Journal of Zhejiang University SCIENCE B
ISSN 1673-1581 (Print); ISSN 1862-1783 (Online)
www.zju.edu.cn/jzus; www.springerlink.com
E-mail: jzus@zju.edu.cn

Effect of perioperative autologous versus allogeneic blood transfusion on the immune system in gastric cancer patients*

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Received Apr. 25, 2007; revision accepted June 28, 2007

Abstract: Background: Allogeneic blood transfusion-induced immunomodulation (TRIM) and its adverse effect on the prognosis of patients treated surgically for cancer remain complex and controversial. However, the potential risk associated with allogeneic blood transfusion has heightened interest in the use of autologous blood transfusion. In the present study, the serum concentrations of neopterin, interferon-gamma (IFN-γ), T lymphocyte subsets ($CD3^+$, $CD4^+$, $CD8^+$, $CD4^+/CD8^+$) and a possible association between these variables were investigated. The purpose was to further evaluate the effect of autologous versus allogeneic blood transfusion on immunological status in patients undergoing surgery for gastric cancer. Methods: Sixty ASA I~II (American Society of Anesthesiologists) patients undergoing elective radical resection for stomach cancer were randomly allocated to receive either allogeneic blood transfusion (n=30) or autologous blood transfusion (n=30). Serum concentrations of the neopterin, IFN-γ and T lymphocyte subsets in the recipients were measured before induction of anesthesia, after operation, and on the 5th postoperative day. Results: Both two groups, serum neopterin, IFN-γ, percentages of T-cell subsets ($CD3^+$, $CD4^+$), and $CD4^+/CD8^+$ ratio had significantly decreased after operation, but decreased more significantly in group H (receiving allogeneic blood transfusion) than those in group A (receiving autologous whole blood transfusion) (P<0.05). On the 5th postoperative day, serum neopterin, IFN-γ, $CD3^+$, $CD4^+$ T-cells, and $CD4^+/CD8^+$ ratio returned to the baseline values in group A. In contrast, the above remain decreasing in group H, where there were no significant relations between serum neopterin and IFN-γ. Conclusion: Perioperative surgical trauma and stress have an immunosuppressive impact on gastric cancer patients. Allogeneic blood transfusion exacerbates the impaired immune response. Autologous blood transfusion might be significantly beneficial for immune-compromised patients in the perioperative period, clearly showing its superiority over allogeneic blood transfusion.

Key words: Transfusion-induced immunomodulation (TRIM), Autologous blood transfusion, Allogeneic blood transfusion, Neopterin, Interferon-gamma (IFN-γ), $CD3^+$, $CD4^+$, $CD4^+/CD8^+$ ratio

doi:10.1631/jzus.2007.B0560 **Document code:** A **CLC number:** R614.1

INTRODUCTION

Cancer radical resection is often associated with greater surgical trauma and longer operative time, as well as more blood loss. It is not surprising that patients demand blood transfusion support to save life during the perioperative period. Unfortunately, an untoward effect of blood transfusion is immunosuppression, which has been postulated to result in promoting tumor recurrence and metastasis, etc. Several studies indicated that immunosuppressive effect is mediated by the leukocytes presence in allogeneic blood (Kirkley, 1999; Chu, 1999). The infusion of incompatible major histocompatibility complex antigens between donor and recipient may be responsible for this deleterious effect (Lin *et al*., 2002). Perioperative decrease in the interleukin-2 (IL-2) and natural killer (NK) cells activity suggested that allogeneic blood transfusion compromises the immune response (Bar-Yosef *et al*., 2001; Yan *et al*., 2005). In summary, there are many transfusion-induced immunomodulatory complications, whose exact mechanism has not been fully elucidated. Nevertheless, these above-mentioned potential risks have

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* Project supported by the Health Department of Zhejiang Province (No. 2004A040) and the Education Department of Zhejiang Province (No. G20030486), China

heightened interest in the use of autologous blood transfusion. Many researches showed that autologous blood transfusion was a cost-effective alternative to reduce allogeneic blood consumption in perioperative period (Carless *et al*., 2004; Vanderlinde *et al*., 2002; Lewis *et al*., 2005). But up to now, there are few data available on the effects of autologous blood transfusion on immune function.

Recently, it has become clear that an effective immune response is largely dependent on the activation of T lymphocytes. The various cytokines secreted by activated T lymphocytes are involved in immune regulation. Hereinto, interferon-gamma (IFN-γ) is one of cytokines produced by T lymphocytes of the $CD8^+$ cytotoxic/suppressor cells and $CD4^+$ helper cells, which plays an important role in innate and adaptive immunity. IFN-γ may upregulate the expression of major histocompatibility complex (MHC) to contribute to the immunological responses (Nagao *et al*., 2000). It also activates macrophages to kill tumor cells by releasing reactive oxygen intermediates and TNF-α (tumor necrosis factor-α) (Ray and Kirschner, 2006). Additionally, neopterin is mainly synthesized by activated monocytes/macrophages in response to induction by IFN-γ. Measurement of neopterin concentrations in body fluids like serum, cerebrospinal fluid or urine provides information about T helper cell 1-derived cellular immune activation (Murr *et al*., 2002). Due to its early and highly change to various pathological situations, neopterin in human body fluids is considered a reliable marker as monitoring processes, development and prognosis of many diseases including immune disorders, inflammations, and coronary artery diseases (Murr *et al*., 2002; Melichar *et al*., 2006). Taken together, these findings indicate that cell-mediated immune response is highly related to the activity of IFN-γ and neopterin. Therefore, in the present study, we hypothesize that the levels of IFN-γ and neopterin may reliably and sensitively reflect autologous or allogeneic blood transfusion-associated immunomodulation. We measured the serum concentrations of neopterin, IFN-γ, T lymphocyte subsets ($CD3^+$, $CD4^+$, $CD8^+$, $CD4^+/CD8^+$) in the perioperative period and investigated a possible association between neopterin and IFN-γ. The aim was to further evaluate the effect of autologous versus allogeneic blood transfusion on immunological status in patients with gastric carcinoma.

MATERIALS AND METHODS

Patients

After the protocol for the present study was approved by the Ethics Committee of Zhejiang University (Hangzhou, China), sixty ASA I~II (American Society of Anesthesiologists) patients, aged 40~65 years (33 male, 27 female), undergoing elective radical resection for stomach cancer were studied. All the patients' preoperative hemoglobin (Hb) was >110 g/L, and hematocrit (Hct) >33%. The patients with cardiovascular, respiratory, hepatic, renal diseases, endocrinopathy and immune disease were excluded. Patients with a history of blood transfusion were excluded from this study to avoid the effects of previous transfusion. The patients undergoing radiotherapy, chemotherapy and hormone therapy were not included. They were divided randomly into two groups: group A and group H. Group A received autologous whole blood transfusion, whereas group H received allogeneic blood transfusion.

Anesthesia

Patients were premedicated with intramuscular injection of diazepam (10 mg) and atropine (0.5 mg). Anesthesia was induced with intravenous midazolam (0.1 mg/kg), fentanyl (5 μg/kg), propofol (1 mg/kg) and vecuronium (0.12 mg/kg), and was maintained with isoflurane inhalation (1%~2%) and intermittent boluses of fentanyl and vecuronium. The patients were ventilated mechanically. Monitoring consisted of electrocardiogram, heart rate, respiratory rate, invasive arterial pressure (systolic, diastolic, and mean), and peripheral arterial oxygen saturation by pulse oximetry (SpO_2), end-tidal CO_2 ($EtCO_2$). The patients were subjected to radical gastrectomy (for cancer) and gastroenterostomy. When operation was over, all patients recovered in the PACU (post anesthesia care unit).

Methods

Group H received 400 ml of allogeneic blood during the period of gastroenterostomy only if the loss of blood exceeded 500 ml and Hb was below 100 g/L. In group A, 400 ml of autologous blood was removed from radical artery before surgery and 500 ml of plasma substitute was given via internal jugular vein at the same time. Mean arterial pressure was maintained at 70~90 mmHg on average all along. Group A

also received autologous blood at the time of gastroenterostomy.

Sample collections

Blood samples (5 ml venous blood) were taken from internal jugular vein before induction of anesthesia (baseline), after operation (day 0), and on the 5th postoperative day (day 5) into Vacutainer tubes containing or not ethylenediaminetetraacetic acid (EDTA), respectively.

Determination of serum neopterin, plasma IFN-γ

All blood samples without EDTA were centrifuged immediately and supernatants were stored at −70 °C till assay. The concentrations of serum neopterin and IFN-γ were determined by enzyme-linked immunosorbent assay (ELISA). The kits of neopterin were provided by the tumor graduate school of the Second Affiliated Hospital of Zhejiang University. The kits of IFN-γ were produced by Genzyme Company in Cambridge, MA, USA.

Determination of T lymphocyte subsets

T lymphocyte subsets were determined using whole-blood flow cytometry. A hundred microlitres anticoagulated blood samples with EDTA were incubated with 20 μl of relevant monoclonal antibody reagent containing CD3-peridnin-chlorophyll protein (PerCP), CD4-fluorescein isothiocyanate (FITC), and CD8-phycoerythrin (PE) at room temperature for 45 min, followed by adding 2 ml erythrocytes lysis solution and incubation at room temperature for 10 min. The mixture was centrifuged at 4 °C for 12000 r/min×5 min and the supernatant was removed. The cell pellets were washed with 1×PBS (phosphate-buffered saline) solution, collected by centrifugation, resuspended in 1 ml 1% paraformaldehyde (pH 7.3) immediately, then measured with a FACSCalibur™ flow cytometer and analyzed with CellQuest 3.1™ software (Becton Dickinson Immunocytometry Systems) that counts 10000 cells.

Statistics

The patients' data were recorded and calculated with SPSS 11.0. *t*-test was used to compare the character of patients between different groups. After test of homogeneity (all $P>0.05$), one-way analysis of variance (ANOVA) was used to compare the difference of time within A or H group; LSD (least significant difference) was used to test the difference for day 0 and day 5 compared with baseline. When compare the difference between A and H groups, two-way ANOVA was used. Linear correlation analysis between neopterin and IFN-γ was also conducted. $P<0.05$ was considered statistically significant.

RESULTS

Patient characteristics

There were no significant differences between the two groups in age, weight, sex, time length of surgery and amount of blood loss (Table 1).

Serum neopterin and IFN-γ analysis

In the group H, serum neopterin and IFN-γ decreased significantly after operation and on the 5th postoperative day as compared with the baseline (before induction of anesthesia) values ($P<0.05$ or $P<0.01$). In the group A, serum neopterin and IFN-γ also decreased significantly after operation as compared with the baseline values ($P<0.05$). However, on the 5th postoperative day, they returned to the baseline values. Between the two groups there were no differences in serum neopterin and IFN-γ before induction of anesthesia ($P>0.05$). Serum neopterin in the group H decreased significantly compared with that in group A ($P<0.05$) after operation. On the 5th postoperative day, in the group H, serum neopterin and IFN-γ decreased significantly compared with those in the group A ($P<0.05$ or $P<0.01$) (Fig.1).

Correlation analysis: In the group H, the

Table 1 Biometric and clinical data of the patients

	Sex (male/female)	Age (year)	Weight (kg)	Operation time (h)	Total blood loss (ml)
Group H (n=30)	17/13	51.3±6.5	61.0±3.1	3.3±0.4	604.2±178.2
Group A (n=30)	16/14	49.3±6.5	64.2±5.9	3.1±0.5	583.3±182.5

Values are expressed as mean±*SD*. No significant changes between the groups. Group H received allogeneic blood transfusion; Group A received autologous whole blood transfusion

correlation coefficients of serum neopterin and IFN-γ before operation, after operation, and on the 5th postoperative day were: r=0.082, P>0.05; r=0.089, P>0.05; r=0.092, P>0.05. There were no significant differences.

T lymphocyte subsets analysis

Before induction of anesthesia, no significant differences were observed in the percentages of T-cell subsets ($CD3^+$, $CD4^+$, $CD8^+$) and $CD4^+/CD8^+$ ratio between the two groups. After operation (day 0), $CD3^+$, $CD4^+$ T-cells and $CD4^+/CD8^+$ ratio decreased significantly relative to baseline (before induction of anesthesia) values in both groups (P<0.05 or P<0.01), but decreased more significantly in group H than in group A (P<0.05). On the 5th postoperative day (day 5), $CD3^+$, $CD4^+$ T-cells and $CD4^+/CD8^+$ ratio were basically close to the baseline values in group A (P>0.05) but remained low in group H. $CD8^+$ T-cells had no significant changes in both groups during the perioperative period (Table 2).

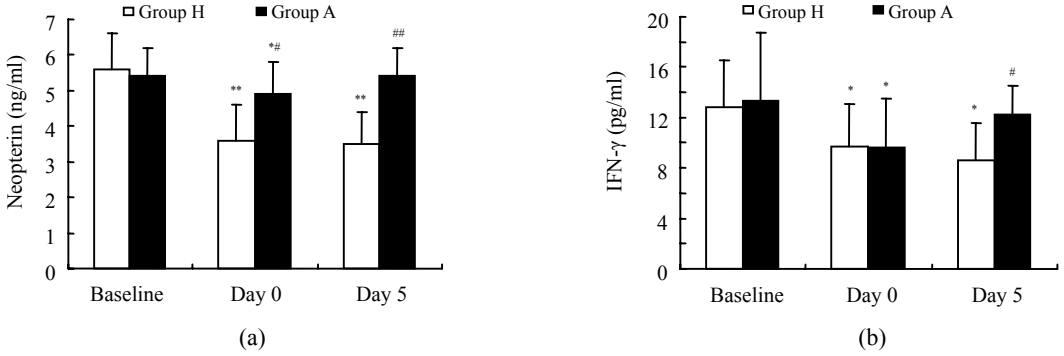

Fig.1 Serum concentrations of neopterin (a) and IFN-γ (b) before induction of anesthesia (baseline), after operation (day 0) and on the 5th postoperative day (day 5) in the two groups

Group H received allogeneic blood transfusion; Group A received autologous whole blood transfusion. $^*P$<0.05, $^{**}P$<0.01, compared with before operation; $^{\#}P$<0.05, $^{\#\#}P$<0.01, compared with group H

Table 2 T lymphocyte subsets during perioperative time

	Group (n=30)	$CD3^+$ (%)	$CD4^+$ (%)	$CD8^+$ (%)	$CD4^+/CD8^+$
Baseline	H	65.0±4.1	38.2±5.1	28.6±4.9	1.36±0.31
	A	67.2±5.1	39.9±4.9	27.3±4.1	1.44±0.23
Day 0	H	53.7±4.5*	27.0±5.2**	26.4±4.2	1.02±0.33*
	A	59.7±5.3$^{*\#}$	31.7±5.0$^{*\#}$	27.1±3.9	1.15±0.29*
Day 5	H	52.1±5.4**	26.5±3.9**	26.7±4.3	0.99±0.21**
	A	63.1±4.7$^{\#\#}$	37.4±4.9$^{\#\#}$	26.9±4.6	1.32±0.26$^{\#\#}$

Values are expressed as mean±SD. Expression of $CD3^+$, $CD4^+$, and $CD8^+$ before induction of anesthesia (baseline), after operation (day 0) and on the 5th postoperative day (day 5) in the two groups. Group H received allogeneic blood transfusion; Group A received autologous whole blood transfusion. $^*P$<0.05, $^{**}P$<0.01, compared with before operation; $^{\#}P$<0.05, $^{\#\#}P$<0.01, compared with group H

DISCUSSION

Recently, multiple proposed pathologic mechanisms that account for the allogeneic blood transfusion-induced immunomodulation (TRIM) were complex and surrounded by controversy in the perioperative period. In this study, we showed that in gastric cancer patients undergoing allogeneic blood transfusion concentrations of serum neopterin significantly reduced after surgery (day 0) and remained in decreasing trend on the 5th postoperative day (day 5). Such a reduction has been paralleled to the drop of IFN-γ cytokine, $CD3^+$, $CD4^+$ T lymphocyte cells and the $CD4^+/CD8^+$ ratio. As compared with autologous blood transfusion, these results demonstrate that allogeneic blood transfusion causes long-term cell-mediated immunosuppression and suggest that various immune cells and cytokines are involved in TRIM complications. Several investigations have indicated that an observed reduction of $CD3^+$, $CD4^+$ T lymphocyte subsets after allogeneic blood transfusion is likely to be mediated by leukocyte alloantigens and

plasma components (Claas *et al*., 2001; Biedler *et al*., 2002). Patients with documented recurrence and metastasis of cancer at the time of blood drawing had the lower T cell counts of many of those studied (Kuss *et al*., 2004). Accordingly, we consider the possibility that the decrease in $CD3^+$, $CD4^+$ T cells, as well as $CD4^+/CD8^+$ ratio, might adversely influence antitumor response. Furthermore, not $CD8^+$ T cells, but reduction of $CD4^+$ T cells showed imbalance in lymphocyte differentiation and proliferation. This disturbed lymphocyte homeostasis might be especially detrimental in malignant patients with poor prognosis (Kuss *et al*., 2004). The depressed secretion of IFN-γ cytokine in the postoperative period accorded with the findings of other investigators. We have demonstrated a decrease in IL-2 production in stimulated lymphocytes after allogeneic blood transfusion (Yan *et al*., 2005). Such a decrease could be simply related to the impaired $CD4^+$ Th1 (T help) of which IFN-γ and IL-2 are products. In contrast, a preponderance of $CD4^+$ Th2 cytokines (IL-4, IL-5, IL-10) over $CD4^+$ Th1 cytokines (IFN-γ, IL-2) in posttransfusion stimulated lymphocytes was found in both mice and humans (Potter *et al*., 2003; Kirkley *et al*., 1995; Babcock and Alexander, 1996). The Th2 cytokine pattern is associated with decreased cytotoxic cell functions and inhibition of macrophage activation (Potter *et al*., 2003; Kirkley *et al*., 1998). These studies also seem to show a hypothetical mechanism of TRIM that Th2 cells can produce immunosuppression in transfusion recipients by down-regulating the activity of Th1 cells.

Increasing amounts of neopterin synthesis in body fluids are associated with activated cell-mediated immunity. Jin *et al*.(2005) and Altindağ *et al*.(1998) showed that increased productions of neopterin are proportional to the progression of autoimmune disorders such as rheumatoid arthritis and systemic lupus erythematosus (SLE). In other words, the results of the present study indicate that down-modulation of serum neopterin sensitively reflects immunosuppressive situation. Additionally, Wirleitner *et al*.(2002) suggested that monocytes/macrophages were thought to be the unique source of neopterin biosynthesis. In vitro IFN-γ is usually a potent inducer (Murr *et al*., 2002). Interestingly, in this study despite a significant decrease in serum neopterin and IFN-γ cytokine in patients undergoing allogeneic blood transfusion, no correlation was observed between the two phenomena. This investigation was supported by Sghiri *et al*. (2005), who demonstrated that IFN-γ is not the only stimulus in vivo needed for the synthesis of neopterin in patients with impairment of the IFN-γ pathway. Therefore, rather than the depressed secretion of IFN-γ cytokine, we speculate that allogeneic blood transfusion directly and/or by other cytokines down-regulates monocytes/macrophage function to exert their inhibitory and regulatory properties on neopterin production. Certainly, further studies need to be done to substantiate this.

In the autologous blood group, the decrease in serum neopterin, IFN-γ cytokine and T lymphocyte subsets shows immune suppression after surgery (day 0). However, its inhibitory effect significantly dropped as compared with allogeneic blood transfusion. This observed decreased immune response is mainly attributed to surgical trauma in the autologous blood group (Schneemilch *et al*., 2005). Moreover, anesthetic agents, blood loss and psychological stress may contribute to perioperative immunosuppression (Andersen *et al*., 1998; Bar-Yosef *et al*., 2001). The above-mentioned assumptions were also confirmed by the present findings. On the 5th postoperative day, once trauma and stress reactions weakened, cell-mediated immune response basically got its breath again. Thus, evidence from the present study suggests that autologous blood transfusion had no significant immune suppression effects. Whereas, Heiss *et al*.(1997) reported that autologous blood transfusions probably stimulated a Th1 pattern with decreased IL-10 and increased IL-2 plasma levels to up-regulate cell-mediated immune function.

Several evidences exist that in patients with malignant tumor, anti-tumor cellular responses are suppressed (Yan *et al*., 2005; Ordemann *et al*., 2002). Immunosuppression further increases undergoing surgical procedures. Significant immunosuppression was suggested to contribute to cancer recurrence and metastasis, which brings high morbidity and mortality (Kuss *et al*., 2004; Angele and Faist, 2002; Biedler *et al*., 2002). Unfortunately, in the present study, all results indicate that perioperative allogeneic blood transfusion exacerbates the impaired immune response. In contrast, autologous blood transfusion might be significantly beneficial for immune-compromised patients in the perioperative period, clearly showing its superiority over allogeneic blood transfusion.

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