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Impact of intraoperative allogenic and autologous transfusion on immune function and prognosis in patients with hepatocellular carcinoma

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Abstract

The effect of intraoperative blood transfusion on the immune function and prognosis of hepatocellular carcinoma (HCC) has not been fully investigated. The aim of this study was to evaluate the effects of intraoperative autologous blood transfusion and allogeneic blood transfusion on immune function and prognosis in surgically treated HCC patients. One hundred fourteen primary hepatic carcinoma patients who would undergo selective operations were divided into two groups, 35 patients in the experimental group received intraoperative autologous blood transfusion and 79 patients in the control group received allogeneic blood transfusion. The amount of serum T lymphocyte subsets, natural killer (NK) cells and immunoglobulin before and after operation, as well as the recurrence-free survival (RFS) were compared. Results shown that, there was no significant difference in the level of immunocytes and immunoglobulin between the two groups before treatment ($P > .05$). At 1 day after surgery, there were significant differences in T lymphocyte, NK cells and immunoglobulin levels before and after transfusion. CD3+, CD4+, CD4+/CD8+, and NK cells in autologous transfusion group were significantly higher than those in allogeneic transfusion group ($P < .05$); the level of IgG, IgM, and IgA in allogeneic transfusion group were significantly lower than those before operation ($P < .05$), the level of IgG, IgM, and IgA in autologous transfusion group did not significantly fluctuate, and significantly higher than those of allogeneic transfusion group ($P < .05$). At 5 days after surgery, all indexes of autologous transfusion group recovered to the preoperative level, the levels of CD3+, CD4+, CD4+/CD8+, NK cells, IgG, IgM, and IgA were significantly higher than those of allogeneic transfusion group ($P < .05$). The follow-up results showed that the RFS of autologous transfusion group was significantly higher than that of allogeneic transfusion group ($P < .05$). In conclusion, compared with allogeneic blood transfusion, intraoperative autologous blood transfusion possessed less impact on immune function, it may even improve immune function and RFS in HCC patients after surgery. Therefore, HCC patients should be recommended to receive autologous blood transfusion instead of allogeneic blood transfusion when they need blood transfusion during the perioperative period.

Abbreviations: AFP = α-fetoprotein, BMI = body mass index, HCC = hepatocellular carcinoma, HGB = hemoglobin, NK cell = natural killer cell, RFS = recurrence-free survival, SD = standard deviation.

Keywords: allogeneic transfusion, autologous transfusion, hepatocellular carcinoma, immune function, prognosis

1. Introduction

Hepatocellular carcinoma (HCC) is the world's third leading cause of cancer deaths, and new cases of HCC are on the rise.[1] There are many therapies for liver cancer, and surgical resection is thus far the most efficient treatment for HCC. However, recurrence and metastasis of cancer cells after surgical resection may result in bad prognosis.[2] During surgical operation for HCC, many patients require blood transfusions to ensure safe surgery. In traditional application, the main method used is emergency allogeneic blood transfusion, but allogeneic blood transfusion can inhibit the immune function of patients with tumors, resulting in a higher postoperative infection rate, but also extend the hospital stay of patients, in serious cases can lead to death.[3] Meanwhile, allogeneic transfusion may transmit hepatitis virus and human immunodeficiency virus, and may also cause immune transfusion reaction.[4] In particular, perioperative allogeneic transfusion may be associated with an increased recurrence rate after tumor resection.[5] Therefore, the question of how to carry out blood transfusion has attracted wide attention.

Autologous transfusion refers to collection or recycling of the patient's own blood for re-transfusion during operation or after

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major blood loss. Currently, autologous transfusion can be divided into pre-stored autotransfusion, dilution autotransfusion and intraoperative recovery autotransfusion. With the increase of clinical blood and the shortage of blood supply, autologous blood transfusion is more and more widely used in clinic. It can save 40% to 50% of the blood resource and is clinically safe and reliable.[6] Autologous blood transfusion can effectively reduce the amount of blood used in perioperative allogeneic blood transfusion, and it has been reported that it can prevent blood-borne diseases, avoid fever, hemolysis and transfusion-related acute lung injury and other adverse reactions.[7,8] In particular, studies have shown that autologous transfusion did not significantly inhibit the immune function after operation, and even enhanced the immune function.[9,10]

The effect of perioperative autotransfusion on immune function and prognosis in patients with HCC is unclear. In this study, we compared the effects of autologous versus allogeneic blood transfusions on immune function in patients undergoing surgery for HCC. We further investigated the effects of these two different blood transfusion methods on the prognosis of HCC patients. Our work suggests that intraoperative autologous transfusion possessed less impact on immune function, may even improve immune function and relapse free survival in HCC patients.

2. Methods

2.1. Patient selection

Between January 2016 and September 2019, 152 HCC patients underwent curative hepatic resection and received intraoperative blood transfusion in the Guangxi Medical University Cancer Hospital were selected according to the following selection criteria:

1. the patients who were diagnosed with stage I to III HCC and underwent open radical surgery;
2. during operation, patients excessive bleeding needed transfusion and received transfusion;
3. patients who had no infection history in 3 months before operation;
4. patients who had no other tumor history;
5. the clinical and pathological data of the patients were complete and accurate;
6. the starting date, ending date and ending state of the follow-up were clear.

The exclusion criteria were:

1. patients with a history of previous blood transfusion;
2. patients with other malignant diseases;
3. patients with distant metastasis before surgery.

According to the method of transfusion, patients were divided into autologous transfusion group and allogeneic transfusion group.

This study was approved by the Ethics Committee of the Guangxi Medical University Cancer Hospital. All study participants provided written informed consent.

2.2. Intraoperative blood transfusion

During operation, all patients received combined anesthesia, the same induction methods and drugs, and the same type and amount of drugs and liquids. Patients with acute hemorrhage and hemoglobin (HGB) < 7 g/dL received red blood cell transfusion. In autologous transfusion group, blood was collected 2 to 3 days before the operation, the volume of blood collection was 200 to 400 mL. After collection, the blood was kept at 4°C and transfused intraoperatively. Patients in allogeneic transfusion group were given red blood cell suspension appropriately according to the amount of blood loss during operation and the monitoring of HB/HCT.

2.3. Measurements of T lymphocyte subsets, NK cells and immunoglobulin

Peripheral venous blood was collected preoperatively, 1 day postoperatively and 5 days postoperatively. The blood samples were subjected to EDTA anticoagulation and the T lymphocyte CD3+, CD4+, CD8+, and NK cell counts were measured by flow cytometry and the CD4+/CD8+ ratio was calculated. The levels of IgG, IgA, and IgM in serum were detected by immunoturbidimetry.

2.4. Following-up

All patients were followed up for 3 years to observe the long-term effect of blood transfusion. During the follow-up period, physical examination and blood AFP were performed every 3 months, and abdominal ultrasound and computed tomography were performed every 3 months. Recurrence, metastasis, and treatment were recorded for all patients. The end date of the follow-up was January 31, 2020. For recurrent patients, RFS is calculated as the time from the start date of the follow-up to the time when the recurrence is detected. For patients without recurrence, RFS is calculated as the time between the start date of the follow-up and the last clinical visit.

2.5. Statistical analysis

The quantitative data are expressed by the mean ± standard deviation (SD). Variance analysis was used for normal distribution data and rank sum test was used for non-normal distribution data. The counting data were analyzed by chi-square test or Fisher precision test. The recurrence rate was calculated by Kaplan–Meier method. Data processing using SPSS22.0 statistical analysis software, and $P < .05$ was considered statistical significance.

3. Results

3.1. Baseline characteristics

Of the 152 patients who underwent curative hepatic resection and received intraoperative blood transfusion between January 2016 and September 2019, 38 patients were excluded: 5 patients had a history of blood transfusions, 19 patients' postoperative outcome was uncertain and 14 patients' clinical data were incomplete. Finally, 114 patients were included in the present study (Fig. 1). Of the 114 patients included, 35 underwent autologous transfusion, 79 patients underwent allogeneic transfusion. The baseline characteristics of the 114 patients are shown in Table 1. There were no significant differences in sex, age, body mass index (BMI), stage of HCC, pre-operative blood tests and cancer characteristics between autologous transfusion patients and allogeneic transfusion patients (all $P > .05$).

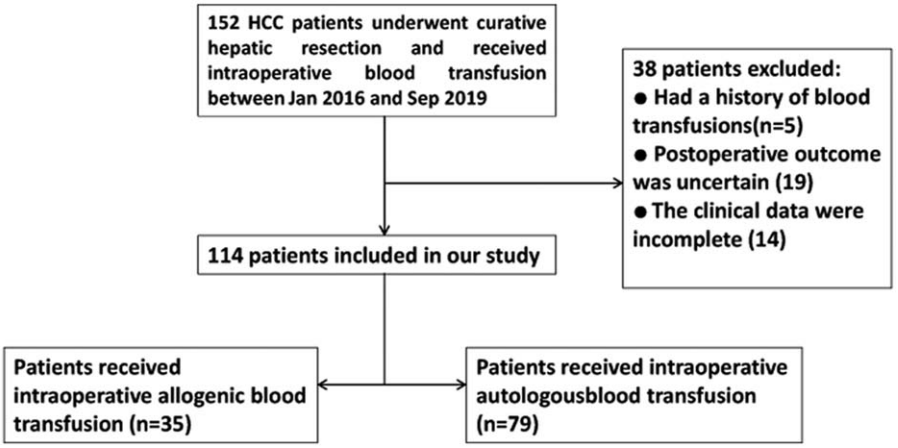

Figure 1. Flow diagram of the patients identified, included, and excluded.

3.2. Comparison of change of T cell subsets and NK cells

The CD3+, CD4+, CD4+/CD8+, and NK cells count decreased on the first day after operation in each group of patients. Meanwhile, after operation, the levels of CD3+, CD4+, CD4+/CD8+, and NK cells in autotransfusion group were significantly higher than those in allogeneic transfusion group ($P < .05$). On day 5 after operations, the levels of CD3+, CD4+, CD4+/CD8+, and NK cells in autologous transfusion group were recovered, and there was no significant difference compared with pre-operation ($P > .05$), while significantly higher than those in the allogeneic transfusion group at the same time point ($P < .05$) (Table 2).

3.3. Comparison in the change of Immunoglobulin level

The levels of IgG, IgM, and IgA in autologous transfusion group had no obvious fluctuation in perioperative period ($P > .05$), and

Table 1

Clinicopathologic factors of hepatocellular carcinoma patients with autologous and allogeneic transfusion.

		Intraoperative blood transfusion		
Characteristics	**No. of patients**	**Autologous transfusion (n = 35)**	**Allogeneic transfusion (n = 79)**	***P***
Mean age (years)	114	48.9	51.6	.742
BMI (kg/m^2)	114	23.8	23.6	.816
Gender				1.000
Male	101	31	70	
Female	13	4	9	
Aetiology of cancer				1.000
HBsAg positive		30	68	
Anti-HCV positive		0	1	
Alcoholism		2	5	
Background liver				.818
Cirrhosis		25	59	
Non-cirrhosis		10	20	
Pre-operative blood tests				
AST (IU/L)		72.1	73.6	.813
Haemoglobin; g/dL		12.8	13.2	.402
platelet count ($\times 10^4$/mm^3)		168	173	.542
Lymphocyte count (mm^3)		1625	1646	.458
AFP (ng/mL)		2093	2146	.371
Albumin (g/dL)		32	34	.703
Total bilirubin (mg/dL)		32	30	0.853
Cancer characteristics				
Tumour diameter >5 cm		15	32	.694
Portal invasion				
Microvascular invasion		19	43	.483
Intraoperative blood loss (g)		600	630	.827
Child-Pugh class				.545
A		3	11	
B or C		32	68	
Anaesthesia time (min)		138	146	.311

AFP = alpha-fetoprotein, anti-HCV Ab = hepatitis C antibody, AST = aspartate aminotransferase, HBsAg = hepatitis B surface antigen.

Table 2

Changes of T cells subsets and NK cells of patients in each group before and after operation.

Index	Groups	n	Before operation	1 d after operation	5 d after operation
CD3+(%)	Autologous transfusion	35	65.2±6.24	60.3±4.68*,a	64.6±5.81*,b
	Allogeneic transfusion	79	67.5±6.83	53.2±4.65	55.6±5.11
CD4+ (%)	Autologous transfusion	35	40.6±5.71	35.6±3.56*,a	40.2±5.87*,b
	Allogeneic transfusion	79	41.18±5.96	20.6±2.67	21.18±5.12
CD8+ (%)	Autologous transfusion	35	20.75±3. 15	22.75±5. 12*,a	20.15±2. 87*,b
	Allogeneic transfusion	79	20.98±3. 02	26.75±4. 65	25.95±2. 57
CD4+/CD8+	Autologous transfusion	35	2. 18±0. 15	2. 02±0. 76*,a	2. 15±0. 21*,b
	Allogeneic transfusion	79	2. 08±0. 22	1. 58±0. 28	1. 60±0. 56
NK cells (%)	Autologous transfusion	35	14.58±1. 43	6.78±1. 21*,a	12.89±1. 05*,b
	Allogeneic transfusion	79	15.08±1. 48	2.78±0. 18	5.14±0. 42

[a] Compared with allogeneic transfusion group, $P<.05$.
[b] Comparison with 1 day after operation, $P<.05$.
* Comparison with before operation, $P<.05$.

the level of IgG, IgM, and IgA in allogeneic transfusion group were significantly lower than those before operation on the first and fifth days after operation ($P<.05$). At the same time, the levels of IgG, IgM, and IgA in autologous transfusion were significantly higher than those in allogeneic transfusion group ($P<.05$) (Table 3).

3.4. Recurrence-free survival

Mean follow-up for survival was 38 months. The median RFS was 30.1 months in autologous transfusion group and 25.2 months in allogeneic transfusion group. The RFS of the autologous transfusion group was significantly higher than that of the allogeneic transfusion group, and the difference between the two groups was statistically significant ($P=.039$) (Fig. 2).

4. Discussion

Surgical resection remains the most important treatment option for most patients with HCC. However, patients with HCC often experience anemia and intraoperative blood loss and may require blood transfusions.[11] While blood transfusion can save lives in many cases, it can also carry significant medical risks, such as incompatibility, transmission of infectious pathogens and allergic reactions.[12] In addition, blood products may alter immune function, including inhibiting the activity of cytotoxic cells and monocytes, releasing immunosuppressive prostaglandins, inhibiting the production of Interleukin-2, and increasing the activity of inhibitory T cells.[3] Perioperative blood transfusion is also associated with cancer recurrence and metastasis.[13] Perioperative blood transfusion has been reported to increase postoperative mortality, local recurrence, and distant metastasis in patients with colorectal cancer.[14] It was also reported that blood transfusion was associated with poor prognosis in patients with esophageal cancer.[13] One study also reported that the immunosuppressive effects of blood transfusions increased the recurrence of head and neck tumors, resulting in a poor prognosis.[15] Another study showed an association between recurrence of advanced ovarian cancer after cytoreductive surgery and perioperative allogeneic blood transfusion.[16] A previous study showed that perioperative blood transfusions did not affect overall and disease free survival in patients with HCC,[17] however, a recent study has shown that allogeneic blood transfusions are an important cause and independent variable of cancer recurrence and death in patients undergoing hepatic resection.[18]

Autologous blood transfusion has been used for more than a century. It was originally used to save blood resources, more importantly, it can reduce unnecessary blood transfusion reaction, reduce the spread of diseases caused by the banked blood. In addition, it can effectively avoid mistakes and accidents of allogeneic blood transfusion, and can avoid other adverse reactions of blood transfusion, such as hemolytic reaction, allergic reaction, microthrombosis, and toxic reaction.[19,20] Moreover, autotransfusion has little effect on the immune function of tumor patients after operation, and may even improve the immune function to some extent,[9] and it may decrease the risk of tumor recurrence in HCC patients.[21] Therefore, it is worth discussing whether autologous blood transfusion should be used instead of allogeneic blood transfusion in perioperative period of cancer patients. In our present study, we compared the effects of autologous versus allogeneic blood transfusions on immune function and clinical outcome in patients with HCC.

Table 3

Changes in plasma of patients in each group of immunoglobulin before and after operation.

Index	Groups	n	Before operation	1 d after operation	5 d after operation
IgG	Autologous transfusion	35	13.82±1.61	12.54±1.21*,a	13.16±1.34*,b
	Allogeneic transfusion	79	13.62±2.21	8.67±1.23*	10.68±1.31
IgA	Autologous transfusion	35	3.18±0.54	2.91±0.38*,a	3.02±0.43*,b
	Allogeneic transfusion	79	3.16±0.41	1.16±0.29*	1.32±0.22
IgM	Autologous transfusion	35	1.36±0.22	0.96±0.21*,a	1.16±0.17*,b
	Allogeneic transfusion	79	1.35±0.18	0.65±0.19*	0.73±0.15

* Comparison with before operation, $P<.05$.
[a] Compared with allogeneic transfusion group, $P<.05$.
[b] Comparison with 1 d after operation, $P<.05$.

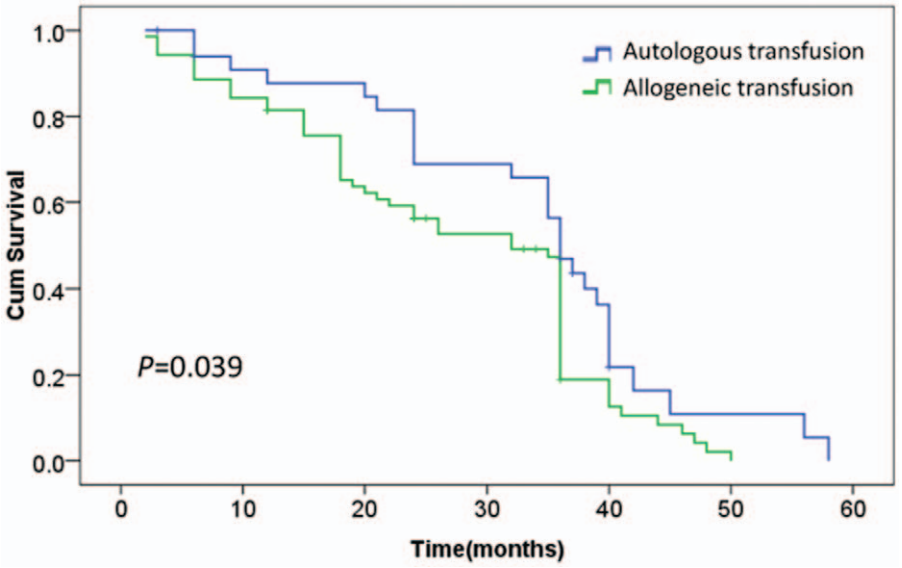

Figure 2. Kaplan–Meier curves comparing recurrence-free survival in the autologous transfusion group and allogenic transfusion group.

The results showed that the amount of CD3+, CD4+ cells, and NK cells decreased significantly on the first day after operation in all patients, suggesting that some certain common factors lead to the changes of cellular immune function in patients after operation. The level of CD3+, CD4+, CD4+/CD8+, and NK cells in autologous transfusion group returned to the baseline on the 5th day after surgery, but not in the allogeneic transfusion group, this suggests that allogeneic transfusion may reduce T lymphocyte and NK cells in cancer patients, which reduces cellular immunity. By observing the effect of allogeneic blood transfusion on immune function of HCC patients, Sugita et al found that allogeneic blood transfusions significantly reduced the absolute number of lymphocyte in peripheral blood. Our results are largely consistent with those of Sugita.[22] The results also showed that the levels of CD3+, CD4+, NK cells, and CD4+/CD8 + ratio in autologous transfusion group were significantly higher than those in allogeneic transfusion group on the 1st and 5th day postoperatively, suggesting that autotransfusion had little effect on immune function in HCC patients, it may even improve immunosuppression.

Human immunoglobulin is a glycoprotein produced by the proliferation and differentiation of B cells after antigen stimulation, and in tumor patients, IgG, IgA, and IgM are the main immunoglobulins involved in the immune process. Antibody is an important effective molecule of humoral immunity, which plays an important role in humoral immunity by binding specific antigen. Therefore, measuring the level of Immunoglobulin in blood can be used to evaluate the humoral immune function of tumor patients, and can also be used as an effective index of anti-infection immunity. The results of this study showed that the levels of IgG, IgM, and IgA in autologous transfusion group did not significantly fluctuate during perioperative period, but the levels of IgG, IgM, and IgA in allogeneic transfusion group were significantly lower than those before operation on the 1st and 5th days after operation. At the same time, the levels of IgG, IgM, and IgA level in autologous transfusion group were significantly higher than those in allogeneic transfusion group. These results suggest that perioperative autotransfusion has little effect on humoral immune function in HCC patients, and allotransfusion can significantly inhibit the immunoglobulin level in HCC patients. Although this is a temporary inhibition, it is very detrimental to the treatment of postoperative anti-infection and complications in cancer patients. Several studies have observed the effect of autotransfusion and allotransfusion on the immune function of cancer patients. it was found that allotransfusion can inhibit the immunoglobulin level significantly, but autotransfusion did not.[9,10,23] The possible reason of this result is, after allogeneic blood transfusion, allogeneic red blood cells and their degradation products can be regarded as antigens by the body,[24] thus producing antigen-antibody reaction and consuming a lot of immunoglobulins, while autotransfusion blood usually has a shorter storage time and produces fewer metabolites, therefore, the effect on Immunoglobulin secretion is slight.

In the outcome of the prognostic analysis, we found that the RFS of autotransfusion group was longer than that of allogeneic transfusion group, and the difference was statistically significant. The results revealed that the preoperative autologous blood transfusion possessed less impact on survival in patients with HCC. By observing the effect of blood transfusion on the prognosis of patients with liver cancer, Tai et al found that perioperative allogeneic blood transfusion had a significant negative effect on the recurrence of liver cancer.[18] Sugita et al[22] have also demonstrated that intraoperative allogenic blood transfusion was associated with decreased survival after surgery for HCC patients, and suggested that autologous blood transfusions should be actively recommended for HCC patients who require blood transfusions, and intraoperative allogeneic blood transfusions should be avoided.

The study has some limitations. First, although we controlled for clinical and other therapeutic factors, there may be other unmeasured or unmeasurable factors that influence the patient's clinical outcome. Second, in addition to red blood cells, some patients received transfusions of other blood products, such as frozen plasma and platelet concentrates, whose effects on immune function and prognosis were not evaluated.

In conclusion, our study revealed that, compared with allogeneic blood transfusion, intraoperative autologous blood transfusion possessed less impact on immune function, it may even improve immune function and survival in HCC patients. Therefore, HCC patients should be recommended to receive autologous blood transfusion instead of allogeneic blood transfusion when they need blood transfusion during the perioperative period.

Author contributions

Youwei Gong and Ling Chen designed the overall project. Youwei Gong and Yonglian Tang collected and analyzed the data and wrote the manuscript. Yinghong Xue did the statistical analysis. All the authors have read and approved the final manuscript. All authors contributed toward data analysis, drafting and revising the paper and agree to be accountable for all aspects of the work.

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