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Immunologic changes to autologous transfusion after operational trauma in malignant tumor patients: Neopterin and Interleukin-2[*]

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Abstract: Objective: To estimate the impact of autologous transfusion on the status of perioperative immune activation in malignant tumor patients. The Serum Neopterin and Interleukin-2 (IL-2) were measured. Methods: Sixty patients undergoing elective radical resection for malignant stomach tumor were enrolled in the prospective study and assigned to the following groups: (1) Group A received autologous transfusion; (2) Group H received allogeneic transfusion. The perioperative course (Before induction of anesthesia, after operation and 5 d after operation) of Neopterin and IL-2 was compared. Results: In group A, Serum Neopterin was significantly lower than baseline after operation and IL-2 had no significant changes. In group H, both Serum Neopterin and IL-2 were significantly lower than baseline after operation and 5 d after operation. Compared with group A, Serum Neopterin was significantly lower than baseline after operation and 5 d after operation and IL-2 was significantly lower than baseline 5 d after operation. Conclusion: Autologous transfusion decreased the perioperative immune suppression in malignant stomach tumor patients.

Key words: Autologous transfusion, Allogeneic transfusion, Serum Neopterin, INF-gamma, Immunologic change
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INTRODUCTION

Allogeneic blood transfusion therapy is associated with many risks, including major and minor blood transfusion reaction, non-A non-B hepatitis, hepatitis B and HIV infection, but autologous blood transfusion can easily avoid the above risks and resolve the shortage of blood transfusion resources. It is considered as a safe and effective method for reducing allogeneic blood use. Recent years clinical studies showed that perioperative allogeneic blood transfusions can also induce immunosuppression which directly has adverse effect on cancer recurrence, postoperative infection and poor prognosis of the malignant patients. Some domestic and overseas scholars reported that allogeneic blood transfusion could decrease cellular immune function by inhibiting differentiation of T lymphocytes and impairing natural killer (NK) cells activity (Landers *et al.*, 1996; Heiss *et al.*, 1997). But up to now, there are few data available on the effects of autologous blood transfusion on immune function. The aim of this study was to explore perioperative immunomodulation in malignant patients by investigating the clinical effect of changes in Serum Neopterin and Interleukin-2 (IL-2) after allogeneic and autologous blood transfusion.

MATERIALS AND METHODS

Selection of cases

After the protocol for the present study was approved by the Ethics Committee of Zhejiang Univer-

sity (Hangzhou, China), sixty ASA I–II patients from March 2000 to December 2000, aged 40–65 yr (33 male, 27 female), undergoing elective radical resection for stomach cancer were studied. They were divided randomly into two groups: group A and group H. All the patients' preoperative Hb was >110 g/L, and Hct>33%. The patients with cardiovascular, respiratory, hepatic, renal diseases, endocrinopathy and immune diseases were excluded. The patients undergoing radiotherapy, chemotherapy and hormone therapy were not included. Group A received autologous 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. The patients were subjected to radical gastrectomy (for cancer) and gastroenterostomy.

Methods

Perioperative average blood loss of all patients was (550±150) ml. Group H received 400 ml of allogeneic blood during the period of gastroenterostomy only if the loss of blood exceeded 500 ml. In group A, 400 ml of autologous blood were removed from radial artery before surgery and 500 ml of plasma substitute were 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 CVP line before induction of anesthesia, after operation (day 0) and on the 5th postoperative day (day 5). The collected blood was rapidly transferred to the central laboratory to test the concentrations of Serum Neopterin and IL-2 by ELISA method. The concentration of Serum Neopterin was measured by Sigma full-automatic enzyme labelling instrument,

with ng/ml unit. The tumor graduate school of Second Affiliated Hospital of Zhejiang University provided us with kits. The concentration of IL-2 was measured by double antibody method, with pg/ml unit (kits produced by Genzyme Company in Cambridge, Massachusetts, USA).

Statistics

The patients' data were recorded and calculated with SPSS 11.0. Differences between points of time within a study group were evaluated by the paired *t*-test. Significance of differences between study groups was tested by ANOVA. Linear correlation analysis was also conducted. *P* value of <0.05 was considered statistically significant.

RESULTS

Group A included 30 patients (16 males and 14 females), with mean age (49.3±6.5) yr and mean body weight (64.2±5.9) kg, group H also 30 patients (17 males and 13 females), with mean age (51.3±6.5) yr and mean weight (61.0±3.1) kg. There were no significant differences between the two groups in age, weight, sex, time length of surgery, amount of blood loss and fluid infusion.

In the autologous group, Serum Neopterin was much lower after operation (4.88±0.86) ng/ml as compared with the baseline (before operation (5.44±0.80) ng/ml, *P*<0.01). On the 5th postoperative day, it basically rose close to the preoperative level. There were no significant changes in IL-2 during the whole perioperative stage (Fig.1a).

In the allogeneic group, Serum Neopterin and IL-2 were (3.64±0.97) ng/ml and (9.62±1.36) pg/ml at the time point after operation, respectively. On the 5th postoperative day, Serum Neopterin was (3.48±0.89) ng/ml and IL-2 was (9.13±0.75) pg/ml. Compared with the baselines Serum Neopterin (5.61±1.00) ng/ml and IL-2 (10.11±0.81) pg/ml, they were significantly lower (Fig.1).

There were no differences between the two groups in Serum Neopterin and IL-2 before operation. Serum Neopterin (3.64±0.97) ng/ml in group H was significantly lower than that (4.88±0.86) ng/ml in group A (*P*<0.01) after operation. There was no significant difference between the two groups in IL-2

after operation. On the 5th postoperative day, in the group H, Serum Neopterin and IL-2 were much lower than those in the group A ($P<0.01$) (Fig.1).
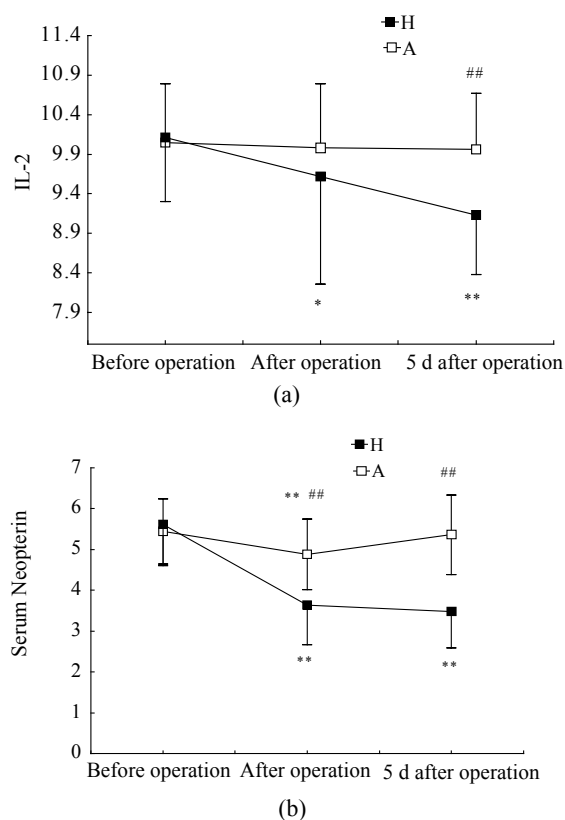
Fig.1   IL-2 (a) and Serum Neopterin (b) during Perioperative time
[*]$P<0.05$ versus before operation time point; [**]$P<0.01$ versus before operation time point; [##]$P<0.01$ versus group H

Correlation analysis: In the group H, the correlation coefficients of Serum Neopterin and IL-2 before operation, after operation and on the 5th postoperative day were: $r=0.076$, $P>0.05$; $r=0.099$, $P>0.05$; $r=0.045$, $P>0.05$. In contrast, in the group A, the correlation coefficients of Serum Neopterin and IL-2 before operation, after operation and on the 5th postoperative day were $r=-0.270$, $P>0.05$; $r=-0.350$, $P>0.05$; $r=-0.260$, $P>0.05$. There were no significant differences.

DISCUSSION

Monocyte-macrophage lineage cells are important in the development of the immune response. During the course of the immune response, activated monocytes and macrophages release various lymphokines to regulate maturation and differentiation of T and B lymphocytes. Expression on the monocyte membrane of HLA-DR molecules is crucial for antigen presentation to T cells (Haupt *et al*., 1998). Monocytes/macrophages participate in immunomodulation in different aspects, inhibiting interaction of immunologic cells and regulating the intensity of immune response.

Neopterin is synthesized mainly by monocytes/macrophages after induction by gamma-interferon and is considered to be a marker for activation of cellular immune system. Increased concentrations of Neopterin in human serum indicate activation of cell-mediated immune response (Wachter *et al*., 1989; Immanuel *et al*., 1997). It is a simple, reliable and sensitive parameter of cell-mediated immunity. In various groups of patients with malignant diseases, the concentrations of Neopterin correlate to the stage of disease development (Lissoni *et al*., 1997). So it can be used for non-specific laboratory monitoring of cancer progression and dissemination. In this study, Serum levels of Neopterin significantly decreased after surgery (day 0) in the allogeneic group ($P<0.01$). On the 5th postoperative day (day 5), the trend of decreasing concentration was maintained. This indicated allogeneic blood had directly inhibited the function of monocytes/macrophages after surgery and 5 d after surgery. Clinical studies showed that leukocytes and plasma in allogeneic whole blood were main factors in immunosuppression. Weisbach *et al*.(1999) considered that leukocytes in allogeneic blood released various kinds of substances, such as histamine and cytokines, to induce immunosuppression by free radicals, tissue damage, etc. Wangshiduan reported that patients who had accepted allogeneic blood because of radical gastrectomy for cancer had depressed CD_4^+/CD_8^+ T cell ratio on the 1st postoperative day, and that at the same time NK activity and LAK activity also decreased. In summary, allogeneic white cells and plasma would further complicate cellular immune reaction by inhibiting the function of monocytes/macrophages. In this study, although Serum Neopterin decreased in the autologous group after surgery, it was still higher than that in the allogeneic group. This decline is associated with stress reactions such as operative trauma. Differences in Serum Neopterin between the baseline

and the 5th day postoperatively were not obvious. This indicated that on the 5th postoperative day, cell-mediated immune response basically got its breath again and stress reactions weakened. Autologous transfusions probably stimulated a TH1 pattern with decreased IL-10 and increased IL-2 plasma levels to regulate cell-mediated immune function (Heiss *et al*., 1997).

IL-2 is an important cytokine produced by activated CD_4^+ lymphocytes, which plays critical roles in various immunological phenomena. It stimulated proliferation and differentiation of T and B cells, enhancing antibody secretion, increasing natural killer (NK) cells activity and quantity and activating lymphokine activated killer (LAK) cells activity (Rubin, 1995; Liberman *et al*., 2003). NK cells are believed to play an important role in host defence against certain cancers. LAK cells also have broad cytotoxic potential against both autologous and allogeneic tumor cells. The main functional roles of IL-2 are considered to be the development and maintenance of cytotoxic responses including natural killer cells and cytotoxic T lymphocyte activity. It triggers T cells to produce various lymphokines such as gamma-interferon and B cell growth factor. It also increases antibody-dependent cell-mediated cytotoxicity. Hence, a decrease in IL-2 production will result in a decrease in B-cell stimulation, antibody production and impaired NK activity. The lack of IL-2 induced a significant inhibition of immunity and a higher recurrence rate in transfused patients. Our study showed that there was no significant difference in IL-2 between the postoperative day and the preoperative day in the autologous group, whereas in the allogeneic group, IL-2 remained at low level after operation. PGE2 is known to down-regulate macrophage class II antigen expression and inhibit IL-2 production and target cell response to IL-2 (Gurlo *et al*., 1998). Some researchers considered PGE2 production by monocytes was increased after transfusion; others considered the decreasing mononuclear-macrophage activity reduced the quantity of lymphoblasts and inhibited the response to mitotic factor. Decreasing IL-2 adversely affects the prognosis of sick patients.

In this study, there was no correlation between Neopterin levels and IL-2 after blood transfusion. It indicated the regulatory mechanism of immunosuppression induced by allogeneic blood transfusion is complicated. Only one immunocyte or one cytokine cannot be responsible for the intricacies of various immunological phenomena. This study showed Serum Neoperin's potential value as a new marker for activation of cellular immune system and a simple, reliable and sensitive parameter of cell-mediated immunity.

In conclusion, the results of this study suggest that perioperative allogeneic blood transfusion can seriously inhibit cellular immune function, whereas autologous blood transfusion can reduce immune inhibition and other complications. Neopterin was found to be an early marker for monitoring immune function and to have reference value for assessing the prognosis of sick patients.

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