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Management of intra-operative major bleeding during single-port video-assisted thoracoscopic anatomic resection: two-center experience

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Abstract

Background Our objective is to report on two centers' experience of intra-operative management of major vascular injury during single-port video-assisted thoracoscopic (SPVATS) anatomic resections, including bleeding control techniques, incidence, results, and risk factor analysis.

Methods Consecutive patients ($n=442$) who received SPVATS anatomic lung resections in two centers were enrolled. The different clinical parameters studied included age, previous thoracic surgery, obesity (BMI > 30), tumor location, neoadjuvant therapy, and pleural symphysis. In addition, peri-operative outcomes were compared between the groups, with or without vessel injury.

Results There were no intra-operative deaths in our study. Overall major bleeding incidence was 4.5%, whereby 70% of major bleeding episodes could be managed with SPVATS techniques. In order to determine risk factors possibly related to intra-operative bleeding, we used case control matching to homogenize our study population. After case control matching, pleural symphysis was significantly related in the univariate ($p=0.005$, Odds ratio 4.415, 95% CI 1.424–13.685) and multivariate analysis ($p=0.006$, Odds ratio 4.926, 95% CI 1.577–15.384). Operative time ($p<0.001$), blood loss ($p<0.001$), and post-operative hospital stay ($p=0.012$) were longer in patients with major vascular injury. There were no differences in 30-day mortality and 90-day morbidity.

Conclusions In summary, major intra-operative bleeding episodes during SPVATS anatomic lung resections are acceptable and most such bleeding episodes can be safely managed with SPVATS techniques.

Keywords Single-port VATS · Complications · Bleeding episodes · Risk factor analysis · Management

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Abbreviations

SPVATS	Single-port video-assisted thoracoscopic surgery
PET	Positron emission tomography
CT	Computed tomography
MRI	Magnetic resonance imaging
EBUS-FNA	Endobronchial ultrasound biopsy fine needle aspiration

For most minimally invasive thoracic surgeons, massive intra-operative bleeding is the most dangerous complication to deal with. Despite the potential benefits of minimally invasive surgery, including fewer complications, less pain, shorter post-operative hospital stay, attenuated post-operative inflammatory response, and non-inferior survival outcomes [1–6], minimally invasive surgery is still less common than thoracotomy [7]. Fear of major intra-operative vessel injury could be one reason why the adoption rate of minimally invasive surgery remains limited [7]. Over the last five years, single-port video-assisted thoracoscopic surgery (SPVATS) has been gradually adapted for the treatment of lung and mediastinal disease [8–10]. However, despite numerous publications demonstrating SPVATS as a feasible and safe surgery [10–12], very few articles have specifically addressed how to solve unexpected intra-operative bleeding episodes in SPVATS. Two of these studies were our own, in which we described in detail the techniques for managing unexpected bleeding accidents during the operation [14, 15]. However, intra-operative bleeding episodes are very hard to predict. A cohort study with data from different centers could help to validate a treatment's feasibility and outcomes. Hence, the primary objective of this article is to summarize the experience of two centers in which we manage major vessel injury intra-operatively, during single-port video-assisted thoracoscopic surgery (SPVATS). The second objective is to describe the incidence and outcomes of major vascular injury and analyze the possible risk factors after case control matching of patients in two centers.

Materials and methods

Patients

A retrospective observation cohort study was performed using prospectively established databases from two medical centers. The study was approved by the institutional review board of each institution (IRB No.: 2013/092, 20170805B0). Patients who underwent SPVATS anatomic resection in the Minimally Invasive Thoracic Surgery Unit at Coruña Hospital (Spain) and Chang Gung Memorial Hospital (Taiwan) between January 2014 and February 2017 were all enrolled in this study. Enrolled patients underwent different anatomic resections depending on their clinical factors, such as predicted pulmonary function reserve, tumor size, and tumor location. Segmentectomy was selected for patients who had marginal pulmonary function or tumor size equal to or less than 2 cm. Lobectomy, bilobectomy, or pneumonectomy was chosen based on the tumor location and size and what the patient's clinical condition permitted. Preoperative workup for each enrolled malignancy patient included chest computed tomography, pulmonary function test, and detailed distant metastasis survey, such as bone scan, positron emission tomography (PET), brain computed tomography (CT), or brain magnetic resonance imaging (MRI). The clinical mediastinal node survey was based mainly on the PET finding and selectively performed node staging with mediastinal scope or endobronchial ultrasound biopsy fine needle aspiration (EBUS-FNA) to rule out multiple N2 station metastasis disease. The concept of major bleeding usually varies among surgeons, but here we define a major vascular bleeding event as an injury to a vessel requiring immediate compression due to significant blood loss, unstable vital signs, or bleeding that cannot be managed simply with a sponge within 1–2 min.

Surgical techniques

The surgical approach for SPVATS anatomic resections is the same as we described before [15, 16] for two different centers. From January 2014, one self-taught consultant began to perform SPVATS in CGMH. Due to a lack of relevant SPVATS anatomic resection experience in CGMH, he participated in a SPVATS training course in Shanghai Pulmonary Hospital (SPH, Shanghai). The consultant had previous multiport VATS experience, and gained proficiency by following the steps of SPVATS anatomic resection in gradual increments under Dr. Gonzalez-Rivas's guidance in the SPH training course. The first action when major bleeding occurs is to compress the bleeding area directly with sponge stick, gauze or with the help of lung parenchyma for at least 1–2 min. If the first attempt to compress the bleeding site fails, try to use suction tube to remove the blood clot to get better vision and then compress the bleeding site again. Once the bleeding site has been confirmed, surgeons have many options available today, such as metal clips or polymer vascular lock for segmental vessel laceration or bleeding. The best way to apply these to a bleeding vessel is to use two clips or locks for the proximal end of injured arteries while the distal end of the vessel can be ligated with an energy device, another clip or lock. During the placement of clips or locks, surgeons should be extremely cautious because misplacement of clips or locks could cause more damage to the pulmonary artery. A curved or flexible lock applier might be helpful for placing locks safely at the base of an artery. In addition, topic coagulants, such as FloSeal®, Tissel®, TachoSil®, Hemopatch®, and so on, can be used for minor vessel oozing or bleeding problems. Their efficacy depends on the choice of the product for each indication and their correct application. Beyond the above-mentioned options, performing a direct suture repair on the pulmonary artery presents a risky and challenging possibility for any surgeon and should only be done once preparation for conversion to thoracotomy is complete. Before execution, surgeons should try to use the suction tip to compress the proximal end of the injured vessel and suck away surrounding blood clots to make the operation field clearer. If this maneuver fails, proximal artery base control should be considered first to reduce afferent vascular flow. This allows better assessment of the defect and precise and safe suture stitching. Given the fragility of the pulmonary artery, caution cannot be overemphasized with regard to the traction of the sutures, especially when surgeons try tying the knot over the pulmonary artery defect (Fig. 1A). In the event of situations such as a huge tumor surrounding the inter-lobar area, or dense adhesion over the hilar area, a precautionary procedure such as main pulmonary artery sling with vessel loop to prepare for possible accidents, might be useful to prevent catastrophic bleeding (Fig. 1B). Left main pulmonary artery control is easier than right side control because the left main pulmonary artery is smaller and does not branch off extrapericardially as early as the right main pulmonary artery. Sometimes, intrapericardial artery control is needed. However, it is not recommended to perform this procedure on every patient since it may increase the probability of uncontrollable vascular injury.

Statistics

Clinical records of each patient were reviewed for demographic and clinical data including age, gender, body mass index (BMI), peri-operative parameters, and post-operative outcomes. All collected clinico-pathologic factors were analyzed by SPSS version 19.0 (SPSS Inc, Chicago, IL, USA). Continuous variables were expressed as mean ± standard deviation. Statistical analyses were performed using the independent *t* test for continuous variables and Pearson Chi-square test for categorical variables. A probability value < 0.05 was considered statistically significant. In order to mitigate demographic bias between the two centers, patients' characteristics (gender, smoking status, disease diagnosis, acute coronary syndrome history, forced expiratory volume in 1 s (FEV1), and neoadjuvant chemotherapy (yes/no)) were used for case control matching.

Results

Between January 2014 and February 2017, 442 consecutive operations were scheduled for SPVATS anatomic resections by the Minimally Invasive Thoracic Surgery Unit at Coruña Hospital (Spain) and Chang Gung Memorial Hospital (Taiwan). Among these scheduled SPVATS anatomic resections, there were 356 lobectomy cases, 49 segmentectomy, 21 bilobectomy, and 16 pneumonectomy cases. Of the 442 patients, 364 patients (82.4%) had primary lung cancer, 45 (10.2%) had secondary lung cancer, and 33 (7.4%) were diagnosed with non-malignant lesion. The clinical and demographic characteristics of the patients before and after case control matching are presented in Table 1. Twenty intra-operative bleeding episodes were recorded. One episode occurred during segmentectomy, seventeen occurred during lobectomy; one occurred during bilobectomy, and one episode occurred during pneumonectomy. Four cases were converted to thoracotomy due to uncontrolled bleeding and two cases were shifted to two-port VATS to manage intra-operative bleeding. In our series, the overall thoracotomy conversion rate was 3.8% (17/442). 4.5% of patients (20/442) had unexpected major vascular bleeding events, while 0.9% (4/442) had bleeding-related thoracotomy conversion in our retrospective two-center cohort study. The intra-operative death rate was zero. The detailed mechanism and repair outcomes of major bleeding episodes during SPVATS anatomic resection are shown in Table 2.

After case control matching, 364 patients (327 from CHUAC, 37 from CGMH) were enrolled for risk factor analysis of intra-operative vascular injury. Eighteen

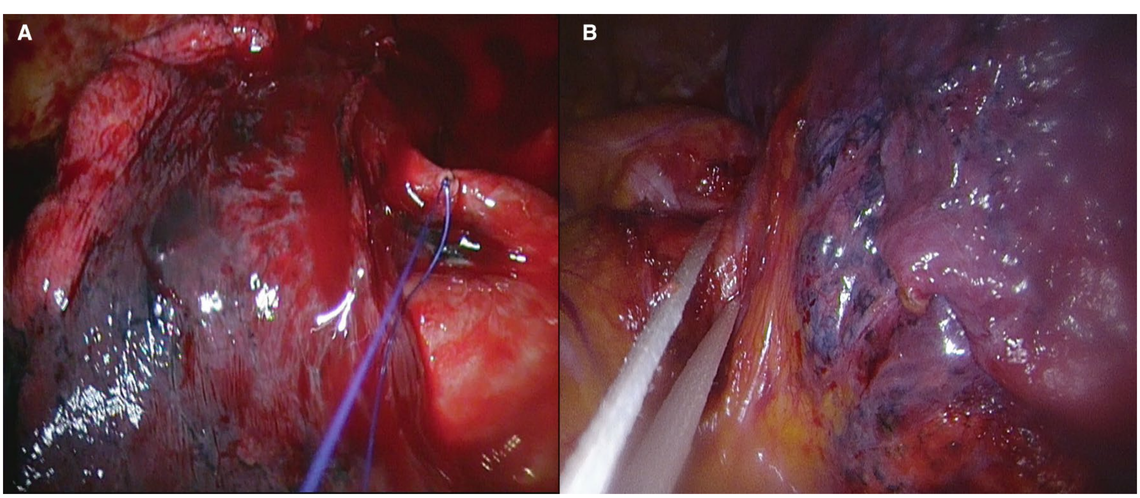

Fig. 1 Advanced bleeding control strategies: **A** repair vascular injury area with suture, **B** Left side proximal main pulmonary artery control

Table 1 Clinical and demographic characteristics of the patients before and after case control matching

Variable	CHUAC ($n=327$)	CGMH ($n=115$)	Total ($n=442$)	p value
Age	65.01 ± 11.54	60.89 ± 10.56	63.94 ± 25.90	0.001
Gender				< 0.001
Male	247	59	306	
Female	80	56	136	
Smoking status				< 0.001
No	80	79	159	
Former	124	16	140	
Current	123	20	143	
ACS history				0.088
Yes	49	10	59	
No	278	105	383	
COPD				0.189
Yes	47	11	58	
No	280	104	384	
Additional primary malignancy				0.891
Yes	86	31	117	
No	241	84	335	
Body mass index	26.16 ± 3.99	23.71 ± 2.67	25.50 ± 3.84	< 0.001
ECOG				0.244
0	196	76	272	
1	131	39	170	
FEV1	2.33 ± 0.65	2.19 ± 0.59	2.30 ± 0.63	0.043
Neoadjuvant chemotherapy				0.014
Yes	49	7	56	
No	278	108	386	
Diagnosis				< 0.001
Primary malignancy	283	81	364	
Secondary malignancy	31	14	45	
Benign	13	20	33	
Tumor location				0.899
RUL	113	35	148	
RML	23	8	31	
RLL	64	27	91	
LUL	77	27	104	
LLL	50	18	68	
Tumor sizes (cm)	3.15 ± 1.78	2.56 ± 1.36	2.99 ± 1.7	0.002
Major bleeding episodes				0.250
Yes	17	3	20	
No	310	112	422	
Operation time (min)	206.36 ± 67.75	197.29 ± 61.24	204 ± 66.17	0.206
Drainage duration (day)	3.96 ± 3.04	3.96 ± 2.70	3.96 ± 2.95	0.990
Post-operative hospital stay (day)	5.34 ± 3.86	5.12 ± 3.22	5.28 ± 3.7	0.582
Variable	CHUAC ($n=327$)	CGMH ($n=37$)	Total ($n=364$)	p value
Post case matching				
Age	65.01 ± 11.54	60.49 ± 7.87	64.55 ± 11.30	0.021
Gender				0.291
Male	247	25	272	
Female	80	12	92	

Table 1 (continued)

Variable	CHUAC ($n=327$)	CGMH ($n=37$)	Total ($n=364$)	p value
Smoking status				0.10
No	80	10	90	
Former	124	15	139	
Current	123	12	135	
ACS history				0.495
Yes	49	4	53	
No	278	33	311	
COPD				0.294
Yes	47	3	50	
No	280	24	304	
Additional primary malignancy				0.891
Yes	86	31	117	
No	241	84	325	
Body mass index	26.16 ± 3.99	24.38 ± 2.59		0.010
ECOG score				0.793
0	196	23	219	
1	131	14	145	
FEV1	2.33 ± 0.65	2.30 ± 0.61		0.807
Neoadjuvant chemotherapy				0.112
Yes	49	2	51	
No	278	35	313	
Diagnosis				0.904
Primary malignancy	283	32	315	
Secondary malignancy	31	4	35	
Benign	13	1	14	
Tumor location				0.942
RUL	113	12	125	
RML	23	2	25	
RLL	64	7	71	
LUL	77	5	82	
LLL	50	11	61	
Tumor size (cm)	3.15 ± 1.78	2.24 ± 1.22	3.05 ± 1.75	0.004
Major bleeding episodes				
Yes	17	1	18	
No	310	36	346	0.507
Operation time	206.36 ± 67.75	193.03 ± 55.04	205 ± 66.63	0.250
Drainage duration (days)	3.96 ± 3.04	3.54 ± 1.38	3.92 ± 2.91	0.514
Post-operative hospital stay (days)	5.34 ± 3.86	4.48 ± 1.48	5.25 ± 3.70	0.183

ACS acute coronary syndrome, *COPD* chronic obstructive pulmonary disease, *ECOG* eastern cooperative oncology group, *FEV1* forced expiratory volume in 1 s, *RUL* right upper lobe, *RML* right middle lobe, *RLL* right lower lobe, *LUL* left upper lobe, *LLL* left lower lobe

episodes occurred in the case matched cohort, with pleural symphysis significantly related in the univariate ($p = 0.005$, Odds ratio 4.415, 95% CI 1.424–13.685) and multivariate analysis ($p = 0.006$, Odds ratio 4.926, 95% CI 1.577–15.384) (Table 3). In addition, we analyzed the peri-operative parameters of patients with or without major vascular injury. Patients without major bleeding episode had significantly less intra-operative blood loss, a shorter operative time, and a shorter post-operative hospital stay. Detailed peri-operative data are given in Table 4.

Table 2 Major bleeding episodes during single-port video-assisted thoracoscopic anatomic resection ($n = 20$)

Case	Vessel injured	Mechanism of injury	Techniques of repair	Conversion to thoracotomy	Conversion to two ports	Blood loss (ml)	Blood trans-fusion
1	Right middle lobe artery	Dissection of PA	Hemolock proximally	No	No	220	N
2	Posterior ascending artery	Dissection of PA	Hemolock proximally	No	No	220	N
3	Posterior ascending artery	Open incomplete fissure	Hemolock proximally	No	No	530	Y
4	Interlobar trunk	Dissection of PA	PA loop proximally loop, then staple proximally	No	Yes	550	Y
5	Anterior trunk	Stapling of PA	Oversewn	Yes	No	1000	Y
6	Anterior trunk	Stapling of PA	Oversewn	Yes	No	1500	Y
7	Anterior trunk	Dissection of PA	PA loop proximally, then staple proximally	No	No	650	Y
8	Anterior trunk	Staple line hematoma	Compression	No	No	150	N
9	Interlobar trunk	Dissection of PA	PA loop proximally, then staple proximally	No	Yes	650	Y
10	Left upper lobe vein	Staple line disruption	Oversewn	Yes	No	600	Y
11	Anteromedial basal artery	Dissection of PA	PA loop proximally, then staple proximally	No	No	550	Y
12	Anterior trunk	Thermal injury	PA loop proximally, then staple	No	No	210	N
13	Anterior trunk	Dissection of PA	Oversewn	Yes	No	630	Y
14	Interlobar trunk	Dissection of interlobar lymph node	Compression, then hemopatch	No	No	150	N
15	Azygos vein	Thermal injury	Hemolock proximally	No	No	650	Y
16	Right middle lobe vein	Hemolock dislodge	Relock with hemolock	No	No	530	Y
17	Right lower lobe vein	Thermal injury	Staple proximally	No	No	170	N
18	Lingular artery	Hemolock crushed by endostaple	Hemoclip proximally	No	No	250	N
19	Posterior ascending artery	Open incomplete fissure	Compression, then hemo-clips proximally	No	No	250	N
20	Superior segmentectal A	Open incomplete fissure	Oversewn	No	No	670	Y

PA pulmonary artery

Table 3 Univariate and multivariate analyses of intra-operative bleeding risk factor after case matching

Risk factors	Odds ratio	95% CI	*p* value
Elderly patients (age > 65)	1.113	0.429–2.889	0.825
Previous thoracic surgery	1.138	0.143–9.066	0.902
Obesity (BMI > 30)	1.338	0.372–4.805	0.654
Tumor size(> 3 cm)	1.009	0.389–2.619	0.985
Tumor location (upper lobe/non-upper lobe)	1.898	0.662–5.441	0.226
Neoadjuvant therapy	0.351	0.046–2.676	0.289
Pleural symphysis	4.415	1.424–13.685	0.005
Risk factors	**Hazard ratio**	**95% CI**	***p* value**
Tumor location	1.927	0.660–5.618	0.229
Neoadjuvant therapy	0.214	0.027–1.686	0.144
Pleural symphysis	4.926	1.577–15.384	0.006

BMI body mass index

Table 4 Comparison of peri-operative outcomes of patients with/without vessel injury post case control matching ($n = 364$)

Peri-operative parameters	Vessel injury ($n = 18$)	No vessel injury ($n = 346$)	p value
Operative time (min)	293.55 ± 78.01	200.40 ± 62.78	< 0.001
Tumor size (cm)	3.03 ± 2.01	3.06 ± 1.74	0.948
Intra-operative blood loss (ml)	535.00 ± 338.92	47.85 ± 29.92	< 0.001
Drainage day (day)	4.72 ± 2.60	3.88 ± 2.93	0.202
Post-operative hospital stay (day)	7.39 ± 5.12	5.14 ± 3.59	0.012
30-day mortality rate	0%	0.8%	0.692
90-day morbidity rate	11.1%	24.3%	0.2

Discussion

Surgeons preparing for minimally invasive surgery, especially for SPVATS, and explaining the risk of operations in the outpatient clinic, are often asked by patients whether the operation is safe when done by only one incision wound. Similarly, other surgeons who are beginning with SPVATS ask how to manage unexpected bleeding accidents during surgery and whether these bleeding episodes can be dealt with by SPVATS technique. To address these questions, we have shared our experiences in a previous article [13]. However, lack of clinical data to support the feasibility is a weakness of the previous article. Hence, in this report, we gathered experiences from two different medical centers to see whether this kind of endoscopic surgery is safe, feasible and as universal an experience as we described before. Our cohort showed that the overall incidence of major vascular injury during elective SPVATS anatomic resection was 4.5%, similar to robotically assisted thoracoscopic surgery (RATS) and multiport video-assisted thoracoscopic surgery (VATS) [17–21]. Among 20 cases of major intra-operative bleeding, anterior trunk injury was the leading cause of bleeding (6/20). All six patients were previous smokers or are active smokers and pleural symphysis was encountered during the operation. Three patients had tough anthracotic perivascular lymph nodes in their operation record, which might have increased the difficulty of isolating the anterior trunk, resulting in vessel injury. In addition, we focused on the bleeding-related conversion rate in our cohort study. Strictly speaking, only 4 cases needed conversion to thoracotomy, 2 cases needed shifting to two-port VATS, while 14 major bleeding episodes could be managed with SPVATS techniques. Therefore, our experience has affirmed that SPVATS anatomic pulmonary resection is sufficiently safe and feasible. Even though vessel injury did occur during the operations, most bleeding accidents could be treated speedily and adequately with an expert hand. As expected, blood loss and operative time were significantly greater in the group with vascular injury than in that without injury (Table 4). Although post-operative hospital stay was longer in the vascular injury group, there were no differences in 30-day mortality and 90-day morbidity between the two groups. Our management did not appear to compromise short term outcomes for vascular injury patients. Long-term follow-up was warranted.

With the accumulation of surgical experience, the rate of operation-related complications generally decreases. However, the incidence of intra-operative vascular injury may be an exception to this rule, since, with increasing experience, surgeons perform more complex cases, involving more risk of intra-operative bleeding. Our results indicate that the occurrence of vascular injury is inevitable, but a well-trained surgical team can treat it appropriately. The decision regarding repair by single-port technique, conversion to multiport VATS or thoracotomy depends on many factors: the severity of vascular injury, the chief surgeon's experience, the first assistant's experience, the anesthesiologist and nursing staff's cooperation and so on. Obviously, such parameters are very difficult to analyze in a retrospective study. However, one guiding strategy overrides: keeping calm and compressing the bleeding site directly, then calling for help from experienced surgeons, or converting to thoracotomy directly if the bleeding accident might threaten the patient's life. Usually, if the patient's vital signs permit, we try to find out where the bleeding point is, and use the technique mentioned above to control intra-operation bleeding (Supplementary Material Video 1, Video 2 and Fig. 2). Furthermore, it is very safe and easy for a SPVATS surgeon to perform an anterior thoracotomy for vascular bleeding control if the bleeding site is compressed adequately. In our limited data analysis, pleural symphysis was significantly associated with intra-operative vascular injury. It may further be associated with an ambiguous field of operation, or easy oozing, and erosion of the surgeon's patience, etc. When encountering severe adhesion, surgeons should be attentive and maintain patience. However, pleural symphysis remains a vague risk factor. The extent of pleural symphysis associated with intra-operative bleeding needs to be further explored in the future.

Surprisingly, we found neoadjuvant therapy showed no positive association with intra-operative bleeding episodes in univariate and multivariate analysis. Augustin et al. found that induction therapy was an independent risk factor for conversion to thoracotomy in VATS lobectomy [22]. In general, neoadjuvant therapy tends to cause perihilar fibrosis

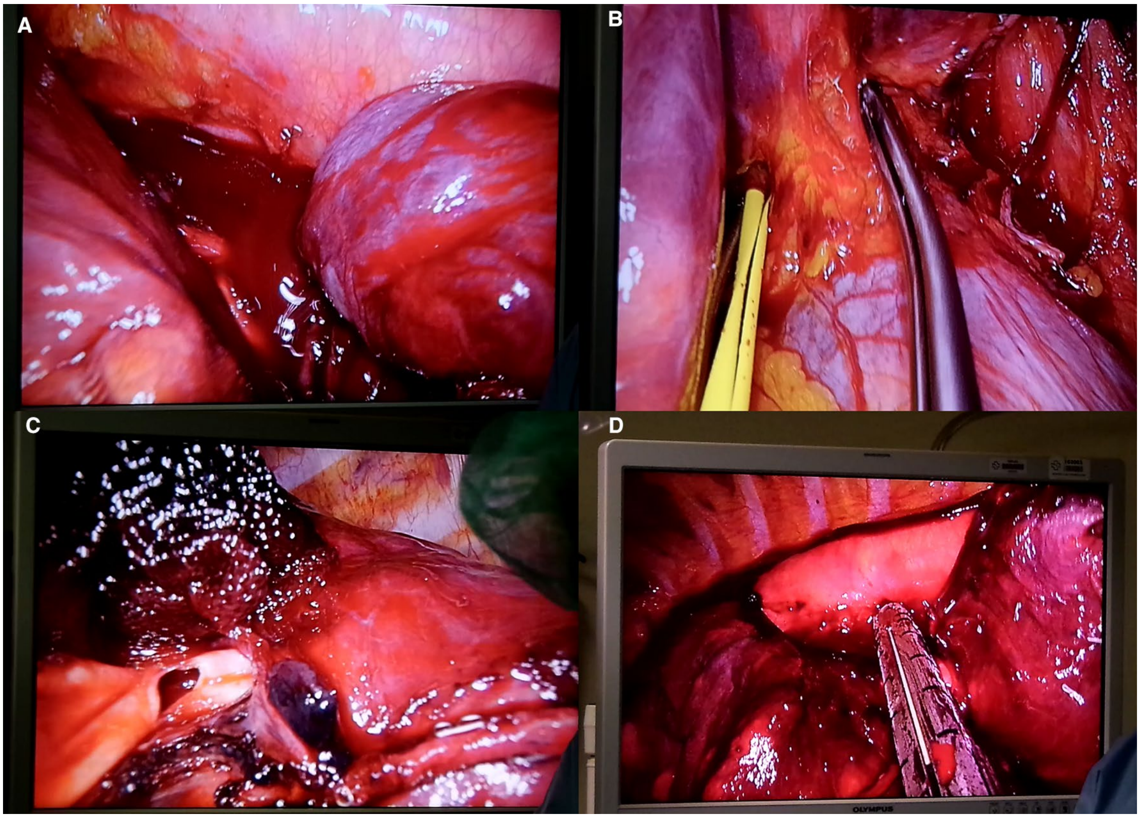

Fig. 2 Management of left side anteromedial basal artery injury. **A** Appropriate compression of the bleeding site. **B** Use vascular loop (yellow strip) for left side pulmonary artery root control to reduce inflow and resect pulmonary vein to reduce backflow. **C** Examine the extent of vessel injury. **D** Resect the pulmonary artery more proximally. (Color figure online)

and anatomic distortion. The dissection planes surrounding vessels are often thicker and more adherent. In fact, the operation time was significantly different when comparing patients with or without neoadjuvant therapy in our series (237.75 ± 83.88 min vs. 199.10 ± 61.81 min, respectively, $p < 0.001$). However, SPVATS did indeed provide a direct and clear view to the hilar structure, which might help surgeons to dissect the hilar structure safely during a challenging operation.

For any kind of research, the key element and limitation are methodology. First, limited by the unpredictability and scarcity of major intra-operative bleeding, a retrospective observation study in different institutes is but one of the numerous research options, and could compensate for its shortcomings in terms of finite numbers of enrolled patients by lending credibility and validation to a specific treatment, but is likely to be associated with selection bias and inconsistent methodology. For example, the incidence of intra-operative bleeding episodes might be underestimated as a result of our definition of a major vascular bleeding event as an injury to a vessel, requiring immediate compression because of significant blood loss, unstable vital signs, or bleeding that cannot simply be managed with gauze packing within 1 min. Second, all of the SPVATS anatomic resections were performed by surgeons with abundant VATS experience, but there might be minor differences in surgical techniques and varying SPVATS experience among surgeons, which might have resulted in different strategies for dealing with intra-operative bleeding. Martin et al. found that the conversion rate of SPVATS lobectomy differed among surgeons with varied previous VATS experience [23]. However, our cohort lacked a complete record of each surgeon's VATS experience. Third, although we found neoadjuvant therapy did not associate with intra-operative bleeding in our series, we lacked sufficient data to determine the optimal timing between neoadjuvant therapy and surgery. It is a topic worth thinking about deeply. Further evaluation is warranted. Fourth, we acknowledge a further limitation of this study lies in different diagnosis and different operative approaches, which inevitably led to bias in our results. Nevertheless, despite having such challenging cases in our cohort, our conversion rate (4.5%) was consistent with Hsu (2.5%) [12] and Xie's study (4.6%) [24] and better than Tam's cohort (15.8%) [25]. Fifth, although all operations were conducted by different surgeons, the principles for management of intra-operative bleeding were always the same, confirming that the strategy is not limited to a single surgeon or dependent on personal technique, but is a useful, feasible and universal approach for the management of intra-operative bleeding in SPVATS anatomic resections.

Conclusion

In summary, the retrospective observation cohort study revealed that the strategies for major intra-operative bleeding control in SPVATS anatomic resection are effective, practical and not limited to personal experience if surgeons keep calm during the bleeding accident and have well-rehearsed SPVATS techniques.

Author contributions Study conception and design: CFW, DG-R, TM, RF, CYW. Acquisition of data: CFW, TM, RF, MD, EF, CYW, MJH, MP, YHL, YKC, DG-R. Analysis and interpretation of data: CFW, MD, CYW. Drafting of manuscript: CFW, TM, RF, DG-R. Critical revision: CFW, TM, DG-R.

Compliance with ethical standards

Disclosures Ching Feng Wu, de la Torre Mercedes, Ricardo Fernandez, Maria Delgado, Eva Fieira, Ching Yang Wu, Ming Ju Hsieh, Marina Paradela, Yun Hen Liu, Yin Kai Chao, and Diego Gonzalez-Rivas have no conflicts of interest or financial ties to disclose.

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