.82
	GroupA	20	76.75±8.66	81.58±7.40	83.05±6.06	82.20±8.25	82.11±8.53
	GroupH	20	82.00±5.76	79.78±7.36	81.07±5.06	80.81±4.87	78.98±9.77
	Group A+H	20	81.65±6.64	77.02±7.51	80.52±4.39	77.66±6.11	82.16±4.84

Note: comparison with T_0, [a]P<0.05; compared with group C, [b]P<0.05; compared with group A, [c]P<0.05; compared with group H, [d]P<0.05. [a]P<0.05 vs. T_0; [b]P<0.05 vs. group C; [c]P<0.05 vs. group A; [d]P<0.05 vs. group H

Results

Comparsion of patients' basic information

There was no statistically significant difference in the sex, age, weight, operation time of patients and case constitution between groups (P>0.05) (Table 1).

Hemodynamics and Hb/Hct test results of patients in each group

Compared with values before bloodletting, the Hb and Hct of the patients in group H and group A+H decreased immediately after bloodletting (P<0.05), and the Hb and Hct of the patients in all groups decreased immediately after operations (P<0.05). There was no significant difference between the Hb, Hct in each group before bloodletting and those immediately after operations (P>0.05). Immediately after bloodletting, the Hb and Hct in group H and group A+H were lower than those in group C and group A (P<0.05). During operations, HR and MAP changes of patients in all groups were not significant, and there was no statistical difference between groups or within any group (P>0.05) (Table 2).

Test results of T cell subsets and NK cells in each group of patients

The $CD3^+$ and NK cells in each group of patients decreased at T_1 and T_2 points. Among them, compared with other groups, the $CD3^+$ in group A decreased significantly at T_2 (P<0.05), and the $CD3^+$ and NK cells only in group A decreased significantly at T_3 and T_4 (P<0.05). The $CD4^+$

Table 5. Changes in Plasma of Patients in Each Group of Immunoglobulin Before and after Operation ($\bar{\chi}$±s, g/L)

Index	Groups	n	T_0	T_1	T_2	T_3	T_4
IgG	Group C	20	11.49±2.02	10.21±1.69[a]	11.05±1.64	11.68±1.66	12.02±1.81
	Group A	20	11.65±1.97	10.26±1.53[a]	9.73±1.79[ab]	10.47±1.61[ab]	11.18±1.71
	Group H	20	12.09±1.75	10.76±1.65[a]	11.67±1.67[c]	12.03±1.84[c]	11.55±1.67
	Group A+H	20	11.78±2.16	10.57±1.69[a]	11.58±1.86[c]	11.94±2.13[c]	11.36±1.99
IgA	Group C	20	2.92±0.87	2.40±0.66[a]	2.88±0.68	3.01±0.77	2.82±0.67
	Group A	20	2.83±0.87	2.33±0.59[a]	2.27±0.67[ab]	2.29±0.71[ab]	2.75±0.56
	Group H	20	2.99±0.81	2.45±0.67[a]	3.00±0.69[c]	3.02±0.64[c]	2.74±0.54
	Group A+H	20	2.85±0.84	2.35±0.68[a]	2.94±0.68[c]	2.99±0.60[c]	2.98±0.69
IgM	Group C	20	1.21±0.44	1.11±0.31	1.22±0.30	1.31±0.37	1.27±0.25
	Group A	20	1.24±0.34	1.14±0.30	1.23±0.25	1.19±0.49	1.21±0.31
	Group H	20	1.18±0.38	1.11±0.27	1.29±0.34	1.25±0.31	1.21±0.28
	Group A+H	20	1.25±0.34	1.14±0.28	1.27±0.26	1.27±0.27	1.24±0.28

Note: comparison with T_0, [a]P<0.05; compared with group C, [b]P<0.05; compared with group A, [c]P<0.05; compared with group H, [d]P<0.05. [a]P<0.05 vs. T_0; [b]P<0.05 vs. group C; [c]P<0.05 vs. group A; [d]P<0.05 vs. group H

and $CD4^+/CD8^+$ ratio in group A, group C and group A+H of patients reduced at T_1 and T_2 (P<0.05), while $CD4^+$ in group H increased at T_2 (P<0.05) and the $CD4^+/CD8^+$ ratio did not significantly change (P>0.05). At T_3 and T_4, the $CD4^+$ and $CD4^+/CD8^+$ ratio only in group A decreased significantly (P<0.05), and there was no significant difference between other groups (P>0.05). There was no significant difference in $CD8^+$ within any group of patients or between four groups (P>0.05) (Table 3).

Changes of plasma IL-2, IL-10, TNF-α and perforin of patients in each group

Postoperative IL-2 of patients in group C decreased at T_1 and T_2 (P<0.05), postoperative IL-2 of patients in group A decreased at all time points (P<0.05), and postoperative IL-2 of patients in group A+H decreased only at T_1 (P<0.05). There was no significant difference between groups at T_1 (P> 0.05); at T_2, T_3 and T_4 postoperative IL-2 of patients in group A was lower than other groups (P <0.05) and there was no significant change between other groups (P>0.05). The IL-10 of patients in each group increased at T_1 and T_2 (P<0.05), including the fact that the IL-10 of patients in group A increased significantly, and at T_3 and T_4 the IL-10 of patients only in group A increased (P<0.05) while the IL-10 of patients in other groups dropped to levels at T_0. TNF-α increased at T_1 (P<0.05) and there was no significant difference in perforin within each group or between groups (P>0.05) (Table 4).

Changes of patients' plasma immunoglobulin in each group

IgG and IgA of patients in four groups were lower at T_1 than those at T_0 (before operation) (P <0.05), while IgM did not significantly change (P>0.05). At T_2 and T_3, the IgG and IgA of patients only in group A decreased and the difference was statistically significant (P<0.05). There was no significant difference in IgG, IgA and IgM of patients in each group at T_4 and before operation (P> 0.05). There was no significant difference in IgG, IgA, IgM between groups at T_0, T_1, T_4 (P>0.05), while at T_2 and T_3, the IgG and IgA levels in group A were significantly lower than other three groups (P<0.05), but IgM did not significantly change (P>0.05) (Table 5).

Discussion

Dilute autotransfusion does not require blood type test and cross matching test, and avoids the potential risk of allogeneic blood transfusion-transmitted diseases. Meanwhile, it may reduce the loss of red blood cells, reduce blood viscosity and improve microcirculation perfusion. In this study, after blood collection, the minimum mean Hb in patients who would receive autotransfusion was 100.8 ± 7.1, and the minimum mean Hct in the patients was 28.2 ± 2.5. Both of them were within the acceptable ranges. All patients responded well after blood collection, autotransfusion was smooth and hemodynamics was stable during operation, and there were no adverse reactions from the autotransfusion.

During the process of the body's immune response, T lymphocytes play an important role (Wang et al., 2013). Animal experiments and clinical studies have shown that T cells are helpful to control the growth of tumor cells (Hod et al., 2010; Bogdan et al., 2011). $CD3^+$ is the main recognition unit for T cells to recognize endogenous antigens and almost all mature T cells in the body express $CD3^+$. $CD4^+$ T cells can be recognized by antigen peptides presented by particular major histocompatibility complex (MHC) moleculesII. $CD4^+$ T cells can activate natural killer cells, $CD8^+$ T cells and macrophages, and enhance killing capacity of corresponding cells. Meanwhile, they can promote B lymphocyte proliferation and differentiation, resulting in producing corresponding antibodies. Portion of $CD4^+$ T cells in vivo can identify antigen peptides presented by MHC II molecules to directly kill target cells (Leal-Noval et al., 2010). After $CD8^+$ T cells are activated in vivo and differentiate into mature effector cells, they can only identify antigen peptides presented by specific MHCImolecules and kill relatively sensitive target cells with high selectivity and specificity. They can release effect molecules with particle exocytosis to kill target cells, and can also induce apoptosis of target cells through Fas-Fasl death signal transduction pathway (Piconese et al., 2008). NK cells can not only remove malignant cells in the body, but also can play an immune surveillance role in tumorigenesis, directly inhibit tumor cell growth and prevent metastasis of tumor and tiny cancer embolus

so that they play a very important role in the body's anti-tumor immunity (Holt et al., 2011). Therefore, the detected percentages of T cell subsets and NK cells in plasma can represent changes of lymphocytes in circulation and are effective indicators to reflect postoperative cellular immune function in tumor patients and to have important value for determining the prognosis of tumor patients after operations (Liu et al., 2013).

The results of this study showed that compared with the preoperative values, the postoperative $CD4^+$ and $CD4^+/CD8^+$ ratio of patients in each group decreased, indicating that certain common factors lead to postoperative cellular immune function changes of patients. The results of this study also showed that the $CD3^+$, $CD4^+$, $CD4^+/CD8^+$ ratio and NK cells in allogeneic blood transfusion group began to decline immediately after operation and had not recovered on postoperative day 7, indicating that allogeneic blood transfusion can reduce postoperative T lymphocyte subsets and NK cells number of tumor patients thus allogeneic blood transfusion can reduce cellular immune function. By observing impact of allogeneic blood transfusion on immune function in gastric cancer patients, Maeta et al found that allogeneic blood transfusion can significantly reduce the number of NK cells in gastric cancer patients, leading to inhibition of patients' cellular immune function (Barnett et al., 2010). Our study result is basically consistent with the study result of Maeta. The test result also showed that number of the $CD4^+$ T cells of patients in autotransfusion group was higher on postoperative day 1 than before operation ($P<0.05$), while there was no significant difference in other indicators between autotransfusion group and control group ($P>0.05$), indicating that impact of autotransfusion on immune function of tumor patients is small and autotransfusion even improves immune inhibition. In this study, there was no significant difference in postoperative T cell subsets and NK cells of patients between hemodilution + allogeneic blood transfusion group and control group ($P>0.05$), probably because upregulation of cellular immunity caused by hemodilution covers up immune function inhibition caused by blood transfusion.

In recent years, many studies have found that Th1/Th2 balance in tumor patients shifts to Th2 and some people think that this may be one of the mechanisms of tumor immune escape (Lopez et al., 2011). Because there are no specific markers of Th1 and Th2, we chose the most representative IL-2, IL-10 and TNF-α. Changes of their plasma concentrations were measured to observe impact of transfusion and allogeneic blood autotransfusion on Th1/Th2 balance.

The study results showed that immediately after operation the IL-2 in four groups had declined, especially in allogeneic blood transfusion group it decreased significantly. From postoperative day 1 to 7, the IL-2 in all groups had slightly rebounded except the IL-2 in allogeneic blood transfusion group continued to decline. The fact suggests that allogeneic blood transfusion can significantly inhibit plasma IL-2 level and autotransfusion has slight impact on IL-2 secretion. IL-2 has many biological functions. It can stimulate naive T cells to go into cell division cycle rapidly, promote the proliferation of NK cells in vivo, enhance killing activity of lymphoid toxicity cells in vivo, and prolong survival time of T cells and NK cells. In addition, IL-2 can promote IL-2 receptor expression of B cells, promote differentiation and maturation and immunoglobulin production of B cells, and enhance phagocytosis of macrophages (Pipkin et al., 2010; Boyman et al., 2012). Therefore, decrease of IL-2 plasma concentration can inhibit function of T cell and B cell, leading to poor prognosis in tumor patients.

The results of this study showed that the IL-10 in allogeneic blood transfusion group began to increase immediately after operation and had not been recovered on postoperative day 7, while the IL-10 in the remaining three groups increased on the postoperative day 1 and recovered to the preoperative levels soon on the postoperative day 3, indicating that allogeneic blood transfusion can increase the secretion of IL-10 and autotransfusion has less impact on IL-10. IL-10 is an important anti-inflammatory cytokine and can inhibit anti-tumor immunity of the body: on the one hand, it can inhibit Th1 differentiation and the corresponding cytokine synthesis through the antigen-presenting cells. On the other hand, it can inhibit cytotoxicity of effector cells such as cytotoxic T cells, NK cells and γδT (Howell et al., 2007; Kourea et al., 2008). Increase of IL-10 can cause differentiation of Th0 to shift to Th2, resulting in a poor prognosis in tumor patients.

The results of this study showed that on postoperative day 1, compared with preoperative levels, the plasma TNF-α level in four groups elevated. The phenomenon may be caused by operative trauma and other stimuli. On postoperative day 3 to 7, there was no significant difference between any groups at any time point, indicating either autotransfusion or allogeneic blood transfusion has comparatively small effect on the plasma concentrations of TNF-α. A study, which researches impact of blood transfusion on tumor necrosis factor in patients with lung cancer, has also found that after allogeneic blood transfusion there is no significant change of patients' plasma TNF-α (Vamvakas et al., 2007). The finding is consistent with the result of this study. TNF-α is quite important for tumor immunity, because it can kill tumor cells directly by cytotoxic effect, destroy epithelial tissue of peripheral vessels of tumor cells and blocks tumor blood supply by the effect of thrombosis. It can also promote the killing effect of cytotoxic T cells and other killer cells to tumor cells by immunomodulatory effects (Balkwill, 2009). From above research, it can be seen that after tumor patients receive allogeneic blood transfusion, IL-2 continuously decreases and IL-10 increases so that balance of Th1/Th2 shifts more to Th2, while the effect of autotransfusion on Th1/Th2 balance is not significant.

Perforin is a kind of glycoprotein, the structure of which is similar to complement C, and mainly exists in the cytoplasm granules of cytotoxic cells. Perforin-dependent mechanism of cell apoptosis can not only kill tumor cells, but also involves in tumor immune surveillance and tumor metastasis inhibition, and meanwhile perforin also plays a positive role in postoperative infection and related complication prevention of tumor patients (Brudvik et al., 2011; Rajbhandary S et al., 2013).

The results of this study showed that at various

postoperative time points the perforin concentrations of patients in four groups did not change significantly ($P>0.05$). It indicates that either allogeneic blood transfusion or autotransfusion has only slight impact on plasma perforin expression. Recurrent researches suggest that plasma perforin expression may be related to secretion of specific cytokine, i.e. interleukins (Pintaric et al., 2012; Schmidt et al., 2012). In this study, postoperative plasma IL-2 level of patients in allogeneic blood transfusion group significantly decreased, while compared with preoperative secretion level the IL-10 secretion level increased. Theoretically, after allogeneic blood transfusion, activity of cytotoxic lymphocyte to secrete perforin should decrease, but this study did not show this phenomenon. The reasons may be that some other immune cells can also release perforin to show cytotoxicity and other functions, resulting in the fact that change of plasma perforin is not obvious and there is no statistically significant difference.

Human immunoglobulin is glycoprotein produced by plasma cells which after antigen stimulation B cell proliferated and differentiated, and the immunoglobulin involving in the immune process in tumor patients mainly is IgG, IgA and IgM. Antibodies are important effective molecules mediating humoral immunity and perform humoral immune function by binding corresponding specific antigens. Therefore, the determination of immunoglobulin levels in tumor patients can evaluate their humoral immune function and can also be used as effective indicators of their anti-infection immunity.

The results of this study showed that immediately after operation the plasma IgA and IgG concentrations in four groups of patients had slightly declined. This may be because during operation in order to maintain effective blood volume a lot of crystalloid solution and colloid solution were infused, resulting in diluting visible components of blood. On postoperative day 1, the plasma IgA and IgG concentrations in control group, hemodilution group and combined group quickly returned to preoperative levels, while the plasma IgA and IgG concentrations in allogeneic blood transfusion group continued to decline and did not recover until postoperative day 7. The study results showed that conventional infusion and dilute autotransfusion had slight impact on humoral immune function of tumor patients, while allogeneic red blood cell transfusion could significantly inhibit postoperative immunoglobulin levels of tumor patients. Although this is a transient suppression, it is very unfavorable for postoperative anti-infection treatment and complication prevention of tumor patients. By observing effects of blood transfusion on immunoglobulin in patients with intestinal tumor, Changbo Han have found that on postoperative day 1 the plasma IgA, IgG and IgM concentrations of patients receiving allogeneic blood transfusion decreased significantly (Han, 2011). A study shows that by observing impact of autotransfution and allogeneic blood transfusion on immunity of orthopedic patients, it is found that allogeneic blood transfusion significantly inhibits postoperative immunoglobulin levels of patients, but autotransfusion does significantly not (Piao et al., 2013). The analysis for it believes the reason may be that during preservation of blood the red blood cells may age, blood cells produce excessive various antigen substances and immune complexes, and red blood cells are overburdened (Pandey et al., 2010). Combined with the results of this study and based on comprehensive analysis, we consider that impact of different transfusion methods on postoperative immunoglobulin of tumor patients could be caused by two reasons. One, after allogeneic blood transfusion, immune function of the body's T cells which can provide the second signal to activate B cell may be reduced, resulting in B cell differentiation and maturation disorders so that end product of B cells, plasma cells, are produced less, to secrete less immunoglobulin, and meanwhile autotransfusion has less impact on the immune function of T lymphocytes thus its impact of immunoglobulin secretion is not significant (Jin, 2009; Voskoboinik et al., 2010). On the other hand, after allogeneic blood transfusion, allogeneic red blood cells and their degradation products may be considered as the antigens which can produce antigen-antibody reactions to consume a lot of the immunoglobulin, meanwhile blood for autotransfusion is generally stored shorter, produces less metabolites, and is the autologous blood so that its impact on the secretion of immunoglobulin is slight.

In summary, perioperative allogeneic blood transfusion can cause immune dysfunction of tumor patients, resulting in poor prognosis of tumor patients. This immune suppression may be joint action result of reducing number of lymphocytes, reducing secretion of cellular mediators and immunoglobulins and other kinds of mechanisms. Autotransfusion slightly impacts immune function of patients. Therefore, during perioperative period allogeneic blood transfusion should be strictly limited in tumor patients, and when blood transfusion is needed autotransfusion should be the first choice to reduce influence of blood transfusion on postoperative immune function of patients.

Acknowledgements

The authors are grateful to Dr. Jun Yu for statistical support and Dr. Bao-wei Jin for data collection. The study is supported by Outstanding Leaders Training Program of Pudong Health Bureau of Shanghai (Grant No. PWR12013-03).

References

Balkwill F (2009). Tumour necrosis factor and cancer. *Nat Rev Cancer,* **9**, 361-71.

Barnett CC, Beck AW, Holloway SE, et al (2010). Intravenous delivery of the plasma fraction of stored packed erythrocytes promotes pancreatic cancer growth in immuno-competent mice. *Cancer*, **116**, 3862-74.

Bernard AC, Davenport DL, Chang PK, et al (2009). Intraoperative transfusion of 1 U to 2 U packed red blood cells is associated with increased 30-day mortality, surgical-site infection, pneumonia, and sepsis in general surgery patients. *Am Coll Surg*, **208**, 931-7.

Bogdan C (2011). Regulation of lymphocytes by nitric oxide. *Methods Mol Biol*, **677**, 375-93.

Boyman O, Sprent J (2012). The role of interleukin-2 during homeostasis and activation of the immune system. *Nat Rev*

Immunol, **12**, 180-90.
Brudvik KW, Taskén K (2011). Modulation of T cell immune functions by the prostaglandin E(2)-cAMP pathway in chronic inflammatory states. *Br J Pharmacol*, **5**, 411-9.
De Oliveira GS Jr, Schink JC, Buoy C, et al (2011). The association between allogeneic perioperative blood transfusion on tumour recurrence and survival in patients with advanced ovarian cancer. *Transfus Med*, **11**, 1111-41
Han CB (2011). Effect of immunoglobulin on autologous blood transfusion in patient with colocectal cancer. *J Clin Hematol*, **24**, 597-8.
Hendrickson JE, Hillyer CD (2009). Noninfectious serious hazards of transfusion. *Anesth Analg*, **108**, 759-69.
Hod E A, Zhang N, Sokol SA, et al (2010). Transfusion of red blood cells after prolonged storage produces harmful effects that are mediated by iron and inflammation. *Blood*, **115**, 4284-92.
Holt D, Ma X, Kundu N, et al (2011). Prostaglandin E(2) (PGE (2)) suppresses natural killer cell function primarily through the PGE(2) receptor EP4. *Cancer Immunol Immunother*, **60**, 1577-86.
Howell WM, Rose-Zerilli MJ (2007). Cytokine gene polymorphisms, cancer susceptibility, and prognosis. *J Nutr*, **137**, 194S-9S.
Jin BQ (2009). A major breakthrough in T-B cells and collaborative study of follicular helper T cells, a new $CD4^+$ effect of T cell subsets. *Chin J Cell Mol Immunol*, **25**, 1-5.
Kourea K, Parissis JT, Farmakis D, et al (2008). Effects of darbepoetin-alpha on plasma pro-inflammatory cytokines, anti-inflammatory cytokine interleukin-10 and soluble Fas/ Fas ligand system in anemic patients with chronic heart failure. *Atherosclerosis*, **199**, 215-21.
Leal-Noval SR, Muñoz-Gómez M, Arellano V, et al (2010). Influence of red blood cell transfusion on $CD4^+$ T-helper cells immune response in patients undergoing cardiac surgery. *J Surg Res*, **164**, 43-9.
Liu ZM, Wang YB, Yuan XH. (2013). Exosomes from murine-derived GL26 cells promote glioblastoma tumor growth by reducing number and function of $CD8^+$ T cells. *Asian Pac J Cancer Prev*, **14**, 309-14.
Lopez TV, Lappin TR, Maxwell P, et al (2011). Autocrine/ paracrine erythropoietin signalling promotes JAK/STAT-dependent proliferation of human cervical cancer cells. *Int J Cancer*, **129**, 2566-76.
Meng J, Lu XB, Tang YX, et al (2013). Effects of allogeneic blood transfusion in patients with stage II colon cancer. *Asian Pac J Cancer Prev*, **14**, 347-50.
Ojima T, Iwahashi M, Nakamori M, et al (2009). Association of allogeneic blood transfusions and long-term survival of patients with gastric cancer after curative gastrectomy. *Gastrointest Surg*, **13**, 1821-30.
Pandey P, Chaudhary R, Aggarwal A, et al (2010). Transfusion-associated immunomodulation: Quantitative changes in cytokines as a measure of immune responsiveness after one time blood transfusion in neurosurgery patients. *Asian J Transfus Sci*, **4**, 78-85.
Piao YR, Piao LZ, Zhu LH, et al (2013). Prognostic value of T cell immunoglobulin mucin-3 in prostate cancer. *Asian Pac J Cancer Prev*, **14**, 3897-901.
Piconese S, Valzasina B, Colombo MP, et al (2008). Triggering blocks suppression by regulatory T cells and facilitates tumor rejection. *J Exp Med*, **205**, 825-39.
Pintaric M, Gerner W, Saalmüller A (2008). Synergistic effects of IL-2, IL-12 and IL-18 on cytolytic activity, perforin expression and IFN-gamma production of porcine natural killer cells. *Vet Immunol Immunopathol*, **121**, 68-82.
Pipkin ME, Sacks JA, Cruz-Guilloty F, et al (2010). Interleukin-2 and inflammation induce distinct transcriptional programs that promote the differentiation of effector cytolytic T cells. *Immunity*, **32**, 79-90.
Rajbhandary S, Zhao MF, Zhao N, et al (2013). Multiple cytotoxic factors involved in IL-21 enhanced antitumor function of CIK cells signaled through STAT-3 and STAT5b pathways. *Asian Pac J Cancer Prev*, **14**, 5825-31.
Schmidt NW, Khanolkar A, Hancox L, et al (2012). Perforin plays an unexpected role in regulating T-cell contraction during prolonged Listeria monocytogenes infection. *Eur J Immunol*, **42**, 629-40.
Sparrow RL (2010). Red blood cell storage and transfusion-related immunomodulati- on. *Blood Transfus*, **3**, 26-30.
Vamvakas EC, lajchman MA (2007). Transfusion-related immunomodulation (TRIM): An update. *Blood Rev*, **21**, 327-38.
Voskoboinik I, Dunstone M A, Baran K, et al (2010). Perforin: structure, function, and role in human immunopathology. *Immunol*, **235**, 35-54.
Wang WJ, Tao Z, Gu W, et al (2013). Variation of blood T lymphocyte subgroups in patients with non- small cell lung cancer. *Asian Pac J Cancer Prev*, **14**, 4671-3.