Optimizing endoscopic therapy for early Barrett's neoplasia

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CHAPTER 4

THORACOLAPAROSCOPY DISSECTION OF ESOPHAGEAL LYMPH NODES WITHOUT ESOPHAGECTOMY IS FEASIBLE IN HUMAN CADAVERS AND SAFE IN A PORCINE SURVIVAL STUDY


ABSTRACT

Background

High-risk early esophageal adenocarcinoma (i.e. submucosal invasion >500 nanometers, poor differentiation and/or presence of lymphovascular invasion) is currently treated with esophagectomy with lymph node (LN) dissection given the high rates of LN metastasis. However, esophagectomy is associated with substantial morbidity and mortality. Endoscopic radical resection followed by thoracolaparoscopic LN dissection without concomitant esophagectomy could be an alternative. The study aim was to evaluate the feasibility and safety of thoracolaparoscopic dissection of esophageal LNs in a preclinical setting.

Methods

(1) In human cadavers, thoracolaparoscopic dissection of LNs involved in drainage of the esophagus was performed. Subsequently, esophagectomy was performed to be able to detect retained LNs. Outcome parameters included the number of dissected LNs, the number of retained LNs in the esophagectomy specimen (ES), and technical success. (2) In swine, thoracolaparoscopic LN dissection was also performed. After the procedure, the swine survived for 28 days. Thereafter, the swine were sacrificed and esophagectomy was performed. Outcome parameters included the presence of ischemia and/or stenosis in the ES and other complications.

Results

(1) In five human cadavers, a median of 26 LNs (interquartile range 22-46) were dissected. In two ES, one retained LN was found: one high paraesophageal, one low paraesophageal. Technical success rate was 100%. (2) None of the seven porcine ES showed signs of ischemia or stenosis. One swine died because of ventricular fibrillation during surgery; during follow-up no complications were observed.

Conclusions

Thoracolaparoscopic dissection of LNs involved in the drainage of the esophagus is feasible in human cadavers and swine. The porcine survival study suggests that the esophageal vascularity is not severely compromised by the procedure. As anatomy differs between swine and humans, safety of the procedure will have to be investigated thoroughly before applying this new technique as the treatment of choice.
INTRODUCTION

The incidence of esophageal adenocarcinoma (EAC) is increasing in the West [23]. Early EAC can be treated with endoscopic resection techniques, such as endoscopic mucosal resection (EMR) or endoscopic submucosal dissection (ESD) [21, 24]. Both techniques are associated with high curative resection rates and acceptable complication rates [25, 26].

Early EAC can be divided into low-risk and high-risk lesions, based on the histological features of the resection specimen. In low-risk early EAC (i.e. mucosal tumor or submucosal tumor with infiltration less than 500 nanometers, negative resection margins, not poorly differentiated, and absence of lymphovascular invasion), an endoscopic resection is considered to be a curative treatment, since in these lesions spread of tumor cells to the adjacent lymph nodes (LNs) is rare (< 2%) [1, 2, 10]. In high-risk EAC (i.e. >500 nm submucosal invasion, and/or poor differentiation grade, and/or lymphovascular invasion) the risk of concomitant LN metastases is considered to be too high and surgical esophagectomy is indicated in case of acceptable clinical condition [5, 6, 8]. In case of an irradical endoscopic resection (tumor-positive vertical resection margin), an esophagectomy is required to excise residual cancer. In case of a radical endoscopic resection, but a high-risk EAC, the additional yield of subsequent esophagectomy is in the LN dissection, since the primary tumor has been radically resected by endoscopic means.

Surgical esophagectomy however, is a major surgical procedure with substantial morbidity, mortality and associated with an impaired quality of life (QoL) [21, 27–30]. Surgical morbidity is mainly caused by anastomotic leakage and ischemia of the gastric conduit. Moreover, in majority of patients with early EAC who have undergone esophagectomy, no tumor-positive LNs are found [1, 31].

We therefore hypothesized that an algorithm consisting of endoscopic radical (R0) resection of the tumor, followed by thoracolaparoscopic dissection of LNs involved in the drainage of the esophagus, could be an alternative for patients with high-risk early EAC. This treatment algorithm would allow preservation of the structural and functional integrity of the esophagus, and may reduce surgical morbidity and maintain QoL.

In this preclinical study, we evaluated the feasibility and safety of thoracolaparoscopic dissection of LNs involved in the drainage of the esophagus in human cadavers (phase I) and in a porcine survival study (phase II). The human cadaver study was conducted to assess the feasibility, whereas the porcine survival study mainly investigated the safety aspects (i.e. occurrence of esophageal stenosis or ischemia) of the procedure.
METHODS

Phase I: Human cadavers

In fresh human cadavers, a surgical dissection of all LNs involved in the drainage of the esophagus was performed by means of combined thoracoscopy and laparoscopy. All individuals provided written consent during their life that their body could be used for scientific purposes after death. Directly after death the cadavers were deeply frozen and defrosted shortly before the experimental procedure.

The thoracolaparoscopic LN dissection was performed by three surgeons, all experts in the field of minimally invasive upper gastrointestinal (GI) surgery (M.B., S.G. and M.W.). The cadaver was placed on the operation table in prone position. Right thoracoscopy was carried out by the insertion of four trocars along the medial and caudal edge of the right scapula (Figure 1a). The first trocar was placed at the lower edge of the scapula with direct vision. To create optimal visualisation, the thoracic cavity was insufflated with carbon dioxide (8-15mm Hg). Subsequently, in this order, the subcarinal, high paraesophageal (station 2 and 4, left and right), aortopulmonary (AP) window (station 5) and low paraesophageal (station 8 and 9) LNs were dissected. To reach the AP window and the left lymph node station 2 and 4, we first mobilized the esophagus from the main bronchi and carina. The esophagus is completely encircled at the level of the AP window and aortic arch. Subsequently, the esophagus is retracted from the trachea, and the AP window can be reached both from behind the esophagus (esophagus retracted to the right) as between the esophagus and the trachea (esophagus elevated to dorsal). For the left lymph node station 2 and 4, the esophagus is retracted to the right side of the patient. During the removal of the LNs, the vascularization of the esophagus was carefully preserved. After removal of all LNs, the thoracic cavity was inspected for the presence of retained LNs and thereafter, all trocars were removed and wounds were closed. The cadaver was turned to the supine and anti-Trendelenburg position. Four trocars were inserted (Figure 1b) and carbon dioxide (15 mm Hg) was insufflated to create optimal sight. The LNs around the celiac trunk (station 20), left gastric artery (station 17), common hepatic artery (station 18) and splenic artery (station 19) were laparoscopically dissected. In this area, the gastric vascularization was also carefully preserved and the left gastric artery was not ligated. It was attempted to spare most branches of the vagal nerve, while dissecting the left gastric artery LNs. Therefore, during dissection of these LNs, the gastric wall of the lesser curvature was not completely exposed. However, the area was carefully checked for the presence of residual LNs following dissection. After harvesting all targeted LNs, open esophagectomy was performed. The various LN stations and the esophagectomy specimen were send to the pathologist for histopathological evaluation. The number of LNs per LN station were counted and the esophagectomy specimen was investigated for the presence of retained LNs.
Phase II: Animal study

After finishing the first phase of this study, we investigated safety of the procedure in a porcine survival study. The study was performed at the Animal Research Institute AMC, Amsterdam, The Netherlands, after protocol approval by the Animal Experiment Committee. Animal care was in accordance with European Union guidelines.

Eight female swine (Topigs, Van Beek SPF Swine Breeding BV, Lelystad, The Netherlands) were included. All swine entered the animal facilities thirteen days prior to start of the surgical procedure for acclimatizing purposes. They were put on a grid one day before surgery and did not receive any food the day before and the night before surgery to ensure emptying of the upper GI tract and to prevent aspiration during intubation and surgery.

The same surgeons, mentioned earlier, performed thoracolaparoscopic LN dissection. The procedure was carried out in the same way as we did in the human cadavers, starting with dissection of the thoracic LNs, followed by the abdominal LNs. After placement of the trocars, carbon dioxide was insufflated to obtain visualisation of the esophagus (Figure 2a). After removal of all targeted LNs (stations 2,4,5,7,8 and 9), the thorax was drained and the trocar sites were stitched intracutaneously. Thereafter, the swine was turned into supine and anti-Trendelenburg position. Abdominal trocars were placed under sight (Figure 1b) and carbon dioxide was insufflated (12 mm Hg). Subsequently, all visible LNs around the left gastric, common hepatic, splenic artery, and the celiac trunk were laparoscopically removed (stations 17-20). During the procedure, esophageal and gastric vascularization was preserved carefully (preservation of the left gastric artery). Wounds were stitched intracutaneously. To minimize postoperative discomfort, all swine received buprenorphine (0.02mg/kg intramuscular) 30 minutes before the end of the procedure.

Follow-up swine

After the procedure, the swine were detubated and placed on a grid for the following 3 days to prevent sawdust infecting the operation wounds. They all received analgetics...
(buprenorphine 0.02mg/kg and metacam 0.4mg/kg intramuscular daily) up to 3 days after the operation. All swine were checked daily for the presence of food-related problems such as vomiting, diminished food intake, failure to thrive, or other problems such as infection of the operation wounds or decreased mobility. Twenty-eight days after surgery, the swine were euthanized with an intravenous overdose of pentobarbital (>100mg/kg). The esophagus and cardia of all swine were subsequently harvested for histopathological evaluation.

Pathology
Two pathologists, both experts in the field of GI pathology (C.S. and K.K.), examined all specimens. The specimens obtained during surgery and those obtained after follow-up (LNs and esophagectomy specimen) were fixed overnight in buffered formalin 3.6% directly after dissection.

LNs were dissected manually, embedded on paraffin and subsequently sectioned and stained with hematoxylin and eosin. No fat clearing techniques were used. All LNs were counted and recorded according to LN station.

The esophagectomy specimens were investigated for the presence of retained LNs. All retained LNs were counted and the location on the esophagectomy specimen was recorded. Furthermore, the porcine esophagectomy specimens were examined macroscopically to rule out the presence of stenosis. Thereafter, the specimens were investigated microscopically for the presence of ischemia.

Outcome parameters
Phase I: Human cadaver study
1. The number of LNs obtained by thoracolaparoscopic lymph node dissection, subdivided per LN station.
2. The number of retained LNs after thoracolaparoscopic LN dissection (as assessed by counting the number of retained LN in the various stations in the esophagectomy specimen).
3. Technical success, defined as the ratio of dissected LNs and the total number of LNs (dissected LNs plus retained LNs in esophagectomy specimen) ≥ 0.9 (90%).

Phase II: Swine study
Outcome parameters in the phase II study were:
1. Presence of ischemia or stenosis in the esophagectomy specimen after 28-day survival
2. Complications, intraoperative and during follow-up.

Since anatomy (and thus location of the various LN stations) is different in swine, the number of dissected LN was not an outcome parameter in the porcine survival study.
Statistical Analysis
The results are presented as the mean ± standard deviation (SD) for normally distributed data. The median and range were calculated for data with a skewed distribution. Data analysis was performed using SPSS statistical software package (version 19, SPSS Inc., Chicago, USA).

RESULTS
Phase I: human cadaver study, number of dissected LN
In five human cadavers (three male, median age 83 years) thoracolaparoscopic LN dissection was performed. Because of the frosting (and defrosting) of the cadavers, tissue characteristics had changed: colors of the various tissues had changed and some tissues were more stiff than usual (in living humans). In total, 13 LN stations were assessed and a median of 26 LNs (interquartile range 22-46) were dissected. In one cadaver, 62 LNs were removed, which is more than in the other cadavers. Median procedure time was 165 minutes (range 80-225). The first procedure took 225 minutes, the last one only 80 minutes.

Number of retained LN
In two out of five cadavers, one retained LN was found in the esophagectomy specimen: one located high paraesophageal (at the level of station 2) and one low paraesophageal (at the level of station 9). An overview of the dissected (and retained) LNs is shown in Table 1. All procedures were considered technically successful, as determined by the definition that at least 90% of the targeted LNs needs to be dissected.

Phase II: porcine survival study
In eight female swine, thoracolaparoscopic LN dissection was performed. Median procedure time was 190 minutes (range 150-265); the first procedure lasted 245 minutes, the last procedure 150 minutes.

The first swine that was operated, died during the procedure because of ventricular fibrillation (VF). Resuscitation was performed but was not successful. To prevent other swine from acquiring VF as well, metoprolol (3mg, intravenously) was administered during the procedure if heart rate exceeded 90 beats per minute (every 15 minutes, maximum of 3 gifts). None of the other swine experienced any heart arrhythmias during the procedure.

Other complications included accidental common bile duct injury, treated by stitching of the defect (n=1), and injury of the spleen, eventually leading to laparoscopic splenectomy and additional antibiotics postoperatively (n=1). Blood loss during the procedures was minimal, apart from the swine in whom a splenic injury caused an estimated blood loss of 1000 cc. All perioperative adverse events could be managed laparoscopically, there were no conversions to open surgery. No severe vomiting was observed during follow-up: all swine gained weight as expected.
Swine, presence ischemia or stenosis
The esophagectomy specimens showed no signs of stenosis or ischemia neither macroscopically nor microscopically (Figure 2 and 3, respectively).

**DISCUSSION**
This study shows that thoracolaparoscopic dissection of LNs involved in the drainage of the esophagus is feasible in fresh human cadavers. The porcine survival study shows that esophageal vascularization seems not compromised by the procedure: no ischemia or stenosis was observed in the porcine esophagectomy specimen. In human cadavers, a median of 26 LNs was dissected and the procedure was technical successful in all cases. It is of major importance to remove sufficient LNs during lymphadenectomy, because the number of removed LNs is an independent predictor for survival after esophagectomy[32]. This may also account for the thoracolaparoscopic LN dissection without concomitant esophagectomy after an endoscopic R0-resection of the tumor. The median number of resected LNs in our human cadavers is in line with that of esophagectomy series in the literature: Biere et al. and Luketich et al. reported similar rates of a median of 20 and 21 LNs, respectively, which were removed during minimally invasive esophagectomy[33,34].

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**Table 1. Results human cadavers**

<table>
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<td>6</td>
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<td>0</td>
<td>0</td>
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a= lymph node, b = paraesophageal, c = station 2, 4, 5, d = station 7, e = station 8, 9

Swine, presence ischemia or stenosis
The esophagectomy specimens showed no signs of stenosis or ischemia neither macroscopically nor microscopically (Figure 2 and 3, respectively).
Figure 2. Macroscopic view porcine esophagectomy specimen.

Thoracoscopy: patient in prone position. Right side = cranial (head of the patient). The continuous line in the represents the right scapula; the crosses depict the position of the trocars. Laparoscopy: patient in supine position.

Figure 3. Microscopic view porcine esophagectomy specimen.

Macroscopic view porcine esophagus in total (a: left picture) and longitudinally cut (b: same specimen, right picture).
Abe et al. showed that laparoscopic LN dissection is effective and safe in patients with high-risk early gastric cancer. They reported data of 21 patients with high-risk early gastric cancer, who were treated with an ESD (R0-resection), followed by laparoscopic LN dissection with preservation of the stomach. Of the 21 patients, laparoscopic lymphadenectomy revealed LN metastases in 2 patients. The need for additional surgery was pointed out, but patients chose to not undergo those procedures due to the invasive character. During a median follow-up of 61 months (including a follow-up of 76 and 84 months in the two LN positive patients), no recurrent malignant disease was seen[35].

Until now, little is known about the feasibility and safety of thoracolaparoscopic lymphadenectomy in early esophageal cancer. Results of the study of Abe et al. are not transferable to patients with esophageal cancer, as the targeted lymph node stations and moreover, vascularization of the esophagus, are different. The most fearful complication of scopic lymphadenectomy is esophageal or gastric ischemia, which might develop if too many vessels supplying the esophagus are damaged. Therefore, we also investigated the safety of this procedure in phase II, the porcine survival study.

Phase II of this study shows that esophageal vascularization is not severely compromised by the thoracolaparoscopic LN dissection. We observed no ischemia or stenosis in the porcine esophagectomy specimens. Abe et al. reported one patient who developed an ischemic gastritis on post-operative day 1 and this patient directly underwent a distal gastrectomy. In the remaining 20 patients, no procedure-related complications were observed[35].

Complications, other than stenosis or ischemia, occurred in 3 out of 8 pigs in our study, which is substantial. Those complications (i.e. VF, splenic injury, common bile duct injury) however, are observed rarely during esophagectomy in humans and are probably attributable to the differences in anatomy between swine and humans, combined with the relative unfamiliarity of the surgeons with the anatomy in this animal model. During phase I of the study, these complications did not occur. One swine died during surgery because of VF, most likely due to the use of electric stimulation near the heart and the vagal nerve. It is known that swine are prone to develop VF if manipulation of the heart or central vessels is performed[21]. VF is a rare complication observed during esophagectomy in humans, and swine can be particularly susceptible to diathermia in that region, so this complication was not being considered as clinically relevant.

A strength of the study included that all procedures were performed by surgeons all expert in the field of minimally invasive upper GI oncology surgery. In our opinion it is imperative to treat patients with esophageal cancer in tertiary centers with extensive experience in the management of esophageal cancer, because of the complexity and associated morbidity of the (surgical) treatment.
Limitations of this study include the small number of cases described. Second, although cadavers are anatomical identical to living patients, blood loss and physiological functions could not be examined. However, blood loss observed during the animal study was minimal (excluding the swine with the splenic injury) and physiological functions remained stable during surgery and post-operatively in majority of swine. Furthermore, in this study it was feasible to perform resection of LNs around the lesser curvature. However, it is unclear if this causes significant damage to branches of the vagal nerve and if this affects postoperative gastric function.

Second, in two of the five esophagectomy specimens in phase I (human cadavers), one retained LN was found. In one specimen, the retained LN was located high paravesophageal and we are not convinced that this LN would have been removed during regular esophagectomy, because it was located very proximal. The clinical relevance of this retained LN may therefore be minimal. The other esophagectomy specimen contained one retained LN that was located near the distal end of the esophagus. This LN would have been removed during esophagectomy. It is inevitable that there will be a certain amount of retained LNs after thoracolaparoscopic LN dissection in some patients. We accept the possibility of missing a LN during the procedure (<10% of total amount), because in majority of patients with an early EAC, no tumor-positive LNs are found[7,22] . Moreover, thoracolaparoscopic LN dissection allows preservation of the esophagus and this potentially reduces surgical morbidity and maintains QoL.

Future research should focus on the implementation of the sentinel node (SN) concept for patients with early esophageal cancer. Pathological status of the SN is considered to predict pathological status of the locoregional LNs. By performing SN navigation surgery, the extent of the lymphadenectomy can be adjusted according to the pathological status of the SNs. One can theoretically obey extended lymphadenectomy in patients with tumor-negative SNs, whereas in case of a tumor-positive SN in the cervical area, three-field lymphadenectomy is required, following the Japanese guidelines. SN navigation surgery is a promising technique and may lead to tailored treatment and importantly, may minimize the influence of the treatment on QoL. A recent meta-analysis by Filip et al. reported an overall detection rate of 0.97 (95 % CI 0.81–0.99) an accuracy of 0.90 (95 % CI 0.74–0.97)[23] .

Another important issue is the occurrence of metachronous lesions, which were reported in two patients in the study of Abe et al. and were treated by means of an ESD. Also after endoscopic removal of an EAC in pre-existent Barrett’s esophagus this is an important issue, since metachronous lesions are reported to develop in up to 30% of patients[24–26]. It is therefore of utmost importance that in case of successful eradication of an EAC, the remaining Barrett’s segment is eradicated by means of ablation therapy (if no visible lesions are present). Recent studies suggest that in expert hands eradication rates of >90% can be achieved[[27, and Phoa et al., unpublished data].
In conclusion, thoracolaparoscopic dissection of LNIs involved in the drainage of the esophagus is feasible in human cadavers and swine. The procedure seems safe in survival procedures in swine. This technique may obviate esophagectomy in certain patients in whom the tumor can be radically resected by endoscopic means. Further human studies however, are warranted to confirm that the procedure is effective and safe before application of this technique in daily practice.

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