

RESEARCH ARTICLE

Video-assisted Thoracoscopic Surgery for Treatment of Early-stage Non-small Cell Lung Cancer

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Abstract

Objectives: To evaluate the safety, efficacy, and invasiveness of lobectomy by video-assisted thoracoscopic surgery (VATS) in the treatment of stage I/II non-small cell lung cancer (NSCLC). **Methods:** A total of 148 patients presenting with Stage I or II NSCLC were enrolled into our study, comprising 71 who underwent VATS and 77 patients undergoing conventional thoracotomy lobectomy, in combination with systematic lymph node resection. **Results:** It was found that VATS was superior to conventional thoracotomy in terms of the duration of surgery, intraoperative blood loss, frequency of the need to administer postoperative analgesia, thoracic intubation indwelling time, post-operative hospital stay, and survival rate ($P < 0.05$). We saw no obvious difference in the number of resected lymph nodes with either approach. **Conclusions:** VATS lobectomy is a safe and reliable surgical approach for the treatment of Stage I/II NSCLC, characterized by significantly minimal invasiveness, rapid post-operative recovery, and markedly lower loss of blood.

Keywords: Video-assisted thoracoscopic surgery - lung cancer - lobectomy - lymph node dissection - prognosis

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Introduction

Traditionally, thoracotomy was the most common thoracic surgical approach before the advent of minimally invasive devices. The malignant lesions can be fully exposed and resected, however, there are major concerns associated with post-operative and severe pulmonary complications and prolonged wound healing. This is largely due to massive surgical trauma and intraoperative loss of blood loss, all of which have been major concerns for both the surgeon and the patient. The initial clinical report on thoracoscopic surgery was published in the 1990s (Lee, 2010). Thereafter, thoracoscopic techniques have become an important surgical approach for thoracic surgery, especially following the advent of video-assisted thoracoscopic surgery (VATS).

The advantage of VATS over thoracotomy is that the incision to the patient's chest is relatively minor, which leads to reductions in post-operative infection and wound dehiscence. This permits the prompt return of the patient to full activity. Therefore, thoracoscopic surgery was gradually adopted for the diagnosis and treatment of lung malignancies complementing the application of endoscopic gastrointestinal anastomosis (Endo-GIA). In addition, lobectomy and mediastinal lymph node resection using thoracoscopic devices (Flores, 2010) enables VATS to be one of the most feasible alternatives for early stage lung cancer treatment, an approach which has been widely adopted in developed countries (Yim et al., 1996; Ohbuchi et al., 1998; Roviario et al., 1998; McKenna, 2006; Onaitis

et al., 2006; Mahtabifard et al., 2007; Swanson et al., 2012). Prior to 2003, the rate of annual VATS lobectomy in lung cancer was shown to be less than 5% but rose to approximately 10% by 2006. Experts in the field have estimated that approximately 60% of lung cancer surgeries could be performed by adopting VATS in the US over the next several years.

Application of VATS in surgical medicine was first elaborated in the Guidelines of Clinical Oncology of the National Comprehensive Cancer Network (NCCN) in 2006. VATS lobectomy is now considered the standard approach in the treatment of early stage NSCLC by the NCCN guidelines of 2007. However, in China, safety concerns in regard VATS lobectomy have limited application of this approach and it is only performed in developed regions. Surveying the published literature informs us that the major approach for thoracoscopic-assisted lobectomy is by direct visualization and does not rely solely on the use of a fiber optic thoracoscope in VATS lobectomy. Therefore, we conducted a comprehensive assessment of VATS lobectomy in the treatment of early stage NSCLC to elucidate the safety and efficacy of this approach as compared with that of conventional thoracotomy.

Materials and Methods

Patients

We recruited 148 study subjects to this study who presented with NSCLC at stage I or II, without

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radiotherapy or chemotherapy between January 2005 and December 2010. Surgical treatment of the recruited subjects was considered as a first line therapeutic strategy. Thus, 71 study subjects underwent VATS (VATS group) and 77 study subjects (Thoracotomy group) underwent conventional thoracotomic lobectomy respectively. Chest X-ray examination, bronchoscopy, arterial blood/gas analysis, and pulmonary function tests were performed prior to surgery. Inspection of chest CT scans revealed an absence of enlarged mediastinal lymph nodes (defined as a lymph node having a diameter larger than 1.0 cm) in all of the recruited subjects. In addition, the size of the tumors in the study subjects was less than 6.0 cm in diameter. This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of QiLu Hospital, Shandong University. Written informed consent was obtained from each subject before participation in the study.

We found that the clinical data from both groups were comparable. In addition, the inclusion criteria for VATS surgery included a requirement that the study subjects presented with Stage I or II NSCLC and that it was technologically feasible to perform VATS lobectomy. For example, such requirements as an absence of enlarged lung hilum, enlarged mediastinal lymph nodes, absence of extensive pleural thickness and calcification, and a tumor diameter of less than 5 cm needed to be satisfied. Following signed informed consent by the study subjects and their families, VAST lobectomy was initiated (Table 1).

Surgical approach

Surgery was performed under general anesthesia. In addition, for some subjects, continuous high epidural anesthesia was supplemented so that the amount of inhalation anesthetic drugs could be reduced, and thus facilitate recovery of the study subjects from anesthesia, and reduce postoperative pain. It was noted that continuous epidural anesthesia was not necessary in successfully performing VATS lobectomy. In our surgical approaches, left lateral double-lumen endotracheal intubation, unilateral lobar ventilation.

VATS lobectomy

Two 1.5 cm incisions were made at the 8th intercostal axillary line or at the 7th or 8th intercostal subscapular line, to enable access ports for observation and operating devices, respectively. In addition, one 4.0 cm incision was

made at the anterior axillary line, at the 4th intercostal space for the resection of the superior and middle lobes, and at the 5th intercostal space for the resection of the inferior lobe respectively, according to the location of the tumor. The incision location was in accordance with that reported in the literature (Yim et al., 1996; Demmy and Nwogu, 2008; Kim et al., 2010; Swanson et al., 2012).

We suggest that in the first instance massive pleural adhesions should be disassociated in order to retain acceptable mobility of the lobes. Conventional thoracic surgery devices were used to detach the pulmonary veins and artery if the interlobar fissure could be completely differentiated. An additional suture needed to be placed across the large vessel (with a diameter greater than 1.0 cm) using forceps or endoscopic equipment, and served as a traction tool under situations when the vessel was dissected. Endo-GIA or conventional suture ligation of the dissected vessel was used according to the intraoperative or the financial condition of the subject. By contrast, Endo-GIA was used for bronchial treatment in all study subjects. During this procedure, forced lung inflation was usually conducted prior to bronchial dissection in order to confirm involvement of the targeted bronchi in the lesions. Thorough mediastinal lymph node resection should also be performed following lobectomy. The resection strategies used in VATS and conventional thoracotomy were similar in that R2, R4, the 7th, and 9th lymph nodes on the right side, and the 5th, 6th, 7th, and 9th lymph nodes on the left side were routinely resected (D'Amico, 2006). Surgical specimens were removed from the functional incision and placed into a home made specimen bag. Rinsing of the thoracic cavity with pre-warmed distilled water and an air leakage test were conducted before closure of the incision.

Thoracotomic lobectomy

The standard posterolateral thoracotomic incision was adopted for all study subjects. The incision was made between 15 and 20 cm in length and at the 4th or 5th intercostal space according to the location of the lesion. The thoracic cavity was opened layer-by-layer, and the ribs were distracted by use of a thoracic retractor. The adhesions were first disassociated, following which the Endo-GIA or the conventional ligation approach was used for treatment of the interlobar fissures, depending on the economic condition of the study subjects. The pulmonary arteries and veins were detached from the surrounding

Table 1. The Comparison of Baseline Data of Two Groups

Group	VATS	Thoracotomy	<i>P</i> value*
Male	33	48	0.096
Female	36	29	
Age (year)	63.12 ± 9.85	66.23 ± 9.29	0.051
Tumor size (cm)	2.55 ± 1.11	2.89 ± 1.09	0.064
Forced expiratory volume (FEV1, L)	2.29 ± 0.63	2.69 ± 0.89	0.002
Respiratory frequency (times/min)	18.68 ± 3.23	19.12 ± 3.46	0.92
Heart beat (times/min)	67.88 ± 16.78	69.37 ± 17.18	0.94
Systolic and diastolic blood pressure (mmHg)	115.6 ± 9.58 69.6 ± 6.87	114.2 ± 7.58 67.3 ± 4.06	0.94
History (number of individuals)	14	18	0.76
Family history of tumors (number of individuals)	4	6	0.89

Table 2. Comparison of Tumor Localization, Classification of the Clinical Stage of Disease, and Pathological Type

Group	VATS	Thoracotomy
Localization of tumor		
Right upper lobe	22	22
Right middle lobe	13	15
Right lower lobe	10	6
Right upper lobe	17	19
Right lower lobe	7	15
Clinical stage		
Ia	28	8
Ib	32	51
IIa	3	2
IIb	6	16
Pathological type		
Squamous cell carcinoma	10	29
Adenocarcinoma	53	37
Alveolar cell carcinoma	4	10
Adenosquamous carcinoma	2	1

tissues, followed by dissection and silk suture ligation. The bronchial stump was used for closure of the truncated bronchi. Finally, thorough conventional dissection of the regional lymph nodes was performed and selective intercostal nerve block was performed as necessary for some study subjects.

Inspection indicators

The intraoperative and postoperative observations were recorded in detail over the first three days after the operation and extensively compared between the two groups, including the duration of the operation, intraoperative blood loss, daily and total drainage volume, the frequency of administration of postoperative analgesic drugs, postoperative hospital stay, the numbers of removed lymph nodes, and postoperative complications. The tumor recurrence rate, tumor-free survival rate, and overall survival rate were also compared between the two groups during the follow-up study.

Statistical analysis

All quantitative data were expressed as mean \pm SD and Student's T-test was used to compare the means of the different groups. Categorical data were described as absolute frequencies and analyzed by Pearson's Chi-square test or Fisher's exact test. Kaplan-Meier survival plots were generated and comparisons made between survival curves using log-rank statistics. SPSS 13.0 software (SPSS Inc., Chicago, IL, USA) was applied to analyze all of the data and an alpha value of $P < 0.05$ was considered statistically significant.

Results

General information

Perioperative death was not seen in the VATS lobectomy group, although two study subjects were excluded from the study due to the conversion in treatment approach to thoracotomy. The rate of conversion was 2.82% (2/71). One subject in the conventional thoracotomy group

was found demised within 30 days after surgery due to respiratory failure, and the perioperative mortality rate was 1.3%.

Comparison of tumor localization, stage classification, and pathological type

A total of 69 subjects underwent VATS lobectomy and 77 subjects underwent conventional thoracotomy respectively. We did not see any significant difference between the two groups with respect to the anatomical location of the tumor (left upper lobe, lower lobe, left upper lobe, lower lobe) or clinical stage (stage I or II). However, the composition of the pathological type, including squamous cell carcinoma, adenocarcinoma or alveolar cell carcinoma, were different. The type of non-squamous cell carcinoma was more frequent in subjects who had undergone VATS as compared subjects who underwent conventional thoracotomy (Table 2, $P < 0.05$).

Comparison of operation time, intraoperative blood loss, and removal of lymph nodes

The mean operation time and intraoperative blood loss in the VATS lobectomy group were 193.42 ± 43.76 min (range 120 min to 315 min) and 232.03 ± 154.06 ml (range 20 ml to 650 ml), respectively. By contrast, the mean operation time and intraoperative blood loss in the thoracotomy lobectomy group was 48 ± 56.51 min (range 130 min to 435 min) and 412.60 ± 298.76 ml (range 50 ml to 1800 ml) respectively. Both groups underwent thorough lymph node removal. In this procedure, 15.01 ± 6.54 lymph nodes (minimum of 3 and a maximum of 33), and 3.22 ± 0.872 lymph node groups were removed in the VATS group. Similarly, there were 16.74 ± 9.96 lymph nodes (minimum of 4 and a maximum of 51) and 3.16 ± 1.288 lymph node groups were removed in the thoracotomy group, although there was no significant differences between the two groups. In addition, we found that the operation time for the VATS lobectomy group was significantly shorter than that of the thoracotomy lobectomy group ($P < 0.05$). There was also markedly less intraoperative blood loss in the VATS lobectomy group as compared to the thoracotomy lobectomy group ($P < 0.05$).

Comparison of daily and total drainage volumes in the post-operative first three days

The research study subjects presenting with chylothorax were excluded from the statistical analysis. Thus, the daily drainage volumes in the post-operative first three days in the VATS group were 320.81 ± 135.69 ml (day 1), 224.63 ± 115.58 ml (day 2), and 195.85 ± 83.81 ml (day 3) respectively. The total drainage volume was found to be 1621.54 ± 748.27 ml. Comparatively, the daily drainage volumes in thoracotomy group over the post-operative first three days was 412.37 ± 178.33 ml (day 1), 269.29 ± 120.14 ml (day 2) and 201.84 ± 118.73 ml (day 3) respectively. The total drainage volume was found to be 1945.86 ± 1016 ml. The daily drainage volume in the VATS group was significantly less in the post-operative first two days as compared with the thoracotomy group ($P < 0.05$). In addition, there was no significant difference by the post-operative third day.

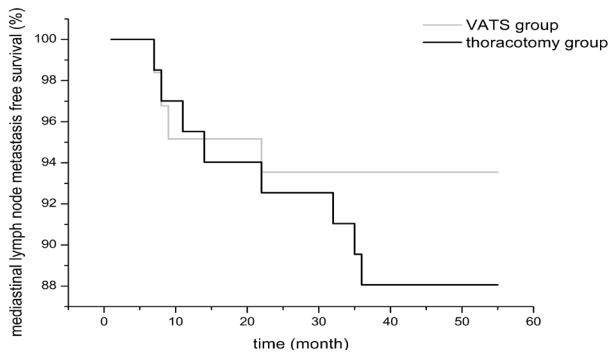


Figure 1. Comparison of Mediastinal Lymph Node Metastases in Both Treatment Groups

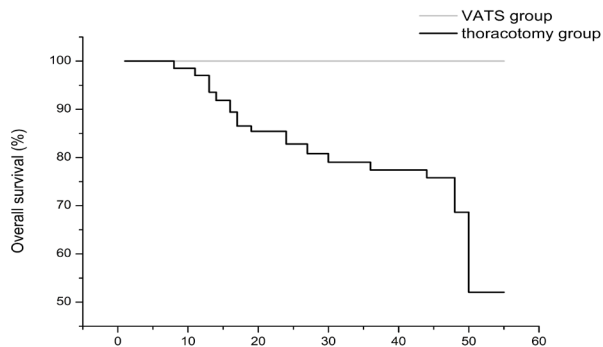


Figure 3. Comparison of Overall Survival Rate in Both Treatment Groups

Comparison of thoracic intubation time, frequency of analgesic administration, and postoperative hospital residence

The chest draining tube was removed when the daily drainage volume decreased to less than 100 ml. The mean thoracic intubation indwelling time in the VATS group was 6.51 ± 3.44 days as compared to a significantly longer time of 9.20 ± 3.91 days in the conventional thoracotomy group ($P=0.035$). The mean frequency of postoperative administration of analgesic was 1 ± 1.07 times per subject for the VATS group, as compared with a significantly greater 3.48 ± 3.29 times per subject for the conventional thoracotomy group ($P<0.05$). Similarly, the mean postoperative hospital stay was 9.20 ± 3.91 days in the VATS group as compared with a much longer stay of 11.46 ± 5.44 days for the conventional thoracotomy group ($P=0.005$).

Comparison of postoperative complications

Common complications from lobectomy, both in the VATS treated group and those subjects treated by the conventional thoracotomy approach, include atrial fibrillation, air leakage, chylothorax, pulmonary atelectasis, and pneumonia. Both atrial fibrillation and chylothorax were found in just one subject (1.45%, 1/69) who had undergone VATS lobectomy. The chylothorax lasted for more than 7 days and was improved by thoracic duct ligation via VATS. Air leakage was found to occur in five subjects (7.25%, 5/69), where one of them had undergone secondary surgery because of air leakage lasting more than five days. Pulmonary atelectasis and pneumonia occurred separately in only three patients where the total incidence was 8.76% (or 6/69 of the total number of subjects). By contrast, in the conventional

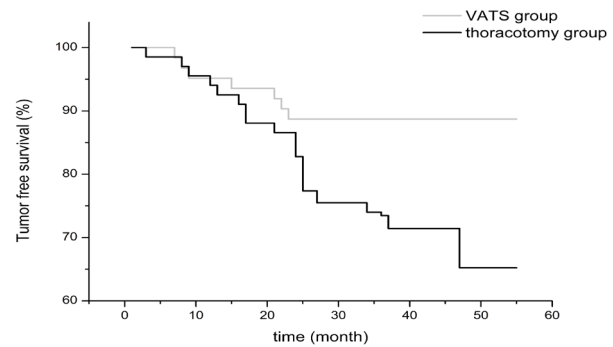


Figure 2. Comparison of the Tumor-free Survival Rate in Both Treatment Groups

thoracotomy group, 11 subjects had atrial fibrillation (14%, 11/77), six subjects had chylothorax (7.79%, 6/77) and one subject was actually cured by thoracic duct ligation via a secondary thoracotomy. Moreover, air leakage occurred in seven subjects (9.09%, 7/77), which was cured by application of talcum powder and 50% glucose to pleural perfusion. Pulmonary complications including atelectasis were found in seven subjects (9.09%, 7/77), and pneumonia was found in 10 subjects (12.99%, 10/77) respectively, with a total incidence of 22.08% (17/77). In general, the incidence of atrial fibrillation and chylothorax were significantly less in the VATS group as compared those in the conventional thoracotomy group ($P<0.05$). Additionally, the incidence of air leakage was not significantly different between the two groups.

Comparison of tumor recurrence, metastasis, and survival rate

The follow-up rate in the VATS group of subjects was 89.6% (62/69) and the follow-up period ranged between one and 35 months. Mediastinal lymph node metastasis developed in four subjects at 7, 8, 9 and 22 months respectively following surgery. In addition, distant metastases were found in three subjects at 15, 21 and 23 months after surgery, respectively. The corresponding 9-month and 23-month tumor-free survival rates were 94% and 78.8% respectively. There were no deaths reported during the follow-up period.

In the thoracotomy group, the follow-up rate was 87.01% (67/77), and the follow-up period ranged between one and 55 months. Development of mediastinal lymph node metastasis was seen in eight study subjects at 8, 9, 12, 17, 25, 34, 36 and 37 months following surgery respectively. In addition, distant metastasis was noted in another eight subjects at 12, 13, 16, 21, 24, 27 and 47 months respectively following the operation. In general, the 9-month and 24-month tumor-free survival was 95.5% and 82.8% respectively. A total of 21 subjects died during the follow-up period, among which 16 subjects died of advanced tumors, and five died of other complications including lung infection, cerebral or myocardial infarction, and pulmonary embolism. The 16-month survival rate was 89.4%. Moreover, the survival rate of the subjects who underwent VATS was significantly greater than those subjects who had undergone conventional thoracotomy ($P=0.04$). However, there was no significant difference in the tumor-free survival rate ($P=0.094$, Figures 1-3).

Discussion

VATS, as a minimally invasive surgical approach, was initially used for the treatment of benign diseases such as at pneumatocele and mediastinal tumors, and in the diagnosis of lung tumors. Today, VATS has been gradually integrated into surgical medicine in the treatment of early stage lung carcinoma (Lewis et al., 1999; Swanson et al., 2012). However, there remain concerns with regard the safety and therapeutic efficacy of VATS-directed lobectomy, an approach which remains controversial. In China, thoracoscope-assisted lobectomy was widely used instead of the video-assisted thoracoscopic lobectomy, which unlike thoracoscopic surgery still relies on direct visualization with the naked eye, whereas the VATS surgical approach is solely visualized with the aid of a fiber optic thoracoscope. Therefore, we aimed to study the safety, efficacy, and invasiveness of VATS lobectomy in the treatment of NSCLC and compared this approach with the more conventional thoracotomy approach.

VATS lobectomy is conducted with the aid of sophisticated devices and a camera attached to the thoracoscope, which is inserted into the pulmonary chest via small access ports. However, the tactile input, feedback and skill of the surgeon can be compromised, which may result in a prolonged duration of surgery and high risk of intraoperative blood loss. However, our findings suggested that the surgical operating time and intraoperative blood loss with VATS were significantly less than that seen by conventional thoracotomy ($P < 0.05$), and this observation was concordant with other studies (Demmy and Curtis, 1999; Sugiura et al., 1999). The visualization of VATS lobectomy is enhanced due to the magnification afforded by the fiber optic thoracoscope, which allows far fewer mistakes during the surgical procedure. This approach also requires a thorough understanding of pulmonary anatomy and the accumulated experience of surgeons practiced in this technique can overcome any compromise associated with tactile feedback.

In principle, lobectomy by the VATS approach should not increase the risk of intraoperative blood loss. Another concern with regard VATS lobectomy is that the hemostasis through the access port could be problematic, especially in the case of a massive hemorrhage from the large blood vessels, and ineffective hemostasis due to blurred thoracoscopic vision and restricted use of hemostatic forceps, all of which pose significant and potential threats on the well-being of patients undergoing this procedure. However, our practice indicates that massive intraoperative blood loss was rare and could be dramatically quelled by employing sufficient preoperative preparation and careful operation during the VATS procedure. Indeed, one subject had a blood volume loss of 200 ml during detachment of interlobar vessels. The surgery was immediately converted to the conventional thoracotomy approach within eight minutes following transient hemostasis treatment by applying gauze pressure. The condition of the patient remained stable during the whole process, and the postoperative recovery was satisfactory. No additional or significant intraoperative blood loss due to rupture of the blood

vessels occurred in any of the other 69 subjects who had undergone VATS lobectomy.

The major muscles of the chest wall are not divided and rib spreaders that can result in rib fractures or costovertebral joint pain, are not used in VATS lobectomy. This permits diminished duration and intensity of pain, and shorter time to achieve full recovery. These were also observed in our study where the frequency of administration of postoperative analgesic was significantly less in the VATS group than that seen for the conventional thoracotomy group (1 ± 1.07 vs 3.48 ± 3.69 , $P < 0.05$). Additionally, most of the subjects who underwent VATS were fully ambulatory and able to walk by foot on the second day following surgery. Their recoveries were significantly more rapid than those who underwent conventional thoracotomy. In addition to benefiting from the minimal surgical trauma, the VATS group subjects experienced lower drainage volumes and tube indwelling time, which collectively translated to a shorter hospital stay as compared to the conventional thoracotomy group.

However, in our study, the chest tube indwelling time and hospital stay were still longer than that of previous reports. It is possible that the indication for drainage tube removal was somewhat different in our study as compared practices reported by others (Mahtabifard et al., 2007; Flores, 2010; Swanson et al., 2012). In our study, the drainage volume was less than 100 ml within a 24 h period, whereas in other studies, this volume needed to be less than 300 ml (Linder et al., 2012).

The minimized surgical trauma seen with VATS lobectomy results in far fewer complications, and permits rapid recovery of patients. Our study was also in agreement with previously published studies that reported the incidence of atrial fibrillation, pulmonary atelectasis, and pneumonia, which were all significantly reduced in the VATS group as compared to the conventional thoracotomy group ($P < 0.05$). In addition, we also found that the incidence of chylothorax was also significantly less in the VATS group than was seen in the conventional thoracotomy group ($1/69$ vs $6/77$, $P = 0.0002$), which hitherto has not been previously reported by others. Thoracic duct injury during surgical removal of mediastinal lymph nodes is a major risk factor in the causation of chylothorax (Le et al., 2002; Shimizu et al., 2002; Paul et al., 2010). Moreover, enhanced visualization provided by VATS lobectomy, due in part to the magnification provided by the fiber optic thoracoscope could permit elegant manipulation and avoid thoracic duct injury. Whether the VATS lobectomy could reduce the incidence of chylothorax requires additional study and confirmation with much larger cohorts of recruited study subjects.

According to the NCCN guidelines for the surgical treatment of lung cancer, at least three groups of mediastinal lymph nodes require removal. Our study showed that the removed lymph nodes were similar for both the VATS group and the conventional thoracotomy group in terms of lymph node group number or in the context of the individual lymph node number (3.22 ± 0.872 groups vs 3.16 ± 1.288 groups; 15.01 ± 6.54 vs 16.70 ± 9.922 lymph nodes, $P > 0.05$). This finding was consistent with the previous work published by others (Hokschi et

al., 2002; Hurtgen et al., 2002; Gharagozloo et al., 2003; West, 2007; Demmy and Nwogu, 2008; Hokschi and Alam, 2008; McKenna, 2008).

The occurrence of lung cancer and mediastinal lymph node metastasis are believed to be associated with incomplete resection of the tumor and mediastinal lymph nodes during surgery, both of which are critical for prognosis (Doddoli et al., 2005). The tumor-free survival rates at nine months after the operation were 94% for the VATS group and 95.5% for the conventional thoracotomy group, respectively. It should be noted that both groups had different follow-up periods, which may actually result in a bias in the survival rates that were reported. However, the statistical analysis indicated that there was no significant difference in terms of follow-up period between the two groups ($P=0.764$). With regard the recurrence rate, we observed a rate of 6.5% for the VATS group seen within 12 months of follow-up. This was a recurrence rate that was similar to the 6% rate reported by Sugi (Sugi, 2000), and which was also concordant with the recurrence rate of 7% that we saw following conventional thoracotomy.

In Conclusion: In the past six years, we treated 148 study subjects who presented with early stage NSCLC and who had undergone surgical treatment at our hospital. Sixty-nine subjects had successfully undergone VATS lobectomy surgeries, and two of these subjects were converted to conventional thoracotomy due to intraoperative blood loss and mediastinal lymph node calcification. In addition, 77 subjects underwent conventional thoracotomic lobectomy. Thus, based on the above clinical information, we conducted a retrospective analysis to extensively compare the VATS group and the conventional thoracotomy group with regard to differences in the duration of the operation, intraoperative blood loss, the number of lymph nodes that were removed, the frequency of treatment with postoperative analgesics, the duration of the chest tube indwelling time, duration of hospital stay, recurrence rate, tumor-free survival rate, and survival rate. In summary, we submit that VATS lobectomy is a safe and effective surgical approach, and is superior to conventional thoracotomic lobectomy in the treatment of early stage-specific NSCLC. In addition, VATS lobectomy is characterized by minimal invasiveness which permits a reduced incidence of postoperative complications, and thereby should be regarded as a thorough treatment option for Stage I or II NSCLC. Finally, our study indicated that VATS lobectomy can be used as an alternative surgical treatment for early stage NSCLC.

Acknowledgements

Xinglong Fan carried out the study conception and design. Xinglong Fan and Yuxia Liu was responsible for data collecting and manuscript writing. Xinglong Fan, Yuxia Liu and Hui Tian participated in the technique support and results analysis. Hui Tian conceived of the study and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

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