Effect of Thoracic Duct Ligation During VATS Esophagectomy On T Cell Subset and DFS in Squamous Cell Carcinoma Patients With T1b-3N0M0 Stage

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Research article

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Abstract

Objective: To study whether the ligation of thoracic duct during video-assisted thoracic surgery esophagectomy will cause damage to the immune system, thus affecting the disease-free survival (DFS) of patients with cT1b-3N0M0 stage.

Methods: We studied the esophageal squamous cell carcinoma confirmed by endoscopic ultrasound biopsy and PET-CT. They were randomly divided into thoracic duct ligation group and non ligation group. In addition to thoracoscopic resection of esophageal cancer, thoracic duct ligation was also performed in the experimental group. The peripheral blood T lymphocyte subsets were detected by flow cytometry during perioperation. All patients were reexamined regularly after operation in order to find recurrence or metastasis early. The Chi-square test and t-test were employed for statistical analysis with statistical significance at p<0.05. The effect of thoracic duct ligation on DFS curves were calculated by the Kaplan–Meier method and compared by the log-rank test. A Cox regression model with stepwise selection was used for the multivariate analyses.

Result: After early screening and late exclusion, a total of 67 patients entered the study and completed the follow-up. There was no significant difference in gender, age, tumor location, depth of invasion, degree of differentiation and presence of tumor thrombus between the ligation group (32 cases) and the non ligation group (35 cases). There was no significant difference in T lymphocyte subsets before and 3 weeks after operation, but there was significant difference on the 1st days after operation. Cox regression analysis showed that depth of invasion (P= 0.0020), degree of differentiation (P= 0.0262), presence of tumor thrombus (P = 0.0158) and thoracic duct ligation (P= 0.0036) were independent factors affecting DFS.

Conclusion: Thoracic duct ligation can affect the short-term immune function after thoracoscopic esophagectomy in squamous cell carcinoma patients with pT1b-3N0M0 stage, and the thoracic duct ligation, depth of invasion, degree of differentiation and presence of tumor thrombus are independent factors affecting DFS.

Trial registration: Chinese Clinical Trial Registry, ChiCTR-IOR-17010437. Registered 15 January 2017, https://www.chictr.org.cn/edit.aspx?pid=17254&htm=4

Introduction

The immunologists, honored with the 2018 award in Physiology or Medicine, pioneered immunotherapy, which harnesses the body’s immune system to fight cancer and achieved great success [1]. The rapid progress has once again proved the infinite power of immune system, and has become the most promising development direction of tumor therapy. Therefore, we have to look back at all aspects of our treatment for tumor from the perspective of immunology. As we all know, lymphocyte recycling is a process of repeated circulation in which lymphocytes, which are designated to reside in the peripheral immune organs, enter the blood circulation through the lymphatic trunk, thoracic duct or right lymphatic
duct, pass through the blood circulation to the peripheral immune organs, pass through the high endothelial venules and redistribute in the systemic lymphatic organs and tissues [2]. It is because of this kind of lymphocyte recycling that lymphocytes can be reasonably distributed in various lymphoid tissues and organs in the body. T cells and B cells with specific antigen receptors constantly travel around the body, increasing the chance of contact with antigen and antigen presenting cells [2]. As the last terminal pathway of lymphatic system into blood circulation, whether the ligation of thoracic duct will cause serious damage to the immune system, thus affecting the recognition and killing of tumor by the immune system of patients, is a subject worthy of study.

There have been different opinions on whether to preventively ligate the thoracic duct during esophagectomy [3–5]. With the development of medicine, video-assisted thoracic surgery (VATS) has gradually become the mainstream surgical method for the treatment of early and middle stage of esophageal cancer due to its advantages of small trauma and rapid recovery [6]. Under the new operation method, whether the thoracic duct should be ligated is still in front of us.

**Methods**

**Patients**

Patients with esophageal cancer admitted to our hospital from June 1, 2017 to June 1, 2021, without serious cardiopulmonary and immune diseases. Squamous cell carcinoma with cT1b-3N0M0 stage invasion confirmed by endoscopic ultrasonography and biopsy were enrolled. Patients with distant metastasis and lymph node metastasis were excluded by positron emission tomography-Computed Tomography (PET-CT). The general conditions of heart Color Doppler ultrasound, lung function, liver and kidney function, blood routine and coagulation were evaluated. Patients with anastomotic leakage, severe pulmonary infection, heart failure and other serious complications were excluded. Postoperative pathology confirmed that patients beyond T1b-3N0M0 stage should also be excluded. The study has been approved by the Committee on Medical Ethics of Taian City Central Hospital, and written informed consent was obtained from all patients.

**Surgical procedure**

Video-assisted esophagectomy and cervical gastroesophagostomy were performed by the same group of surgeons accordance with the *Chinese standard for diagnosis and treatment of esophageal cancer*. To identify thoracic duct easily, 100–150 ml olive oil was drunk by the patient 8–12 h before operation. During operation all arch of azygos vein need to be ligated and transected. Behind lower segment esophagus, the thoracic duct could be easily recognized and separated between the descending aorta and the azygos vein. According to random grouping, the thoracic duct was separated and ligated between the level of diaphragm and inferior pulmonary vein.

**Detection of T lymphocyte subsets**
Venous blood was drawn before operation and on the 1st day after operation and three weeks after operation. First, the samples were stained with Tritest CD4-FITC/CD8-PE/CD3-PerCP three-color reagents (BD company, USA) and then analyzed by flow cytometer with CellQuest software (BD Biosciences, Franklin Lakes, NJ, USA). By forward scatter and side scatter monitoring, white blood cell was displayed, and lymphocyte populations were gated according to their size and granularity. The nonspecific binding was determined with isotype control tube, and marker was set for distinguishing fluorescence negative and positive cell populations. A minimum of 2,000 lymphocytes were initially acquired. The number of CD3, CD3/CD4, and CD3/CD8 positive lymphocytes could be analyzed based on their Percp, FITC and PE fluorescence respectively. By this flow cytometric analysis, the percentages of T lymphocyte, CD4+ T lymphocyte, and CD8+ T lymphocyte in total lymphocytes could be reported [7].

Follow-up

All patients were reexamined every 3 months in 2 years, every 6 months in 5 years and every year after 5 years. Reexamination contents: blood routine, gastroscope, chest CT (plain scan or enhanced scan), Color Doppler ultrasound for neck and abdominal, etc. when necessary other examinations such as PET-CT, bone scan, MRI, etc. should be supplemented according to the patient’s symptoms, signs and routine examination.

Statistical analysis

In the follow-up study we collect the age, gender, T stage (T1b, T2, T3), degree of differentiation (G1, G2, G3), tumor location (upper, middle, lower), whether the thoracic duct was ligated, whether the postoperative pathology indicated vascular cancer thrombus, etc. The data of the two groups were compared by Chi-square test. The t-test were employed for T lymphocyte subsets statistical analysis with statistical significance at p < 0.05. DFS (status = 1 for tumor recurrence or metastasis, 0 for deletion) was calculated from the date of operation to metastasis or last follow-up. The results were analysed according to Kaplan-Meier and Cox (proportional hazard) regression with SAS9.2 software.

Results

1. After early screening and pathological stage exclusion, a total of 69 patients were enrolled in the study. In the ligation group, 1 case of anastomotic leakage and 1 case of severe pulmonary infection were excluded. No chylothorax occurred in both groups. Finally, 67 patients entered the study and completed the follow-up. There was no significant difference in gender, age, tumor location, depth of invasion, degree of differentiation and presence of tumor thrombus between the thoracic duct ligation group (32 cases) and the non ligation group (35 cases) (P > 0.05). The results are shown in Table 1.
Table 1
Comparison of clinical data between ligation group and control group

| Data                  | classification | PLG (n = 32) | NPLG (n = 35) | x²   | P     |
|-----------------------|----------------|--------------|---------------|------|-------|
| Age                   | ≥ 60           | 18           | 17            | 0.395| 0.5297|
|                       | < 60           | 14           | 18            |      |       |
| Sex                   | Male           | 27           | 29            | 0.0281| 0.867 |
|                       | Female         | 5            | 6             |      |       |
| Location              | Up             | 5            | 6             | 0.3733| 0.8297|
|                       | Middle         | 17           | 16            |      |       |
|                       | Down           | 10           | 13            |      |       |
| Invasion depth        | T1b            | 17           | 16            | 0.2390| 0.8874|
|                       | T2             | 10           | 12            |      |       |
|                       | T3             | 5            | 7             |      |       |
| Differentiation       | G1             | 6            | 7             | 1.1683| 0.5576|
|                       | G2             | 17           | 22            |      |       |
|                       | G3             | 9            | 6             |      |       |
| Cancer embolus        | Yes            | 6            | 8             | 0.1706| 0.6796|
|                       | No             | 26           | 27            |      |       |

PLG: Prophylactic thoracic duct ligation group; NPLG: non-prophylactic thoracic duct ligation group

2. There was no significant difference in T lymphocyte and T lymphocyte subsets before operation and 3 weeks after operation (P > 0.05), but there was significant difference in T lymphocyte subsets on 1st days after operation (P < 0.05). The results are shown in Table 2.
### Table 2
Comparison of monitoring results of T cell subsets before and after operation

| t                  | data      | PLG (n = 32)      | NPLG (n = 35)      | P    |
|--------------------|-----------|------------------|-------------------|------|
| **Before operation** |           |                  |                   |      |
| CD3 + LYM (%)      | 68.8438 ± 6.2171 | 69.3714 ± 7.4165 | -0.31             | 0.7545 |
| CD3 + CD4 + LYM (%) | 42.1563 ± 6.6677 | 42.0000 ± 6.2261 | 0.10              | 0.9213 |
| CD3 + CD8 + LYM (%) | 23.9688 ± 5.2085 | 25.4000 ± 8.4024 | -0.85             | 0.4013 |
| **1st days after operation** |           |                  |                   |      |
| CD3 + LYM (%)      | 55.5000 ± 7.1662 | 59.3143 ± 6.2486 | -2.33             | 0.0231 |
| CD3 + CD4 + LYM (%) | 27.0000 ± 4.3552 | 36.0000 ± 6.6686 | -6.59             | <.0001 |
| CD3 + CD8 + LYM (%) | 26.2813 ± 5.7318 | 21.5143 ± 7.4612 | 2.91              | 0.0049 |
| **3 weeks after operation** |           |                  |                   |      |
| CD3 + LYM (%)      | 64.3750 ± 5.8737 | 66.4571 ± 6.8999 | -1.32             | 0.1902 |
| CD3 + CD4 + LYM (%) | 37.8750 ± 5.9446 | 39.9429 ± 5.8003 | -1.44             | 0.1546 |
| CD3 + CD8 + LYM (%) | 24.5000 ± 4.6835 | 24.5143 ± 7.4769 | -0.01             | 0.9925 |
| LYM: Lymphocyte   |           |                  |                   |      |

3. DFS was calculated and plotted with the Kaplan-Meier method. The results showed that gender, age and tumor location had no significant effect on DFS (P > 0.05), but depth of invasion, degree of differentiation, presence of tumor thrombus and thoracic duct ligation had significant effect on DFS (P < 0.05). The results are shown in Table 3 and Fig. 1.
Table 3
single factor analysis of the influence of clinical and pathological factors on DFS

| Data                | Classification | N   | x2    |
|---------------------|----------------|-----|-------|
| Sex                 | Male           | 56  | 0.1777| 0.6734|
|                     | Femal          | 11  |       |       |
| Age                 | ≥ 60           | 35  | 0.0131| 0.9089|
|                     | < 60           | 32  |       |       |
| Location            | Up             | 11  | 2.2068| 0.3317|
|                     | Middle         | 33  |       |       |
|                     | Down           | 23  |       |       |
| Invasion depth      | T1b            | 33  | 7.7323| 0.0209|
|                     | T2             | 22  |       |       |
|                     | T3             | 12  |       |       |
| Differentiation     | G1             | 13  | 6.0296| 0.0491|
|                     | G2             | 39  |       |       |
|                     | G3             | 15  |       |       |
| Cancer embolus      | Yes            | 14  | 12.1656| 0.0005|
|                     | No             | 53  |       |       |
| Thoracic duct ligation | Yes         | 32  | 5.8120| 0.0159|
|                     | No             | 35  |       |       |

Cox proportional hazard models for DFS was built for those prognostic factors with p < 0.1 in the univariate analysis. The result show that invasion depth [HR = 2.473, 95% CI (1.393, 4.390), P = 0.002], tumor differentiation [HR = 2.409, 95% CI (1.110, 5.231), P = 0.0262], vascular cancer embolus [HR = 4.422, 95% CI (1.322, 14.789), P = 0.0158] and thoracic duct ligation [HR = 4.410, 95% CI (1.625, 11.963), P = 0.0036] were the independent risk factors affecting the DFS. Please see the Table 4 for details.
Table 4  
Cox regression analysis results of DFS

| DATA                      | β     | SE   | Wald x2 | P       | HR(95%CI)       |
|---------------------------|-------|------|---------|---------|-----------------|
| Depth                     | 0.90526 | 0.29285 | 9.5555  | 0.0020  | 2.473(1.393,4.390) |
| Differentiation           | 0.87927 | 0.39558 | 4.9407  | 0.0262  | 2.409(1.110,5.231)  |
| Cancer embolus            | 1.48660 | 0.61597 | 5.8246  | 0.0158  | 4.422(1.322,14.789)  |
| Thoracic duct ligation    | 1.48379 | 0.50919 | 8.4916  | 0.0036  | 4.410(1.625,11.963)  |

Discussion

With the development of medicine, people pay more and more attention to the unlimited potential of immune system in tumor treatment. Ligation of thoracic duct is an effective measure for the treatment of chylothorax [8]. There have been academic disagreements on whether to preventive ligation during operation [3–5]. Our previous study was to explore the effect of thoracic duct ligation on immune function and absorptive function [7, 9]. This time, we not only increased the monitoring time to dynamically record the changes of T cell subsets after ligation but also chose T1b-3N0M0 patients to expand the study population and increase the number of participants. In addition, we conducted a long-term follow-up of the patients to detect early metastasis and explore the impact of thoracic duct ligation on DFS.

According to the guidelines, for intramucosal esophageal cancer, we generally choose endoscopic resection rather than thoracoscopic resection [10]. At the same time, PET-CT combined with postoperative pathology was used to exclude the patients beyond T1b-3N0M0 who often need chemoradiotherapy and then have interference factors on immune function and DFS [10].

Our previous studies for T1N0M0 stage showed that there was no difference between the two groups before operation, the T cells and CD4 + T cell subsets of ligation group were significantly lower than those of non ligation group on 1st days after operation, and the CD8 + T cell subsets of ligation group were significantly higher than those of non ligation group [7]. In this study, the subjects were extended to T1b-3N0M0 stage, and the same results were obtained. This study was carried out in patients undergoing VATS esophagectomy during which the azygos vein arch often needs to be transected for easier to free esophagus and clean lymphonode. We know there are a lot of communicating branches between azygos vein and thoracic duct [11]. When the thoracic duct and azygous arch are transected at the same time, lymph fluid and a large number of lymphocytes in it are difficult return to the blood in a short time. As time goes on, the accumulated lymph always flows back into the blood through the reestablishment of collateral circulation [12]. It's not hard to explain that there was no difference between the two groups again 3 weeks after operation. It can be imagined that the decrease of immune function in a short time after operation might give the residual tumor cells an opportunity to escape and metastasize.
Postoperative follow-up also confirmed this result. Univariate analysis showed that gender, age and tumor location had no significant effect on DFS ($P > 0.05$), but depth of invasion, degree of differentiation, presence of tumor thrombus and thoracic duct ligation had significant effect on DFS ($P < 0.05$). Cox regression analysis showed the same results, especially the preventive thoracic duct ligation was a high risk factor for postoperative metastasis ($P = 0.0036$, $\beta = 1.48379$). Combined with the results of this study, we suggest that prophylactic ligation should be taken when there are signs of thoracic duct injury, otherwise it is not recommended.

The deficiency of this study is that the number of samples is small, and T cell subsets can not be monitored at more time points. Because of the difficulty of follow-up, OS evaluation index was not used. We are also trying to add additional follow-up data to improve and perfect our study.

**Conclusion**

Our study suggests that the prophylactic ligation of thoracic duct during thoracoscopic esophagectomy can lead to the change of T cell subsets, especially the decrease of CD4+ T cells in the early stage after surgery. At the same time, the late follow-up confirmed that the ligation of thoracic duct, the poor differentiation of tumor, the depth of invasion and the vascular tumor thrombus are the independent risk factors for postoperative recurrence and metastasis.

**List Of Abbreviations**

PET-CT: positron emission tomography-Computed Tomography; DFS: disease-free survival; VATS: video-assisted thoracic surgery; CD: Cluster of Differentiation; FITC: Fluorescein Isothiocyanate; PE: Phycoerythrin; PerCP: Peridinin-Chlorophyll-Protein Complex; SAS: Statistical Analysis Software; PLG: Prophylactic thoracic duct ligation group; NPLG = non-prophylactic thoracic duct ligation group; LYM: Lymphocyte

**Declarations**

**Ethics approval and consent to participate**

The study has been approved by the Committee on Medical Ethics of Taian City Central Hospital (Code of ethical approval: NO. 2016 Ethics Approval 0071).

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

**Consent for publication**
Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

All authors declare that they have no competing interests.

Funding

No founding.

Authors' contributions

Rui-feng Yang is the designer of the experiment, the chief surgeon, the main writer (including statistical processing) and submitter, and also the corresponding author. Yan Zhang is mainly responsible for preoperative informed consent, postoperative management and follow-up and examination after discharge. Xiao-mei Li is mainly responsible for pathological examination and T cell subsets test publication. Ya-chen Sun, Run-qi Zhang and Peng Wang participated in operation, perioperative vital signs monitoring and management.

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Figures
Figure 1

1t single factor analysis of the effect of T stage, tumor differentiation, thoracic duct ligation and vascular tumor thrombus on DFS