Review Article
The long-term outcomes of patients with hepatocellular carcinoma after intraoperative autotransfusion: a systematic review and meta-analysis of cohort studies

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Abstract: There still exits the controversy whether autotransfusion could be used during the cancer surgery. Our aim is to evaluate the long-term effects of intraoperative autotransfusion on patients with hepatocellular carcinoma (HCC). Pubmed, Embase and the Cochrane Library databases were searched for cohorts comparing intraoperative autotransfusion with allogeneic transfusion in patients with HCC. Three outcome indicators including cancer recurrence, the three-year recurrence free survival and three-year mortality were compared. A total of 1175 patients from eight cohort studies were included and separated into autotransfusion group (AT group) and non-autotransfusion group (non-AT group). There wasn't a significant difference in recurrence free survival (RR=1.18, 95% CI 1.03-1.36, I^2=43.8%) between two groups. But a slight preponderance were observed in AT group when evaluating the cancer recurrence and the death (RR of the cancer recurrence=0.85, 95% CI 0.71-1.02, I^2=0.0%; HR of mortality =0.80, 95% CI 0.58-1.11, I^2=0.0%). So, our analysis showed that intraoperative autotransfusion will not increase the risk of cancer recurrence and mortality on patients with HCC, and proved the safety of autotransfusion in liver cancer surgery.

Keywords: Meta-analysis, liver neoplasms, autotransfusion, operative blood salvage

Introduction

As we all know, liver cancer is a big threaten to human health. As the most common histological type of primary carcinoma of the liver, hepatocellular carcinoma (HCC) has always been the focus issue of doctors and researchers. Although modern medicine is fully developed, we can not cure it. Patients with HCC still need surgery such as liver transplant (LT) or hepatectomy to prolong their life and relieve their pain. Before the surgery, Hepatic anemia has usually being existed. And during the surgery, serious hemorrhages usually happen. So transfusions are always needed. However, just like cell-transplant, allogeneic blood transfusion in operation has many disadvantages, such as causing adverse effects of blood transfusion and transmitting disease. Otherwise it may lead to immunosuppression on the cancer patients who already have problems with im-mune system which in turn affect the cancer recurrence after treatment [1]. Autotransfusion has existed for a long time to reduce or even substitute the use of allogeneic blood transfusion, while the safety of autotransfusion or intraoperative blood salvage in cancer surgery has still been a controversial issue because it encompasses theoretical risk of cancer recurrence by reintroducing the viable cancer cells into treated patients. After all, more and more researches [2-4] and clinical evidence proved that the intraoperative autotransfusion will not increase the risk of cancer recurrence.

As there is little possibility to conduct the randomized controlled trials (RCTs) in this field, we collected cohort studies published in the recent 30 years to study the association between autotransfusion of salvaged RBCs/ predeposited RBCs and long-term outcomes post-operation.

Materials and methods

Our systematic review and meta-analysis followed the Cochrane Handbook [5] for Syste-

matic Reviews and Interventions, and reported results according to Meta-analysis of Observational Studies in Epidemiology (MOOSE) guideline [6].

Date source and search

Two authors (Tianhong Guo and Binrui Luo) searched Pubmed, Embase and the Cochrane Library databases for relative articles published up to July 1, 2017 comparatively independent. The MeSH terms include "Liver Neoplasms", "Blood Transfusion, Autologous", "Operative Blood Salvage", and the search strategy is presented in **Appendix 1**. An English language restriction was imposed, so did the researches were limited to human subjects and controlled studies. In order to include other potentially eligible trials, we checked the reference lists of indentified studies searched electronic.

Study selection

The following inclusive selection criteria were applied: Firstly, the population should only be the adults with HCC and had operation during the hospitalization, patients diagnosed with secondary liver cancer are not included. The age and gender were not limited. Secondly, the intervention was autotransfusion (including getting predeposited autotransfusion, Hemodilution or operative Blood Salvage alone/ combine with allogeneic blood transfusion) during the operation. Thirdly, the comparison was non-autotransfusion during the operation. Fourthly, the outcomes were cancer recurrence, mortality and recurrence free survival after the surgery.

Date extraction

Two authors (Ling Jiang and Binrui Luo) independently extracted the relevant date according to PICOS (presented in **Appendix 1**): title name, authors, journal, published time, the total number of the subjects, disease and operation, sexual, average age, the method, dose and machines for autotransfusion in test group, transfusion carried in control group, the outcome indicators, follow-up time and so on. Actually, we also use the software (Engauge Digitizer) to extract the curve of Kaplan-Meier analysis as well, the HR and confidence interval (CI) was calculated later by the date process-

ing form (cal-methods-for-incorporating 1745-6215-8-16, presented in **Appendix 2**). What's more, Authors were also contacted for missing data.

Assessment of evidence

Two authors (Tianhong Guo and Binrui Luo) assessed the included articles using the Newcastle-Ottawa Scale (NOS) independently, where up to 9 points were awarded based on cohort selection. Studies with scores <6 were considered to have low quality and would be excluded from our analysis; on the contrary, the studies with scores ≥6 would be thought to have a good quality and would be included.

Statistical analysis

The cumulate cancer recurrence and recurrence free survival over 36 months after surgery can be extracted from the studies directly, so we use this information as two-category data, and choose the risk ratio (RR) with 95% CIs as the effect size (ES). Using command "metan" to conduct a fixed-effect model (Mantel-Haenszel method) to get pooled ES. Heterogeneity across studies was tested by using the I^2 statistic (use the fixed-effect model to get), which is a quantitative measure of inconsistency across studies. Those with $I^2 < 50\%$ were considered to have low heterogeneity, and a fixed-effect model would be proper. When the heterogeneity is high (those with $I^2 \geq 50\%$), two models (fixed-effect model and random-effect model) would be conducted meanwhile to examine the small sample effect. What's more, the overall pooled estimate by omitting one study in each turn would be investigate as well to find out the influence of every single study. As for the survival data extracted from the curve of Kaplan-Meier analysis, we choose the hazard ratio (HR) with 95% CIs as the effect size for mortality. Using the random-effect model (DerSimonian-Laird method) to calculate pooled ES.

Because there were only 8 articles included in our analysis, tests for funnel plot asymmetry is unreliable (stated in cochrane handbook). So we only use a result of visual funnel plot to show the potential publication bias in our study. All statistical analysis was performed on STATA, version 12.0.
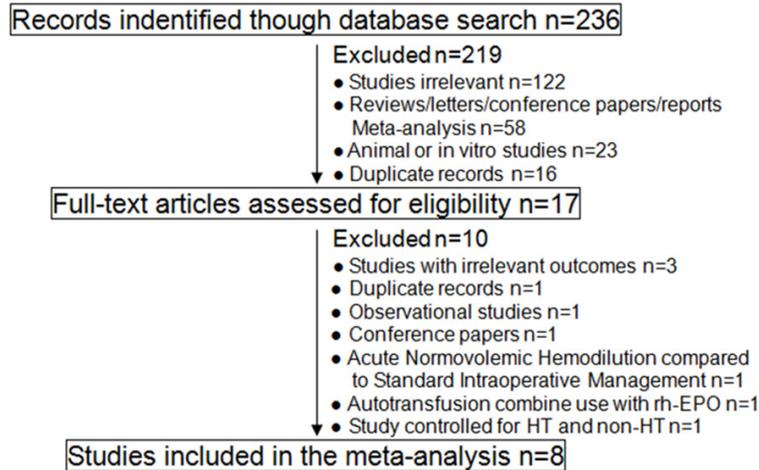
Figure 1. Summary of evidence search and selection.

Results

Figure 1 shows the flow chart of our literature search and selection. A total of 236 online articles were searched out. 122 of them were topic irrelevant (based on the title and abstract); 58 articles were not full-text; 23 articles were studied on animals or *in vitro*; and 16 of them were duplicate records. During the time of scanning the whole articles, we find there were three articles using the irrelevant compares; one compared Acute Normovolemic Hemodilution to Standard Intraoperative Management; one used Autotransfusion in combination with rh-EPO when compared with allogeneic blood transfusion; and one controlled for homogenic transfusion (HT) and non-HT. So, a total of 229 records were excluded, and only 8 studies met the inclusive selection criteria. Manual searching was further conducted, but there was no other studies met the standard.

Table 1 is a brief summary of the characteristics of included studies. As we can see, there were totally 8 pairs of cohorts from 5 countries. The subjects all were patients who planned to have operation and were diagnosed HCC beforehand or during the surgery. The average age of the patients in test group was 55.2 (which were similar in control group with 55.8). And there were more males than females in both groups. Intraoperative autotransfusion (alone or combined with allogeneic transfusion) was carried out in test group, while non-autotransfusion (only allogeneic transfusion) was used in control group. The outcome indica-

tors included events of recurrence, the overall survival and recurrencefreesurvival.Although the follow-up time varied from studies, it was enough for us to evaluate the outcomes. (More details can be seen in **Appendix 1**).

When it comes to the study quality assessment, most articles (7/8) lost point in "assessment of outcome", and some lacked the "study controls for any additional factor". Somehow, all studies reached the 6 points. (Applied in **Appendix 1**).

The cancer recurrence and intraoperative autotransfusion

Figure 2 shows the connection between the recurrence or metastasis of HCC and the intraoperative autotransfusion. We find that when compared to intraoperative allogeneic transfusion, the intraoperative autotransfusion reduced the risk of 15% of HCC recurrence or metastasis post-operation (RR=0.85, 95% CI 0.71-1.02) with no evidence of heterogeneity (I^2=0.0%). **Figure 3** shows the funnel plot of these seven included articles, through visual inspection we find little evidence of publication bias.

Recurrence free survival and intraoperative autotransfusion

Figure 4 shows the relation between recurrence free survival and intraoperative autotransfusion. As we can see, there is not much difference between the two groups (autotransfusion vs. allogeneic transfusion). Autotransfusion might have a slight advantage over the allogeneic transfusion in increasing the Recurrence free survival (RR=1.18, 95% CI 1.03-1.36) with a low heterogeneity (I^2=43.8%).

Mortality and intraoperative autotransfusion

Figure 5 shows the relation between mortality and intraoperative autotransfusion. As we can see, when compared with intraoperative allogeneic transfusion, autotransfusion could reduce the risk of death by about 20% within three years after surgery (HR=0.80, 95% CI 0.58-

Table 1. The brief summary of data extracted form included trials

Study, year	Country	Total Number of subjects	Average age (T vs. C) yr	Gender (the percentage of male, T vs. C)	Transfusion method in T vs. C	Number (lost number) in T vs. C	The outcome indicators	Follow-up time
Hirano [14], 2005	Japan	104	58.4 vs. 57.2	89.1% vs. 84.3%	AT+OBC vs. non-AT (HT)	46 (8) vs. 50 (0)	a[*]. Events of recurrence b[†]. OS and RFS	10 yr
Foltys [15], 2011	Germany	136	55.9 vs. 60.7	70% vs. 77%	IBSA+OBC vs. non-IBSA (HT)	40 (0) vs. 96 (0)	a. Events of recurrence b. RFS	14-4006 days
Fujimoto [16], 1993	Japan	104	59.3 vs. 57.2	87.7% vs. 84.3%	AT+OBC vs. non-AT (HT)	54 (0) vs. 50 (0)	a. Events of recurrence b. OS and RFS	Till death or 1991-12
Han [17], 2015	Korea	397	52.6 vs. 51.9	87.4% vs. 89.7%	AT+OBC vs. non-AT (HT)	222 (61) vs. 97 (17)	Events of recurrence	5 yr
Muscari [18], 2005	France	47	53 vs. 58	84% vs. 87.5%	IBSA+OBC vs. non-IBSA (HT)	33 (0) vs. 16 (0)	Events of recurrence	1 yr
WJG-19-1625 [19], 2013	Japan	83	52 vs. 51	91.7% vs. 88.1%	CATS vs. non-CATS (HT)	24 (0) vs. 59 (0)	a. Events of recurrence b. OS and RFS	25.8 ± 15.1 M
WJG-8-161 [20], 2016	Brazil	158	58 vs. 58	77.9% vs. 77.5%	IBS+OBC vs. non-IBS	122 (0) vs. 36 (0)	a. Events of recurrence b. OS and RFS	-
Kim 2012 [21], 2012	Korea	230	52.3 vs. 52.6	80.2% vs. 78.9%	IBS+LDF vs. non-IBS+LDF	121 vs. 109	a. Events of recurrence b. OS and RFS	60 M

[*]The first outcome indicator; [†]The second outcome indicator; T=test group; C=control group; AT=autotransfusion; OBC=other blood complements; HT=homogenic transfusion; IBSA=intraoperative blood salvage autotransfusion; IBS=intraoperative blood salvage; CATS=continuous autotransfusion system; LDF=leukocyte depletion filter; OS=overall survival; RFS=recurrence free survival.

A meta-analysis for autotransfusion in HCC

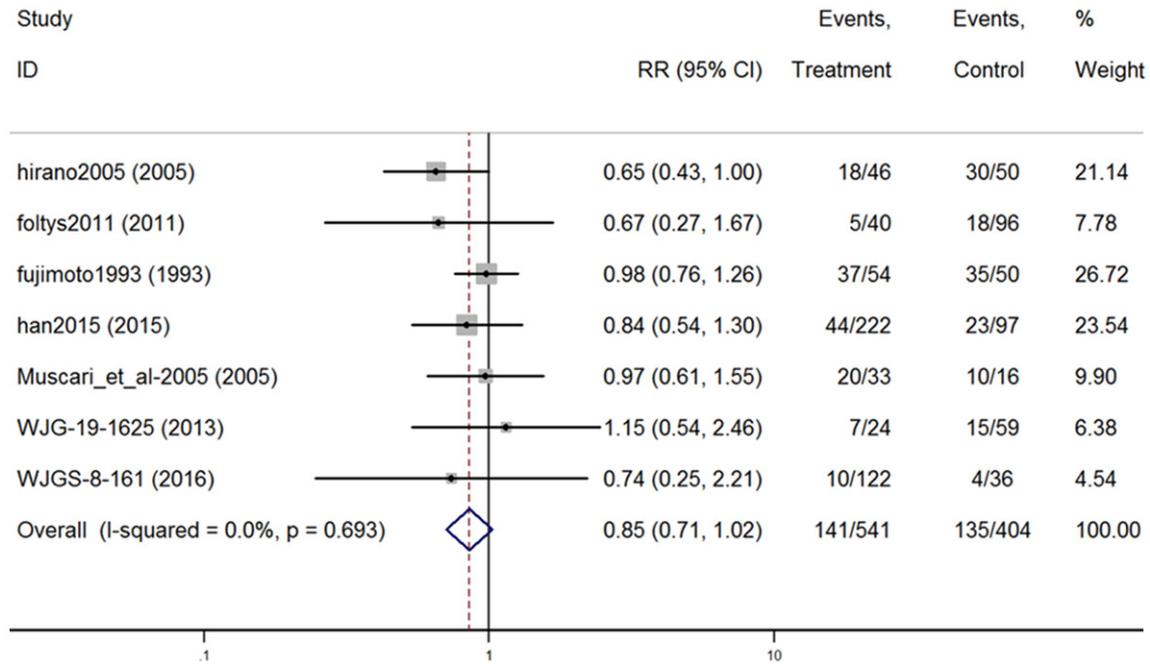| Study ID	RR (95% CI)	Events, Treatment	Events, Control	% Weight
hirano2005 (2005)	0.65 (0.43, 1.00)	18/46	30/50	21.14
foltys2011 (2011)	0.67 (0.27, 1.67)	5/40	18/96	7.78
fujimoto1993 (1993)	0.98 (0.76, 1.26)	37/54	35/50	26.72
han2015 (2015)	0.84 (0.54, 1.30)	44/222	23/97	23.54
Muscari_et_al-2005 (2005)	0.97 (0.61, 1.55)	20/33	10/16	9.90
WJG-19-1625 (2013)	1.15 (0.54, 2.46)	7/24	15/59	6.38
WJGS-8-161 (2016)	0.74 (0.25, 2.21)	10/122	4/36	4.54
Overall (I-squared = 0.0%, p = 0.693)	0.85 (0.71, 1.02)	141/541	135/404	100.00

Figure 2. The relation between the HCC recurrence and intraoperative autotransfusion. The overall RR is represented by the hollow diamond, and the heterogeneity is judged by the I-squared (<50% means low heterogeneity).
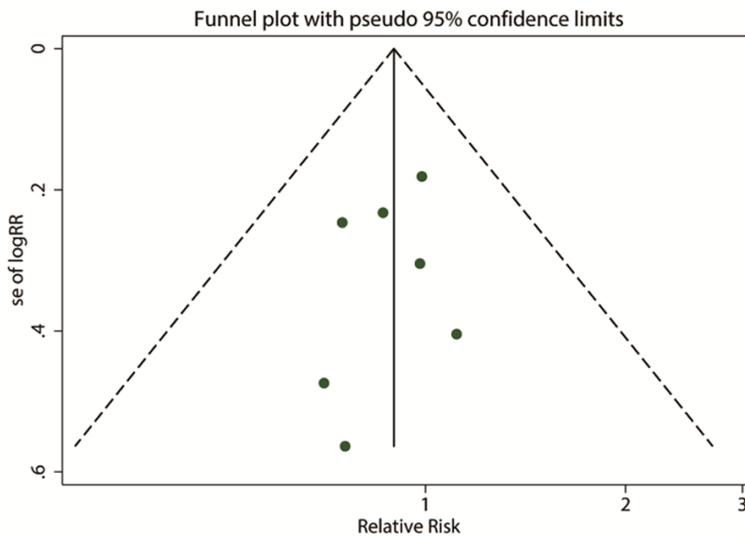

Figure 3. The visual funnel plot of potential publication bias for HCC recurrence and intraoperative autotransfusion. The articles were denoted by the points, and overall logrr was represented by the solid line. The symmetry of points against the line means no published bias.

1.11) with no evidence of heterogeneity (I^2=0.0%).

Discussion

When it comes to malignant tumors, it seems unsafe to transfuse autologous blood to patients themselves during the operation, because there could be potential risks of tumor metastasis. With a better understanding of HCC and the development of cell saver (a machine that can salvage blood or cells lost during operation) and leukocyte depletion filter (LDF), more and more clinical studies try to find out the association between intraoperative autotransfusion and prognosis of HCC patients. Our meta-analysis is aimed to evaluate these studies and demonstrated the overall benefits among them. Finally, we find that intraoperative autotransfusion in deed do much favor in saving resource of blood, at the same time, decrease the risk of cancer recurrence and death rate in HCC patients.

As a matter of fact, there was already some evidence showed that autotransfusion had much advantage over allogeneic transfusion in cancer surgery: immune system does a very im-

Figure 4. The relation between recurrence free survival and intraoperative autotransfusion. The pooled RR is represented by the hollow diamond, and the heterogeneity is judged by the I-squared (<50% means low heterogeneity).

Figure 5. The relation between mortality and intraoperative autotransfusion. The pooled HR is represented by the hollow diamond, and the heterogeneity is judged by the I-squared (<50% means low heterogeneity).

portant job in tumor progression. Jian-Rong Guo [7] came up with that allogeneic transfusion during the perioperative period could obviously decrease the number of T cell subpopulations and the secretion of stimulating cytokines. On the contrary, the secretion of inhibiting cytokines increase in patients with malignant tumors thus causes a Th1/Th2 imbalance. Autologous transfusion has little impact and may even bring about some improvement to

postoperative immune function in patients with tumors. And Shoshana Peller [8] also proved that autotransfusion had an impact on immune parameters that might prove less detrimental to the clinical outcome in oncologic surgery than homologous transfusion. However, there may be other reasonable explanations account for the results of our meta-analysis.

A previous meta-analysis [9] about blood salvage and cancer surgery demonstrated that the use of intraoperative blood salvage (IBS) were not inferior to traditional intraoperative allogeneic transfusion. Several studies revealed a lower cancer recurrence rate among those who received IBS during their surgery (OR=0.65, 95% CI 0.43-0.98), which was consistent with our finding (RR=0.85, 95% CI 0.71-1.02). Both the two meta-analysis did not include the RCTs, which is a limitation. The previous article included the cancer type ranged from low metastatic-potential prostate cancers to much higher risk hepatocellular cancer, on the contrary we only focus on HCC, which may led to a decrease on the protective association but a promotion in homogeneity among studies. Unlike the previous analysis using the odds ratio (OR), we choose RR and HR as the effect size, which can better illustrate the relative risk on survival data between intraoperative autotransfusion and allogenic transfusion. Further more, beyond debating on the cancer recurrence, we also took the three-year mortality and three-year recurrence free survival in.

In this meta-analysis, a total of 945 patients with HCC were observed for the cancer recurrence after operation. Since there was no one using LDF, our finding was in favor of that the salvage RBCs (reinfused by cell saver) did not increase the risk of recurrence, which can be supported by the evidence raised by Donna M. Karczewsk [10]. When it came to the evaluation of death rate and recurrence free survival, 121 HCC patients use intraoperative blood salvage combined with LDF, as the control group use the same transfusion protocol, the allogeneic transfusion, as other studies. This intervention (IBS+LDF) showed a more protective relation to recurrence free survival (RR=1.42, 95% CI 1.12-1.81 vs. pooled RR=1.18, 95% CI 1.03-1.36), but did not make much difference to the association between death rate and intraoperative autotransfusion (HR=0.75 95% CI 0.36-1.54 vs. pooled HR=0.80, 95% CI 0.58-1.11).

The reason may be that LDF can increase the efficiency of stopping the tumor cells from reinfusing to the patients, thus reduce the rate of recurrence after operation, but it can not prevent the patients from death caused by non-tumor factors. As some studies [11-13] have also implied that LDF could remove the tumor cells in the salvage blood effectively, we suggest using IBS combined with LDF during HCC surgeries, which helps to reduce the risk of HCC recurrence to the minimum.

Since the age, disease stage (Child score), and other comorbidities of patients may influence the long-term outcomes, these factor were matched between the test group and control group to the greatest extent in the trials included, which minimize the influence to the least. However, we only included cohort studies, whose level of evidence might be less powerful than RCTs. The follow-up time is different among studies, the reason we choose three-year as the observation period was that all studies surveyed more than three years. As a matter of fact, this time span may be a little shorter for the observation of recurrence free survival and mortality. And we use Kaplan-Meier curves to extract data of recurrence free survival and mortality, which might not as accurate as the original data. But overall, all the results we came out were against the hypothesis theory that intraoperative autotransfusion would raise the risk of cancer recurrence, and would have a bad effect on the outcome of the surgery.

In spite of the limitation, our study confirmed the feasibility of using salvaged RBCs in the operation of HCC. However, we still need more research and RCTs to prove the safety of IBS.

Acknowledgements

Disclosure of conflict of interest

None.

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