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Observational Study

Change of tumor-infiltrating lymphocyte in the perioperative period of associating liver partition and portal vein ligation for staged hepatectomy for massive hepatocellular carcinoma

Wang W *et al*. TIL in ALPPS for massive HCC

Abstract

BACKGROUND

The role of tumor-infiltrating lymphocytes (TIL) in the growth and progression of hepatocellular carcinoma (HCC) has attracted widespread attention.

AIM

To evaluate the feasibility of associating liver partition and portal vein ligation for staged hepatectomy (ALPPS) for massive HCC by exploring the role of TIL in the tumor microenvironment.

METHODS

Fifteen massive-HCC patients who underwent ALPPS treatment and 46 who underwent hemi-hepatectomy were selected for this study. Propensity score matching was utilized to match patients in the ALPPS and hemi-hepatectomy groups (1:1). Quantitative analysis of TIL in tumor and adjacent tissues between the two groups was performed by immunofluorescence staining and further analyses with oncological characteristics. In the meantime, trends of TIL in peripheral blood were compared between two groups during the perioperative period.

RESULTS

Continuous measurement of tumor volume and necrosis volume showed that the proportion of tumor necrosis volume on the seventh day after stage-I ALPPS was significantly higher than pre-operation ($P = 0.024$). In the preoperative period of stage-I ALPPS, the proportion of tumor necrosis volume in the high $CD8^+$ T cell infiltration group was significantly higher than in the low group ($P = 0.048$).

CONCLUSION

TIL infiltration level maintained a dynamic balance during the preoperative period of ALPPS. Compared with right hemi-hepatectomy, the ALPPS procedure did not cause

severe immunosuppression with the decrease in TIL infiltration and pathological changes in immune components of peripheral blood. Our results suggested that ALPPS is safe and feasible for treating massive HCC from the perspective of immunology In addition, high $CD8^+$ T cell infiltration was associated with increasing tumor necrosis in the perioperative period of ALPPS.

Key Words: Associating liver partition and portal vein ligation for staged hepatectomy; Tumor-infiltrating lymphocyte; Multiplexed immunohistochemistry; Tumor necrosis

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Core Tip: This study was conducted to evaluate the feasibility of associating liver partition and portal vein ligation for staged hepatectomy (ALPPS) for massive hepatocellular carcinoma by exploring the role of tumor-infiltrating lymphocyte (TIL) subpopulations in the tumor microenvironment. The ALPPS procedure did not cause severe immunosuppression due to reduced TIL infiltration and pathological alterations in peripheral blood immune components. In addition, high perioperative $CD8^+$ T cell infiltration with ALPPS was associated with increased tumor necrosis.

INTRODUCTION

Primary liver cancer is a common digestive system malignancy, with around 906000 new cases and 830000 deaths occurring globally, with the incidence rate and mortality rate increasing yearly. More than 75% of the pathological types of primary liver cancer are hepatocellular carcinoma (HCC)[1]. According to the newly released diagnosis and treatment specifications, surgery is the primary choice of radical resection of HCC

tumors and the principal treatment strategy for prolonging the survival time of patients with HCC[2,3].

In March 2012, German surgeon Schnitzbauer *et al*[4] was the first to report associating liver partition and portal vein ligation for staged hepatectomy (ALPPS), an innovative hepatectomy, publicly. ALPPS can block part of the blood flow supplying the tumor and completely block the possible collateral circulation between the two hepatic lobes. Thus, ALPPS can effectively stimulate liver hyperplasia and create more favorable conditions for the second-stage surgical resection of the tumor. With the gradual maturity and improvement in ALPPS technology, the clinical application of ALPPS has gone through an early transition, and the incidence of complications and mortality are gradually reducing. HCC patients who have undergone rigorous screening for ALPPS treatment, these risks are comparable to those of traditional hepatectomy and portal vein embolization + hepatectomy, which leads to an increase in the resection rate of massive HCC[5]. As a new method of liver surgery, ALPPS is a promising approach to treating HCC patients.

Tumor-infiltrating lymphocytes (TIL) migrate to the tumor microenvironment (TME) after leaving the peripheral blood circulation system, which involves T and B lymphocytes and natural killer (NK) cells. TIL is an integral part of TME, and its role in HCC tumor growth and progression has attracted widespread attention. Recent studies have focused on the relationship between TIL and the prognosis of liver cancer patients. Anantha *et al*[6] reported for the first time that various immunological components of the future liver remnant (FLR) did not change during the perioperative period of ALPPS. This shows that FLR proliferates rapidly and relatively expands the formation of various immune cells and components to maintain immune functions. However, in the perioperative period of ALPPS, patients need to withstand two surgical insults. The subsequent stress or inflammatory response on the changes and effects of immune cells residing or recruited in TME and their effects are still unclear. More specifically, to understand whether ALPPS could be used as a viable alternative to traditional hepatectomy techniques, it is necessary to study the potential mechanism of ALPPS

complications and the changes and effects of tumor-infiltrating immune cells or components. Here, we investigated the effect of ALPPS surgery on TIL subsets, analyzed the changes in the immune microenvironment of tumor cells during the two-stage ALPPS surgery, and finally evaluated the safety and effectiveness of ALPPS as an alternative to traditional hemi-hepatectomy for the treatment of massive HCC.

MATERIALS AND METHODS

Study design

All subjects were HCC cases from the Department of Hepatobiliary Surgery of the single-center from August 2018 to August 2019. Surgical resection was performed in all cases, with the types of tumors confirmed by postoperative pathological examination. These data have been uploaded to the International ALPPS Registry (www.alpps.net). This study followed the declaration of Helsinki and was approved by the ethics committee of the center. Patients were not required to give informed consent for the study because the clinical data were obtained retrospectively after each patient agreed to treatment by written consent.

Patient criteria

The following inclusion criteria were used for the selection of patients: (1) Patients with FLR/standard liver volume (SLV) < 30%-50% and the patients who have received stage-I ALPPS treatment; (2) Child-Pugh classification A or B; and (3) All subjects were confirmed to be HCC patients by surgery and pathology. The following exclusion criteria were used for rejecting the patients: (1) Incomplete clinical data or histological specimens; (2) Patients without stage-II ALPPS treatment; and (3) Patients undergoing left hemi-hepatectomy.

Multiple immunofluorescence staining

Each specimen was numbered according to the chronological order of the included cases and the site of collection, and hematoxylin-eosin-stained sections of HCC

pathological tissues kept in the case specimen library were retrieved. After the pathologists read the slides, paraffin specimens with typical HCC characteristics of cancerous and paracancerous tissues were selected. The screened tissues were then arranged on the empty white wax blocks in a certain order using a tissue microarray spotter with the assistance of a pathology technician, and the tissue chip was obtained by serially slicing the wax blocks through a slicer, in which each core spot represented a pathological specimen. The prepared tissue chips were placed in slide boxes and refrigerated at 4 °C for storage. Tissue chips are subjected to antigen repair after dewaxing and dehydration. Subsequently, 3% H_2O_2 was added dropwise to block endogenous peroxidase. Primary antibodies (Abcam, United States) were added and kept at 4 °C overnight. Secondary antibodies were added dropwise at room temperature for 50 min, and then horseradish peroxidase reagent was added dropwise. The diluted PANO 7 fluorescent dye (Panovue, Beijing, China) was used to dye CD4, CD56, CD3, CD20, CD8, Foxp3, and DAPI (Sigma-Aldrich, Germany) successively. 1 mL dimethyl sulfoxide was added to the tissue chip at room temperature for 5 min, and the slide was covered. Complete images were acquired with the mantra system (PerkinElmer, Waltham, Massachusetts, United States) to collect multispectral images. The inform image analysis software quantifies the amount of fluorescence excitation for each core site and for each fluorophore. Where the positive expression rate of cells in each sample = number of positive cells/total number of nucleated cells.

Surgical technique

During stage-I ALPPS, the surgeon first opened the abdominal cavity to exclude extrahepatic metastatic tissues. The right portal vein branch would be ligated in the absence of any metastasis. Intra-operative ultrasound-guided anterior hepatic transection was conducted along the middle hepatic vein, and the blood flow of the hepatic artery was preserved. The interval between stage I and stage II of ALPPS depended on the patient's condition and increased FLR. During the stage-II ALPPS, right hepatectomy or enlarged right hepatectomy was performed[7].

Propensity score matching

To add to the control analysis, patients in the ALPPS group were matched 1:1 with those in the right hemicolectomy group using the propensity score matching (PSM) module built into the SPSS 22.0 software. The independent variables of tumor size and number, alpha-fetoprotein (AFP) level, Child-Pugh score, presence of large vessel cancer thrombi, and presence of distant metastases were used as covariate matching items. Age, gender, body mass index, end-stage liver cancer score, and Barcelona clinic liver cancer (BCLC) staging system were used as balanced matches. The caliper value is set to 0.1.

Volume measurement of liver and tumor

The liver volume was analyzed by IQQA-3D liver (EDDA Technology, United States) combined with patient imaging data[8]. SLV was calculated using the Chinese adult standard liver volume estimation formula[9]. FLR/SLV before surgery was used to determine whether FLR was sufficient. The increase in FLR volume confirmed the stage-I ALPPS and stage-II ALPPS. The following conditions were considered acceptable for stage-II ALPPS: (1) FLR/SLV ≥ 50% was accompanied by severe fibrosis or cirrhosis; (2) FLR/SLV ≥ 40% was mild/moderate fibrosis; and (3) FLR/SLV≥ 30% did not contain liver fibrosis or cirrhosis[10]. A complete tumor image was drawn, and the tumor volume was calculated[11]. The tumor necrosis volume was calculated. The percentage of tumor necrosis volume was then calculated = tumor necrosis volume/tumor volume × 100%. Finally, the tumor size and necrotic volume were analyzed before ALPPS, 3 d, and 7 d after stage-I ALPPS.

Follow up

The patients were followed up regularly for 3 mo after discharge and every 3 to 6 mo after that, mainly involving imaging examination (ultrasound, computed tomography and magnetic resonance imaging), liver function inspection, and AFP level test. After

analysis, the overall survival rate of each patient was calculated, with the survival time defined as the time from treatment operation to death. The final events of overall survival included extrahepatic or intrahepatic metastasis, recurrence, and death after primary resection.

Statistical analysis

The data were analyzed and processed with IBM SPSS22.0. The normally distributed measurement data were expressed as mean ± SD, and the count data were defined as quantity (%). The student's *t*-test was conducted to compare the measurement data between two paired groups. Comparison of counting data was made between two groups using the chi-square test or Fisher's exact test, and the R × C chi-square test was used for comparison among groups. Repeated measurement data were compared by repeated measurement analysis of variance. Kaplan-Meier method was used for survival analysis and fitting survival curves. The Log-rank test compared the differences in survival curves among different groups. $P < 0.05$ was considered statistically significant.

RESULTS

Matching results between the ALPPS group and the right hemi-hepatectomy group

The clinical data of 90 patients undergoing hepatectomy in the single-center were collected. 15 HCC patients treated with ALPPS and 46 patients with right hemi-hepatectomy were included for analysis (Figure 1). A 1:1 match was performed between the ALPPS group and the right hemi-hepatectomy group using the PSM module. After matching, the variables such as age, sex, body mass index, the end-stage score of liver cancer, BCLC stage, tumor size and number, AFP level, Child-Pugh score, presence of macrovascular tumor thrombus, and distant metastasis were found to be similar between the two groups, showing no significant difference between them ($P > 0.05$, Table 1). In addition, the ALPPS group's average FLR/SLV measured before the

operation was 36.9% (21.6%-45.4%), and the FLR/SLV value of the right hemi-hepatectomy group was 58.9% (35.3%-77.3%).

Intraoperative and postoperative survey of patients in the ALPPS group and that in the right hemi-hepatectomy group

The average operation time of stage-I ALPPS, that of stage-II ALPPS, and that of right hemi-hepatectomy were 342 min (229-459 min), 293 min (167-400 min), and 338 (140-515) min, respectively, while the mean intraoperative bleeding volumes were 230 (100-500) mL, 619 mL (200-1800 mL), and 344 (190-638) mL, respectively. There was no allogeneic blood transfusion in stage-I ALPPS, with 4 cases in stage-II ALPPS requiring allogeneic blood transfusion and one case receiving De-leukocyte suspension of red blood cells 2 U after right hemi-hepatectomy. All surgical margins were resected with R0. The median interval between the first stage of ALPPS and the second one was 15 d (9-27 d).

No ALPPS group patients experienced postoperative bile leakage, while 2 right hemi-hepatectomy group patients underwent postoperative bile leakage. By the Clavien-Dino criteria[12], for stage-I ALPPS postoperative complications, grade I, grade II, and grade III patients were 13, 1, and 1, respectively. For stage-II ALPPS, grade I, grade II, grade III, and grade IV patients were 8, 4, 2, and 1, respectively. Whereas, for right hemi-hepatectomy postoperative complications, grade I, grade II, grade III, and grade IV patients were 9, 4, 1, and 1, respectively. All other complications were cured, except for a stage-III ALPPS patient rated as grade IV due to postoperative liver failure, and a right hepatectomy patient with respiratory failure rated as grade IV and died during the perioperative period.

The 15 cases of ALPPS patients underwent postoperative liver failure classification by International Study Group of Liver Surgery standards[13]. After stage-I ALPPS, 4 were graded A, 10 for B, and 1 for C and after stage-II ALPPS, 4 were graded A, 9 for B, and 2 for C. For the right hepatectomy group, the number of patients graded A was 6, B was 8, and the one graded C was 1. One patient of the ALPPS group died on the 32nd d after

the second stage, while one of the right hepatectomy group died on the 28th d after the operation (Table 2).

Expression of TIL in HCC microenvironment

TILs are an important component of TME involved in the local immune response, and their degree of infiltration greatly affects tumor growth and progression. In order to determine the infiltration degree and trend change of TIL in the HCC microenvironment, we took tissues from 15 cases of ALPPS and 15 matched patients with right hepatectomy. Cancerous tissues and para-cancerous tissues were used to make tissue microarrays. The specific marker molecules of lymphocyte subsets in TME were stained with polychromatic immunohistochemical staining. The results showed that the infiltration pattern of TIL in cancer tissues was significantly different from that in para-cancerous tissues. The infiltration of TIL in cancer tissues was irregular and diffusely distributed. Whereas, in para-cancerous tissues, TIL was mainly concentrated in the connective tissues of the interlobular portal area, often accompanied by three kinds of ducts: Interlobular artery, interlobular vein, and interlobular bile duct (Figure 2).

The quantitative analysis showed the number of target cells and the total number of all nucleated cells. The positive expression levels of six TIL subsets of T cells, $CD8^+$ T cells, $CD4^+$ T cells, Treg cells, B cells, and NK cells in the same spatial tissues were calculated. Furthermore, the TIL of the right hemi-hepatectomy group, ALPPS group (including stage I and II), and cancer or para-cancerous tissues were compared and analyzed (Figure 3). The results showed that the positive expression level of Treg cells in the cancer tissues was significantly higher than that of the adjacent tissues ($P = 0.043$, Tables 3-6).

Perioperative tumor necrosis in stage-I ALPPS and its relationship with TIL

The proportion of tumor necrosis volume was calculated by analyzing the stage I of ALPPS tumor volume and tumor necrosis volume (Figure 4). The results showed that

the proportion of tumor necrotic volume on the seventh day after stage-I ALPPS was significantly higher than before the operation (P = 0.024, Figure 5). In order to further clarify the relationship between tumor necrosis and TIL in the perioperative period of stage-I ALPPS, the median positive expression level of the 6 TIL subgroups in stage-I ALPPS cancer tissues was used as the cut-off point. The HCC patients receiving ALPPS treatment were divided into a high-infiltration group and a low-infiltration group. We then compared the difference in the proportion of tumor necrosis volume between the two groups. The results showed that the proportion of tumor necrosis volume in the high $CD8^+$ T cell infiltration group was significantly higher than that in the low $CD8^+$ T cell infiltration group (P = 0.048, Figure 6).

Comparison between immune components in peripheral blood of the right hemi-hepatectomy, stage-I ALPPS, and stage-II ALPPS patients

Pairwise comparisons of immune components of peripheral blood were measured between the right hemi-hepatectomy group, stage-I ALPPS group, and stage-II ALPPS group. We found that the components of the complement system, C1q and C3 in peripheral blood in stage-I ALPPS, were significantly higher than those in stage II. (C1q: P = 0.007, C3: P = 0.047, Figure 7). In addition, interleukin (IL)-6 levels in the stage-I ALPPS and stage-II ALPPS increased significantly and reached a peak value on the first day after surgery, and then decreased rapidly but were significantly higher than the preoperative level in stage-I ALPPS and II ($P1$ = 0.000, $P2$ = 0.002). NK cells in stage-I and stage-II ALPPS temporarily increased on the first day after surgery and gradually decreased on the second day after surgery to figures lower than the preoperative level (Figure 8). There was no significant difference in other remaining peripheral blood indicators among the other groups (P > 0.05, Figure 9).

Follow-up results

The ALPPS and the right hemi-hepatectomy group patients were followed up after the surgery. As of May 20, 2020, the median follow-up time of the ALPPS group patients

and that of the right hemi-hepatectomy group patients were 472 d (279-607 d) and 449 d (267-740 d), respectively. There was no significant difference in follow-up time between the two groups (P = 0.528). The survival rate of the ALPPS group and that of the right hemi-hepatectomy group showed no significant difference between the two groups (Figure 10, log-rank test P = 0.733). During the 90-d follow-up, one person died after stage-II ALPPS, and one died after hemi-hepatectomy, respectively, and the mortality rate in each group was 6.67% (1/15).

DISCUSSION

As a planned step-by-step hepatectomy, ALPPS involves strict requirements on liver anatomy, degree of FLR hyperplasia, liver volume evaluation, and patient screening. Stage-I ALPPS separates the left hepatic lobe and the right one and ligates the right hepatic vein, resulting in an inflammatory reaction, hypoxia, tumor necrosis, and other factors, thus leading to a unique and complex immune microenvironment of tumor cells. Therefore, it is necessary to understand such immunological effects of the unique TME formed during HCC treatment with ALPPS from an immunological perspective as anti-tumor effect or tumor-induced immunosuppression. HCC treatment with ALPPS, the subsequent recruitment and change of TIL in the TME, and its effect on the tumor are still not completely understood. To verify the safety of ALPPS in treating massive HCC, more in-depth research on TIL in TME is needed.

In order to determine the perioperative changes of TIL in patients with massive HCC in the right lobe treated with ALPPS and its effect on the tumor, we used PSM analysis on 15 HCC patients treated with ALPPS and 15 HCC patients treated with right hemi-hepatectomy. The results showed that all clinical baseline and tumor nature trends of the two groups were similar. The PSM method was used to reduce the selection deviation and baseline difference to make the sample data of the two groups more comparable[14]. Meanwhile, cancer and para-cancerous histopathological specimens of the right hemi-hepatectomy group and the ALPPS group were collected. The positive expression levels of TIL subsets were detected by polychromatic immunohistochemical

staining. The results showed no significant differences in the 6 main TIL subsets between the ALPPS and right hepatectomy group or between the cancerous and adjacent tissues in the same group. Especially during the "isolated" period of tumor-bearing right hepatic lobe between stage-I ALPPS and stage-II ALPPS, the positive expression level of TIL subsets did not change significantly. It indicated that the degree of TIL infiltration in TME has not changed due to the traumatic stress of ALPPS surgery and the persistence of stage-I ALPPS to II tumors, which provides a basis for the operation of tumor local immune function and the body's resistance to tumor invasion. Previous studies have shown that the decrease in the invasion of TIL could promote tumor immune escape and malignant progression and limit the effect of immunotherapy, leading to a poor prognosis. In contrast, the increase in the infiltration degree of TIL produces the opposite result[15-17].

This study shows that the level of TIL infiltration during the perioperative period of ALPPS maintains a dynamic balance, suggesting that there is no adverse effect on TIL infiltration due to the surgical methods of ALPPS. To further verify the correlation between TIL and HCC, we measured the tumor volume and tumor necrotic volume before stage-I ALPPS operation, 3 d, and 7 d after the stage-I ALPPS operation. We further calculated the ratio of tumor necrotic volume to tumor volume. We found an increase in tumor necrosis volume proportion, gradually from stage I to stage II of ALPPS, which might be caused by ligation of the right hepatic vein during ALPPS operation[18,19].

TIL plays a central role in tumor local immune response, and their infiltration levels largely determine the severity of immune response. This is the main reason for using TIL to evaluate the intensity of immune response induced by ALPPS in this study. T cells not only mediate cellular immune response but also participate in humoral immune response induced by thymus-dependent antigen. $CD8^+$ T cells, also known as cytotoxic T cells, are the primary effector cells of the immune system against tumors. They can kill tumor cells efficiently through the perforin-granzyme pathway, Fas-FasL pathway, and tumor necrosis factor (TNF)-TNF receptor pathway[20,21]. Studies have

shown that the local low level of $CD8^+$ T cell infiltration makes the tumor grow and progress more rapidly. Here, we found a correlation between the infiltration level of $CD8^+$ T cells and the degree of tumor necrosis. The proportion of tumor necrotic volume in the perioperative stage-I ALPPS gradually increased with time. Moreover, the proportion of tumor necrotic volume in the high $CD8^+$ T cell infiltration group was significantly higher than that in the low infiltration group. Based on the fact that there is no difference in the expression levels of $CD8^+$ T cells between the cancer tissues of the ALPPS group and the right hepatectomy group, it can be inferred that after stage-I ALPPS, the right lobe of the tumor-bearing liver is segreagated and the right hepatic vein is ligated, while $CD8^+$ T cells can still effectively infiltrate TME, thus exerting cytotoxicity to kill tumor cells. This result also proves that $CD8^+$ T cells do not reduce their infiltration degree due to the ALPPS operation and maintain the stability of the immune system's killing function.

Components of the peripheral blood circulatory system, including T cells, B cells, Treg cells, NK cells, IL-6, complement components (C1q, C3, C4), and immunoglobulins (IgA, IgG, IgM) can comprehensively reflect the immune function of the body. NK cells are the primary killer cells in innate immunity and can produce cytotoxic effects on tumor cells[20]. Among the peripheral blood immune indicators tested, NK cells temporarily increased on the first day after stage-I and stage-II ALPPS. They then gradually decreased to a lower level than the preoperative one. This trend may be related to the inhibitory effect of Treg cells on NK cells. One study has shown that higher serum IL-6 levels are associated with an increased risk of adverse HCC[22]. In this study, IL-6 in stage-I and II ALPPS increased significantly on the first postoperative day, and reached a peak. However, their levels were consistently higher than the preoperative levels. Stage-I and II ALPPS were significantly higher than those before surgery ($P1 = 0.000$, $P2 = 0.002$) This phenomenon might be related to the "waterfall" inflammation and persistent inflammation stimulus caused by surgical strikes. It is reported that the serum complement C1q increases significantly in the occurrence and development of nonalcoholic fatty liver[23]. In addition, complement C3 is involved in

the occurrence and development of alcoholic hepatitis, thus inducing liver cancer[24]. In our study, the contents of complement C1q and C3 in peripheral blood after tumor removal in stage-II ALPPS were significantly lower than those in stage-I ALPPS. Finally, there was no significant change in serum IgA, IgG, and IgM levels between stage-I and stage-II ALPPS, indicating that the two-stage surgery performed by ALPPS did not cause excessive physiological stress or inflammation. In summary, comparing the changing trend of peripheral blood immune components in different groups showed that the traumatic stress and inflammatory reaction caused by right hepatectomy and ALPPS are similar. The ALPPS procedure did not cause more severe immunosuppression due to the "radical" surgical strategy, consistent with previously reported results[25].

In the past few decades, people have gained a deeper understanding of the importance of TME in the occurrence, development, invasion, and metastasis of HCC[26]. The dynamic changes of TME significantly affect the tumor biological characteristics of HCC. TME is thought to have an active interaction with tumors, not just the passive structural support for tumor growth or survival. Therefore more people are actively studying to understand TME and its interaction with HCC cells. Because each component of TME plays a complex role and influences one another, targeting a specific component of TME is usually of little effect. It can be seen that a better understanding of the biological effects and molecular interactions between each component of TME and tumor cells is crucial for understanding the mechanism and development of tumorigenesis.

In 1988, Rosenberg *et al*[27] invented the TIL therapy. Lymphocytes were isolated and extracted from the patient's body, amplified *in vitro*, and then infused back into the patient's body, opening up a new avenue in the field of tumor treatment. After years of continuous development and improvement, various new therapies based on TIL therapies have come out, such as chimeric antigen receptor T cell immunotherapy (CAR-T) and T cell receptor chimeric T cells immunotherapy (T cell receptor-modified T cell immunotherapy, TCR-T)[28-30]. CAR-T and TCR-T cells are T cells that have been

directionally modified and screened by genetic engineering technology, which strengthens the ability to recognize tumor cells or tumor-associated antigens. They can change the local immune suppression microenvironment induced by tumors and reverse tumor immunity tolerance status, showing good safety and effectiveness in treating various cancers. In the current research, CAR-T therapy has a significant effect on hematological tumors[31,32], and TCR-T therapy has achieved good results in melanoma[33], multiple myeloma[34], lung cancer[35] , and ovarian cancer[36]. The two therapies still face many challenges in treating solid tumors, such as low and uneven treatment response rates, local immunosuppressive effects of the TME, and lack of high-efficiency molecular targets[37,38]. However, the global R&D heat has continued, and several studies on TIL treatment of tumors have entered the clinical trial stage. Given the critical role of TIL in tumor local immunity, various new types of "TIL therapies" have developed rapidly, and significant breakthroughs have been continuously made in the field of tumor treatment. As an essential branch of tumor immunotherapy, TIL therapy is one of the indispensable directions for future medical development. The global multi-center and multi-organization collaboration can promote the standardization of ALPPS surgery and large-scale data statistics. Therefore, it is necessary to deeply understand the trend of TIL changes caused by ALPPS surgery.

From an immunological perspective, this study describes the change in the trend of TME during the perioperative period of ALPPS. We demonstrate that ALPPS is safe and feasible for massive HCC in the right lobe of the liver. However, this study is a single-center study, with a limited number of patients and clinical data, thus, more in-depth discussion on the conclusions is required.

CONCLUSION

The level of TIL infiltration can help maintain the dynamic balance during the perioperative period of ALPPS, which is the basis for the normal operation of tumor local immune response. Compared with the right hepatectomy, ALPPS did not cause a decrease in TIL infiltration and the pathological changes of immune components in

peripheral blood, thus resulting in severe immunosuppression. After stage-I ALPPS, $CD8^+$ T cells effectively infiltrated into TME and played a cytotoxic role in killing tumor cells. Our results suggested that the infiltration of high $CD8^+$ T cells was related to the increase in tumor necrosis.

ARTICLE HIGHLIGHTS

Research background

Associating liver partition and portal vein ligation for staged hepatectomy (ALPPS) is an innovative approach to hepatectomy. The surgical trauma experienced by ALPPS is relatively high. In addition, stage-I ALPPS separates the right and left liver lobes and ligates the right hepatic vein, which causes inflammatory reactions, hypoxia, and tumor necrosis, resulting in a unique and complex immune microenvironment for tumor cells.

Research motivation

The trends and effects of tumor-infiltrating lymphocytes (TIL) residing or recruited in tumor microenvironment (TME) on tumors are still unexplored in studies on ALPPS for hepatocellular carcinoma (HCC).

Research objectives

From an immunological perspective, the immunological effects exerted by the unique TME formed during the treatment of HCC by ALPPS, such as anti-tumor effects or tumor-induced immunosuppression, were investigated. To further evaluate the safety and efficacy of ALPPS in treating massive HCC and conduct an in-depth study of TIL in TME.

Research methods

The exact number of patients of the ALPPS and hemi-hepatectomy groups were screened using propensity score matching. Immunofluorescence staining was performed to detect and quantify TIL in tumors and adjacent tissues in these two

groups of patients. Trends in TIL in peripheral blood during the perioperative period were compared between the two groups.

Research results

The proportion of tumor necrosis volume at postoperative day 7 was significantly higher in stage-I ALPPS than preoperatively (P = 0.024). The proportion of tumor necrosis volume was significantly higher in the high $CD8^+$ T-cell infiltrated group than in the low group before surgery for stage-I ALPPS (P = 0.048).

Research conclusions

From an immunological point of view, ALPPS is safe and feasible for treating the right lobe massive HCC. The level of TIL infiltration during the perioperative period is dynamically balanced, and the ALPPS procedure itself does not lead to severe immunosuppression due to reduced TIL infiltration and pathological changes in peripheral blood immune components.

Research perspectives

Many studies on TIL therapy for tumors have entered clinical trials. As an important branch of tumor immunotherapy, TIL therapy is one of the potential directions for the future development of medicine.

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